CHEMICAL CONSTITUENTS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SELECTED SAPINDACEAE PLANTS

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A Thesis in the Department of Chemistry,
Submitted to the Faculty of Science
in partial fulfilment of the requirement for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

AUGUST, 2023

CERTIFICATION

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DEDICATION

This research is dedicated to Almighty God, the El-Shaddai, the creator of all things, and the author of eternal life for the grace, provision, blessing and sparing my life to begin and finish this programme successfully.

To my late mother, Mrs. Bolanle Grace Iyiade and my father Mr. Gabriel Taiwo Iyiade for their support, encouragement and prayers.

ACKNOWLEDGEMENTS

My gratitude goes to my supervisors: Prof. Dorcas O. Moronkola and Prof. F. A. Dawodu for their advice, patience, immense assistance and encouragement towards the successful completion of this research programme.

My immense appreciation goes to the Head of Department of Chemistry, Prof. P. C. Onianwa and members of staff both teaching and non-teaching for their encouragement, invaluable cooperation and help. Special thanks to Prof. I. A. Oladosu, Prof. Olapeju O. Aiyelaagbe, Prof. O. O. Sonibare, Prof. Sherifat A. Aboaba, Prof. Patricia A. Onocha, Dr. Ganiyat K. Oloyede, Dr. Olaoluwa. O. Olaoluwa, Dr. A. R. Ipeayeda, Mr. Adigun, Mr. Okesanjo, Mr. Owoade and other members of Chemistry Department for the conducive environment and support.

My gratitude also goes to the Dean and members of staff, Faculty of Technology, University of Ibadan; Head of Department, Prof. I. F. Odesola, lecturers, co-technologists and administrative staff of Mechanical Engineering, Faculty of Technology, University of Ibadan including Engnr. Bolade J. S., Oyekale J. O., Rotipin K. I., Adenuga P. A., Mr. Amedu K., Iyiola S. O., Dr. Falade K. I. and Mrs. Osanebi for their cooperation and encouragement. I am most thankful to colleagues on research programme, Dr. Paul Osamudiamen, Dr. Sola Akande, Dr. Modinat Balogun, Dr. Kemi Alade, Mr. Clement Ajiboye and Mr. Emmanuel Ojah for their love and care.

My special gratitude goes to Dr. Awokoya Kehinde and Dr. Olasunkanmi of Obafemi Awolowo University for the links provided on analysis. My immense appreciation to University of Botswana through Prof. Dorcas Moronkola, Indian Institute of interactive medicine, India through Mr. Clement Ajiboye, University of KwaZulu-Natal, Durban, South Africa through Dr. Monsuru T. Kelani and Liverpool John Moore's University, United Kingdom through Dr. Olaoluwa for providing some of their high tech-equipment for the analysis of my research work samples.

My special thanks also goes to the entire members of El-Shaddai Baptist Church, Agbowo, Ibadan, to my Pastor, Rev. Segun Akande and to all other Pastors (Retired and Serving) in the church for their love, prayers and encouragement.

I am deeply indebted to my parents, Mr. Gabriel Taiwo Iyiade and Late (Mrs) Bolanle Grace Iyiade who passed on so suddenly, for their support financially, morally and in prayers. I say thanks to my siblings: Pastor Oluwaseun Iyiade, Dns. Oluwakemi Oyekola, Mrs. Oluwaladun Babatunde and Mr. Oluwafemi Iyiade for their love and encouragements.

To my darling husband, Dn. Akinpelu Oluwaseun John Adekunle, words are not enough to express my heart felt gratitude and appreciation for your love, support and encouragement on this research. I thank God for keeping our home and sustaining the love we share. To the children: Oluwaseunfunmi, Oluwaseyifunmi, Oluwasorefunmi and Oluwasolafunmi, I say thanks for your love and cooperation. May God prosper your ways and grant you speed and spirit of excellence in all endeavours.

ABSTRACT

Blighia sapida (Bs), *Lecaniodiscus cupanioides* (Lc) and *Paullinia pinnata* (Pp) (family Sapindaceae) are ethno-medicinal plants used as antimicrobial and antioxidant agents in the treatment of fevers, skin infections, dysentery and coughs. However, the information on chemical constituents and Essential Oils (EOs) from parts of these plants is limited and the bioactive compounds responsible for their activities have not been fully explored. This study was therefore, designed to extract, isolate and characterise bioactive constituents from these three plants.

The plants Bs, Lc and Pp were collected in Ibadan and authenticated at the University of Ibadan Herbarium as UIH–22407, UIH–23138 and UIH–23139, respectively. The EOs from the plants parts were obtained by hydro-distillation and analysed by Gas Chromatography (GC) and GC-Mass Spectrometry. Hexane, ethylacetate and methanol were used to obtain root extracts of Bs. The active ethylacetate fraction was subjected to chromatographic techniques. The isolated compound from Bs was characterised using spectroscopic methods. The EOs and compound isolated were subjected to antimicrobial assay following standard protocols using bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi*, *Klebsiella pneumonia*) and fungi (*Candida albicans, Aspergillus niger, Rhizopus stolonifer*, *Penicillium notatum*) with gentamicin and tioconazole as standards, respectively. Antioxidant activity was done using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay with α-tocopherol, butylated hydroxyanisole and ascorbic acid as standards. Data were analysed using descriptive statistics.

The yield of EOs from the three plants ranged from 0.3 to 5.6%. Total of 197, 153 and 142 compounds were identified from Bs, Lc and Pp, respectively. Major constituents of the EOs were α-ionone, pentadecanal, geosmin, verticiol, α–caryophyllene and (Z)-11-octadecenoic acid for Bs; (E)-2-hexenal, 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid, tetrapentacontane and 1,2,3,5,7-pentamethyl-1H-pyrrolo [2,3-f] quinolin-9-ol for Lc; caryophyllene and caryophyllene oxide for Pp. However, hexahydrofarnesyl acetone was the most commonly present at 71.4%, 50.0% and 75.0% of EOs from Bs, Lc and Pp, respectively. Infrared absorptions of the compound from Bs at 3331 cm^{-1} (O-H) and 1701 cm^{-1} (C=O); proton NMR signals (ppm) at δ 7.90 (1H, d), 6.23 (1H, d), 7.22 (1H, s), 6.80 (1H, s) and 3.82 (3H, s); carbon-13 NMR with ten carbons (CH_3, $4CH_2$, 5C) ; molecular ion at m/z 192.05 corresponded to 7-hydroxy-6-methoxy-chromen-2-one. Zone of inhibition (mm) of Bs, Lc, Pp EOs and 7-hydroxy-6-methoxy-chromen-2-one at 3.13-100.00 mg/mL ranged from 10±0 to 26±0, 10±0 to 28±0, 10±0 to 28.0±1.0 and 10±0 to 26±0, respectively, while standards ranged from 26±0 to 40±0 mm at 10 µg/mL. The IC_{50} of EOs in antioxidant activity ranged from 0.981 to 1.047 µg/mL, while that of the isolated compound was 1.027 µg/mL compared to 0.950-1.023 µg/mL for standards.

Bioactivities of the essential oils of *Blighia sapida*, *Lecaniodiscus cupanioides*, *Paullinia pinnata* and newly isolated 7-hydroxy-6-methoxy-chromen-2-one from *Blighia sapida* supported the use of the plants in ethno-medicine for treatment of microbial infections.

Keywords: *Blighia sapida*, *Lecaniodiscus cupanioides*, *Paullinia pinnata*, Essential oils, 7-hydroxy-6-methoxy-chromen-2-one

Word count: 460

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LIST OF ABBREVIATIONS

ABTS	2, 2^1-Azinobis (3-ethylbenzthiazoline)-6-sulfonic acid
APT	Attached Proton Test Spectrum
AMDIS	Automated Mass Spectral Deconvolution and Identification System
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CC	Column Chromatography
COSY	Correlation Spectroscopy
DEPT	Distortionless Enhancement of Polarization Transfer
DIZ	Diameter of Inhibition Zone
DMSO	Dimethyl Sulphur Oxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
ESR	Electron Spin Resonance spectrometry
FDA	Food and Drug Administration
GC	Gas Chromatography
GC-FTIR	Gas Chromatography – Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography- Mass Spectrometry
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Correlation Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence Spectroscopy
IC_{50}	Inhibition Concentration value
IR	Infrared Spectroscopy
MDR	Multi Drug-Resistant
MIC	Minimal Inhibitory Concentration
MRT	Maximum Recommended Temperature
MS	Mass Spectrometry
NMR	Nuclear Magnetic Resonance Spectroscopy
NOESY	Nuclear Overhauser Effect Spectroscopy
OC	Oral Candidiasis
PG	Propyl Galate
ROS	Reactive Oxygen Species

RSA	Radical Scavenging Activity
SFD	Staphylococcal Food-borne Diseases
TBHQ	Tertiary Butyl Hydroquinone
TOF-ESI-MS	High Resolution Time of Flight Mass Spectrum
UAE:	Ultrasound-assisted extraction
UTIs	Urinary Tract Infections
UV	Ultraviolet – Visible Spectroscopy
VVC	Vulvovaginal Candidiasis

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Natural products are organic compounds produced by plants, animals and microorganisms which are known as living organisms. These products can be extracted and be useful as food additives, some having various medicinal values including antimicrobial and antioxidant activities which make them to have applications traditionally and industrially as in modern medicine. The detection of these natural products with their usefulness has also encouraged synthesis or modification of existing compounds in order to increase the bioavailability and at the same time, there is increase in the use of natural medicinal plants which is because of the alarming rise in the costs of synthetic drugs apart from side effects these synthetic drugs can cause.

Plants which include shrubs, herbs, mosses, ferns, vines, green algae, grasses and trees are found almost every part of the earth, it has different applications from the beginning of one's life to the end both to man and animal. Some of these plants are common sources of food to animals which may be eaten raw like fruits and some vegetables or as processed food like rice, beans and yam. Many of these plants also have medical, industrial and social applications, these include clothing, provision of oxygen as plants release oxygen to the environment which is needed by any living man or animal, medicine which may be preventive or curative, cosmetics, constructions of furniture, animals' pen, shelter, canoe and even coffin that is used to bury human being at the end of life.

Chemical compounds that have medicinal applications were discovered in some of the herbs and spices for food seasoning (Lai and Roy, 2004; Tapsell *et al.*, 2006). According to Krishnaraju *et al.*, (2005), plants have been used to treat many infections and most of them have minimal side effect (Bibitha *et al.*, 2002; Maghrani *et al.*, 2005; Doughari, 2006). There are plants that possess chemical compounds with potential

values in the treatment of ailments but a number of them could also be poisonous (John-Dewole and Popoola, 2013). Plants are used as therapy for various human disorders and ailments which include burns, snake-bite, conjunctivitis, scalds, abdominal colic, measles, peptic ulcer, diarrhea, arthritis, rheumatism, dysentery, eye injuries, chronic ulcer, hepatitis (Esuoso and Odetokun, 2005; Ogbonnia *et al*., 2008).

The natural products from plants can be grouped into primary metabolites which are found in almost every plant and secondary metabolites which are located in specific plants for specific functions. Examples of primary metabolites are sugars, proteins and fats while examples of secondary metabolites are tannins, saponins, alkaloids, glycosides, flavonoids, terpenoids and steroids.

Crude extracts and essential oils extracted from plants with their chemical constituents have been proved to be useful for thousands of years including biological applications, example of such application is to aid defense against predatory plants (Fraenkel, 1959). Essential oils which are sometimes named volatile oils are concentrated volatile compounds that are secreted by plants which are responsible for their scents. These oils can be extracted from different parts of the plants and they are of many benefits to man which include perfumes, flavourings, antioxidant, antimicrobial, insect repellant, animal repellent, pharmaceutical applications, rituals and embalmment.

There is need to characterise chemical constituents of plants crude extracts and essential oils because of their diverse chemical compositions; also the severity of many infections caused by microorganisms with their resistance to some of the drugs, necessitate use of plants as alternative prevention and treatment strategies in medicine, pharmacy and agriculture once their antibacterial, antifungal or antioxidant activity is established.

Blighia sapida (K. Koenig), *Lecaniodiscus cupanioides* (Planch. ex Benth) and *Paullinia pinnata* (Linn) that are Sapindaceae species were selected for this study based on their wide ethnomedicinal uses. Besides, from the study, the chemical compositions, antibacterial, antifungal and antioxidant activities of various parts of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata* essential oils are limited likewise no report on antibacterial, antifungal and antioxidant activities of *Blighia sapida* root extract and no characterisation of isolate from *Blighia sapida* root

although, there were reports on crude extracts of leaf, stem bark and fruit of *Blighia sapida*, GC-MS and FT-IR analysis of *Blighia sapida* stem bark and root methanol extracts; biological activities on extracts of leaf, stem bark, root and fruit of *Lecaniodiscus cupanioides* and *Paullinia pinnata* with isolations and *Paullinia pinnata*l leaf essential oil; among the reports were antibacterial and antioxidant properties of *Blighia sapida* leaf and stem bark, as well as the isolation and characterisation of vomifoliol from *Blighia sapida* leaf, vomifoliol from *Blighia sapida* stem (Staurt *et al*, 1976) and phenanthrenol from stem bark (Ogunwande and Oladosu, 2011).

Antimicrobial and antioxidant properties of *Lecaniodiscus cupanioides* leaf (Alayande and Ashafa, 2017; Chanda and Dave, 2009; Sofidiya *et al*., 2008), antibacterial properties of the root (Kafu and Adebisi, 2015), also isolation and characterization of 3-O-[α-L-arabinofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-]-hederagenin and 3-O- [α-L-arabinopyranosyl- (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl-]-hederagenin from *Lecaniodiscus cupanioides* stem (Adesegun *et al*., 2014), Cupanioidesosides A, B and C as well as lecanioside A from roots (Messi *et al*., 2020) were reported.

Essential oil of *Paullinia pinnata* leaf was extracted but not characterized nor tested on biological activities (Ajayi *et al*., 2008). Chemical constituents and insecticidal properties of essential oil of *Paullinia pinnata* leaf were reported (Ogunwande *et al*., 2017). Antimicrobial activities of *Paullinia pinnata* leaf extract (Ikhane *et al*., 2015; Roger *et al*., 2015) and its antioxidant properties were established (Jimoh *et al*., 2007; Zamble *et al*., 2006). *Paullinia pinnata* root was reported to show antimicrobial (Annan *et al*., 2005) and antioxidant properties (Zamble *et al*., 2006). From the root, 6-O-β-3-methoxy-4-hydroxybenzoyl derivatives of lupene (Annan and Houghton, 2010), 3-*O*-isovaniolloyl lupene derivatives (Lasisi *et al*., 2015), 6α-(3-methoxy-4-hydroxybenzoyl)-lup-20(29)-ene-3-one (Jackson *et al*., 2015), β-amyrin, paullinomide A, 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthren-6-one, 5α-poriferastane- 3ß, 6α-diol, l-quebrachitol and ß-sitosterol glucopyranoside and β-sitosterol, (Dongo *et al*., 2009) were isolated.

1.2 Statement of the problem

Investigation reported on *Blighia sapida, Lecaniodiscus cupanioides* and *Paullinia pinnata,* the Sapindaceae species is limited despite the use of Sapindaceae species ethnomedicinally as antimicrobials and antioxidant agents. This study therefore aims at extracting and characterising chemical constituents of *Blighia sapida, Lecaniodiscus cupanioides* and *Paullinia pinnata* and their biological activities will be determined.

1.3 Aim of research

The study is designed to characterise the bioactive constituents of *Blighia sapida, Lecaniodiscus cupanioides* and *Paullinia pinnata*.

1.4 Objectives of research

- Extraction and characterisation of essential oils from *Blighia sapida*'s leaf, leaf stalk, stem bark, root, fruit pulp, fruit husk and seed; *Lecaniodiscus cupanioides*'s leaf, leaf stalk, stem bark and root; *Paullinia pinnata*'s leaf, leaf stalk, stem bark and root using GC/GC-MS.
- Isolation of promising chemical constituent of *Blighia sapida* root ethyl acetate by chromatographic methods and characterisation using spectroscopic techniques such as IR, NMR and MS.
- Investigation of antibacterial and antifungal activities of the essential oils of *Blighia sapida, Lecaniodiscus cupanioides* and *Paullinia pinnata*.
- Investigation of antioxidant activities of these oils of *Blighia sapida, Lecaniodiscus cupanioides* and *Paullinia pinnata*.
- Investigation of antibacterial and antifungal activities of the isolate from *Blighia sapida*.
- Investigation of antioxidant activities of the isolate from *Blighia sapida*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Natural product

Natural product is a chemical substance produced by living organisms that are found in nature and this natural product can also be synthesized. It can be a primary or secondary metabolite. Structure and activities of natural product can make it a novel chemical compound which makes it very important in diverse ways including discovery and design of drugs in pharmaceutical industries.

2.2 Secondary metabolites

Metabolism is the sum of all of the chemical reactions that take place in an organism including both primary and secondary metabolism. Molecules like lipids, proteins, nucleic acids and carbohydrates are called primary metabolites, these are formed from carbon, nitrogen and energy, they are common to all cells required for the proper functioning of cells and organisms while some proportion of carbon and energy are diverted to the synthesis of organic molecules that may have no obvious role in normal cell function like growth, photosynthesis and reproduction and these molecules are known as secondary metabolites. These secondary metabolites are present in plant family, genus and species which is sometimes use to classify the plants or determine the uses of plants as medicines, flavorings or recreational drugs. They can be classified base on their chemical structure, composition, solubility in various solvents or the pathway by which they are synthesized. These secondary metabolites have their main function by plants as defences which protect them from predators (Setyorini and Antarlina, 2022).

2.3 Essential oil

Essential oils are concentrated volatile aromatic compounds produced by plants and give plants their wonderful scents, it can be extracted from leaves, leaves stalk, flowers, fruits, wood, stem, roots, bark, peel and resins, although, many plant materials

will be needed for extraction of reasonable amount. They are secondary metabolites in plant that prevent infections and initiate cellular regeneration scents and it can be used in aromatherapy by inhalation for relaxation or stimulation also for tropical applications where they can pass through the skin into the bloodstream and surrounding tissues or ingested by water or in capsules (Virendra and Diwaker, 2007). They have been widely used in antimicrobial, insecticidal, medical and cosmetic applications (Bakkali *et al.,* 2008). These oils are usually liquid at room temperature. Phenolic compounds from essential oils have an aromatic nucleus and phenolic hydroxyl group that is known to be reactive and form hydrogen bonds with active sites of target enzymes (Farag *et al.,* 1989). Aliphatic alcohols are broad-spectrum antifungal agents (Mahmoud, 1994). Chemical composition of essential oils can vary according to the geo-climatic location, growing conditions (Andrade *et al.,* 2011) and the genetic composition of the plant (Abdelouaheb and Amadou, 2012).

2.4 Extraction of essential oil

Common extraction methods of essential oils from plant materials are hydro-distillation, steam and water distillation while others are solvent extraction, aqueous infusion, cold or hot pressing, effleurage, supercritical fluid extraction and phytonic process (Da Porto *et al.,* 2009; Lahlou, 2004; Pourmortazavi and Hajimirsadeghi, 2007).

2.4.1 Hydrodistillation method

In hydrodistillation, the circulatory distillation apparatus (Clavenger-type appratus) is often used, pulverised plant material is placed in the round bottom flask and water is added, then connect to a vertical condenser and a tube for collection of the oil; water is recycled into the round bottom flask by the three-way valve which is at the bottom of the tube since it is a continuous closed-circuit distillation device. The extraction is done between 3-4 hours and the oils obtained are usually stored prior to analysis (Karl-Heinz, 2010).

2.4.2 Steam distillation

Plant material is placed in a glass column; the lower and higher parts are connected to a water flask and a condenser respectively. Water vapour produced in the flask flows through the plant material where the essential oil is extracted and then condensed. The oil is separated from water by decantation (Boutekedjiret, 2003).

2.4.3 Solvent extraction

This method utilises solvents such as hexane, petroleum ether, methanol or ethanol to extract essential oil from the plant. The first product that comes out is called concrete while the final product collected is known as an absolute.

2.5 Chemical constituents of essential oil

Chemical compounds often found as essential oil constituents are hydrocarbons, acids, aldehydes, ketones, alcohols, ester, lactones, oxides, phenol ethers and phenols. (Abdelouaheb and Amadou, 2012; Virendra and Diwaker, 2007).

2.5.1 Hydrocarbons

Azulene, Cadinene, Camphene, Cedrene, Cymene, Farnesene, Fenchane, Menthane, Myrcene, Phellandrene, Pinene, Piperene, Limonene, Sabinene, Storene and Thujane. They are stimulant, decongestant, antiviral, antitumoral, anti-inflammatory, antiseptic, antiviral and bactericidal.

2.5.2 Acids

Citric, Benzoic, Cinnamic, Myristic, Isovaleric and Lactic. Acids are anti-inflammatory.

2.5.3 Aldehydes

Citral, Citronellal, Benzaldehyde, Cinnamaldehyde, Myrtenal and Vanillin. They are anti-fungal, anti-inflammatory, antiseptic, antiviral, bactericidal, disinfectant, sedative, tonic, vasodilators, hypotensive, calming, antipyretic and spasmolytic. They have sweet, pleasant fruity odours.

2.5.4 Ketones

Camphor, Carvone, Fenchone, Jasmine, Menthone, Pinocamphone, Pulegone, Thujone and Verbenone. They are counterirritant, anesthetic, expectorant, antipruritic, mucolytic, cell regenerating, sedative, antiviral, analgesic, digestive and vulnery. They assist the flow of mucus, ease congestion and promote wound healing and encourage the formation of scar tissue.

2.5.5 Alcohols

Geraniol, Citronellol, Menthol, Linalool, Nerol, Santalol, Terpineol and Borneol. Alcohols are antiseptic, anti-fungal, anti-inflammatory, antiseptic, antiviral, bactericidal, germicidal, spasmolytic and tonifying. When terpene is attached with hydrogen and oxygen atoms, it is called alcohol. Alcohols have pleasant fragrance and are the most therapeutic among essential oil without reported contraindications.

2.5.6 Ester

Benzoates, Bornyl acetate, Cinnamates, Eugenol acetate, Geraniol acetate, Geranyl formate, Linalyl acetate and Salicylates. Esters have a sweet, pleasant smell in the oils. Reaction of alcohols with acids forms ester. Esters are antimicrobial because of the presence of alcohol; they are anti-inflammatory, spasmolytic and sedative.

2.5.7 Lactones

Nepetalactone, Bergaptene, Costuslactone, Dihydronepetalactone, Alantrolactone, Einepetalactone, Aesculatine, Citroptene and Psoralen. They are anti-inflammatory, antiphlogistic, antipyretic, expectorant, hypotensive and febrifuge but their contraindication is allergy.

2.5.8 Oxides

Ascaridole, Bisabolone oxide, Cineol, Linalool oxide and Sclareol oxide but the most common one is Cineol. They are expectorant and stimulant.

2.5.9 Phenol ethers

Anethol, Anetol and Safrol. They are diuretic, carminative, stomachic and expectorant.

2.5.10 Phenols

Eugenol, Thymol and Carvacrol. They are antimicrobial, irritant, rubefacient and stimulant. They are crystals at room temperature.

2.6 Non-volatile/ Crude extraction

Crude extraction is the separation of medicinal portions of plant metabolites using solvents selectively through standard procedures or methods (Handa *et al*., 2008).

2.7 Extraction of non - volatile

2.7.1 Soxhlet extraction/ Hot extraction

Pulverised plant sample is placed in a thimble that is located in soxhlet apparatus. The extraction solvent is poured into the round/flat bottom flask and heated to its boiling point using heating mantle. The vapour generated made contact with the sample in the thimble. The non-volatile sample extracted goes to the flask and the solvent vapour condensed by the condenser is refluxed into the soxhlet extractor and the process continues in a cycle.

2.7.2 Maceration/ Cold extraction

Maceration is a technique in wine making and in medicinal plants research. It involves soaking plant materials either coarse or powdered in a stoppered container with a solvent at room temperature for about 3 days with frequent agitation (Handa *et al*., 2008).

2.7.3 Microwave assisted extraction

This method utilises microwave energy to facilitate partition of analytes from the sample matrix into the solvent and heat is transferred by conduction (Trusheva *et al*, 2007). Dipole rotation of the molecules disrupts hydrogen bonding; enhancing the migration of dissolved ions and promotes solvent penetration into the plant matrix (Kaufmann and Christen, 2002).

2.7.4 Ultrasound assisted extraction (UAE)/ Sonication extraction

It uses ultrasound ranging from 20 kHz to 2000 kHz, the mechanic effect from the ultrasound increases the surface contact between solvents and samples and permeability of cell walls. The physical and chemical properties of the samples subjected to ultrasound disrupts the plant cell wall which facilitate release of compounds and enhancing mass transport of the solvents into the plant cells (Handa *et al*., 2008).

2.8 Chemical constituents of non - volatile

2.8.1 Reducing sugar

Reducing sugar is any sugar that has a free aldehyde group or a free ketone group which makes it capable of acting as a reducing agent (Pratt and Cornely, 2013), they form one or more compounds containing an aldehyde group in aqueous medium. Test

reagents are Benedict's reagent (CuSO4 / citrate) and Fehlings reagent (CuSO4 / tartrate).

2.8.2 Alkaloids

Alkaloid is any of a class of naturally occurring organic nitrogen-containing bases and are often classified on the basis of their chemical structure. Examples are tropanes, pyridines, pyrrolidines, pyrrolizidines, indoles, quinolines and the terpenoids, isoquinolines and steroids. Dragendroff, Wagner or Hager's Test can be used to test for alkaloids. Alkaloids are active in the blood vessels, autonomic nervous system, respiratory system, gastrointestinal tract, uterus and aids promotion of diuresis, cure of malaria, malignant diseases and infections (Encyclopedia Britannica, 2017).

2.8.3 Tannins

Tannins are astringents and are polyphenolic biomolecule that bind and precipitate proteins and various other organic compounds like amino acids and alkaloids (Wikipedia, 2018). They are useful as pestisides (Wikipedia, 2018). Braymer or Gelatin Test can be used in screening for tannins. This secondary metabolite is used in the prevention and treatment of inflammation, ulcerated tissues and cancer (Aiyegora and Okoh, 2010).

2.8.4 Glycoside

Glycosides are chemical compounds in which a sugar is bound to another functional group by a glycosidic bond (Wikipedia, 2018); they are compounds containing a carbohydrate (glycone) and a noncarbohydrate (aglycone) residue in the same molecule (Georges-Louis Friedli, 2009). PPresene of this compound can be detected by Modified Borntrager, Keller-Kiliani, Liebermann or Concentrate H_2SO_4 Test. Plants – based laxatives are derived from glycosides (Hietala *et al*., 1987).

2.8.5 Saponins

Saponins are glycosides with foaming and bitter taste characteristics; the ability of saponins to foam is as a result of the combination of the glycone (sugar) which is hydrophilic and aglycone (sapogenin) which is lipophilic. They have anti – diabetic and hypocholesteremic activities. They are also used in the treatment of inflammation (Desai *et al*., 2009).

2.8.6 Steroids

Steroids have four rings arranged in a peculiar configuration of which three of the rings are cyclohexane while the fourth ring is cyclopentane. They are important in pharmacy because of their relationships with sex hormones (Okwu, 2001) and their anti-inflammatory effect (Rhen and Cidlowski, 2005).

2.8.7 Flavonoids

Flavonoids are compounds of 15-carbon skeleton with two phenyl rings and one heterocyclic ring; they have antioxidants effects on human health and fitness which is done by scavenging or chelating (Kessler *et al*, 2003). They prevent deteriation in cognitive performance and neurodegenerative diseases (Calabrece *et al.*, 2003).

2.8.8 Resins

They are organic compounds exuded from plant or tree which are flammable, sticky and insoluble in water and they are used in organic synthesis. They usually exist as gum resins when combined with gum, oleoresins when combined with essential oils, oleo-gum resins when combined with essential oils and gums, balsams when with acid and glycosides when combined with sugar.

2.8.9 Cardiac glycosides

They are compounds containing glycone and aglycone; the aglycone being a steroidal nucleus and are used in the treatment of congestive heart failure and cardiac arrhythmia (Kumar and Shukla, 2023).

2.8.10 Phenols

Phenols are aromatic hydrocarbon bonded with hydroxyl group. They are antioxidants and have properties like anti – diabetics, anti – cancer and anti – inflammatory (Nagavani *et al*, 2010).

2.8.11 Anthraquinones

Anthraquinones are aromatic compounds with formula of $C_{14}H_8O_2$. They have bactericidal, astringent, anti – inflammatory, purgative and moderate anti – tumor activities (Agbafor and Nwachukwu, 2011).

2.8.12 Anthocyanins

Anthocyanins are aromatic compounds and secondary metabolites of plants which include anthocyanins, flavones, flavanones and flavanols. They are health-improving substances which have been detected in grains and regarded as food supplements diet (Dinabandhu and Mahapatro, 2015).

2.8.13 Terpenoids

Terpenoids are the most widespread and largest class of secondary metabolites. Terpene has C5 units as integral number; all terpenoids are known to be derived from the branched C5 isoprene unit known as 2-methyl-1,3- butadiene which is used to classify them into different classes of terpenoids (hemi-, mono-, sesqui-, di-, sester-, tri-, tetra) (Heras *et al*., 2003).

2.8.14 Coumarins

Coumarins belong to benzopyrone family and the principal members are flavonoids. These coumarins have been reported of bacteriostatic, anti-tumor and physiological properties, their biological properties make them important in drug development. The most common coumarins found in nature are esculetin, scopoletin and umbelliferone (Annunziata *et al*., 2020; Jain and Joshi, 2012).

2.9 Techniques in organic chemistry

2.9.1 Chromatographic techniques

2. 9.1.1 Thin Layer Chromatography (TLC)

Glass plates are coated with layers of solid stationary phase which can be silica gel, kieselguhr, and alumina or cellulose powder. Polyamides or sephadex can also be used in special application. TLC plates can also be available as pre-coated TLC plates. The plates are loaded with the spot of samples to investigate using capillary tube; this is then developed in an appropriate solvent. Coloured constituents can be viewed without staining the plates, fluorescent constituents can be viewed under ultra-violet lamp while iodine vapour or vanillin spray can be used to stain fluorescent constituents on the plates in order to locate them.

2.9.1.2 Column Chromatography (CC)

Column chromatography involves the use of cylindrical column packed with solid stationary phase like silica gel or alumina, the column is loaded with sample and

chromatogram is developed by allowing the liquid mobile phase like hexane, ethylacetate or methanol to flow through the column under gravity or using compressed gas as pressure on the top of the column to move solvent down the column for rapid separation. The mobile phase can be combination of two solvents in varied percentage. The eluates are collected as fractions of equal volume, dried and weighed. The residues are spotted on the TLC plate to know the fractions of similar constituents which can be pooled together and those that will need further purification.

2.9.1.3 Gas Chromatography (GC)

Gas chromatography (GC) is used for separating and analyzing compounds that can be vaporized without decomposition. It is technique that is widely used to analyze hydrocarbon mixtures. It is used for separating different components of a mixture or for identifying compounds. Gas chromatography has both mobile and stationary phase, the separation of the chemical constituents depend on the affinity of these constituents to these phases which causes each compound to elute at a different time and this time of elusion is called retention time which is compared to know the compound present. The mobile phase is the carrier gas which is an inert gas for example, helium or unreactive gas for example, nitrogen; it moves constituents of a sample through the stationary phase which is liquid or polymer on an inert solid support which is embedded in a column that is a piece of glass or metal tubing.

Gas Chromatography – Mass Spectrometry (GC-MS): Gas chromatograph is used to separate different compounds while mass spectrometry is coupled with gas chromatography to analyze complex mixture like natural products. Mass Spectrometry is the most widely used spectral isolation detection method for Gas Chromatograph. Coupling a separation technique with mass spectrometry gives advantage of obtaining a spectrum used for identifying the isolated product. Mass spectrometry helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions; the mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. The stream of separated compounds from GC is fed online into the ion source, a metallic filament to which voltage is applied, this filament emits electrons which ionize the compounds and the ions can then further fragment, yielding predictable patterns. Intact ions and fragments pass into the mass spectrometer's analyzer and are eventually detected. For further identification,

computer or manual library search is performed before the spectrum is examined and interpreted to check whether the spectrum belongs to an existing collection (Chauhan *et al*., 2014; Sasaki and Wilkins, 1999).

Gas Chromatograhy – Fourier Transform Infrared Spectroscopy (GC-FTIR): GC-FTIR is used for the separation and identification of complex mixtures of organic compounds by coupling GC with FTIR. It is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. Infrared light is guided through an interferometer and then through the sample (or vice versa); the sample's spectrum is always compared to a reference (Sasaki and Wilkins, 1999).

2.9.1.4 High Performance Liquid Chromatography (HPLC)

HPLC is similar to GC but it is used for samples in liquid phase where pressure is used to force liquid to pass through a column packed with silica gel, alumina or cellulose among others. The mobile phase in this technique is liquid for example, hexane, dichloromethane, methanol and acetonitrile.

2.9.2 Spectroscopic techniques

2.9.2.1 Ultraviolet – Visible Spectroscopy (UV)

Ultraviolet and visible spectroscopy is primarily used to determine the multiple bond or aromatic conjugation within molecules. It requires electromagnetic radiation of high energy where ultraviolet region corresponds to 400-200 nm and 800-400 nm for visible region (Kalsi, 2012).

2.9.2.2 Infrared Spectroscopy (IR)

Infrared Spectroscopy is used for the detection of functional groups for structural identification by passing a beam of infrared light through the sample and recording the infrared spectrum of such sample which is as a result of absorption that occurs when the frequency of infrared is the same as vibrational frequency of the bond in the sample, the transmitted light shows amount of energy absorbed (Kalsi, 2012).

2.9.2.3 Mass Spectrometry (MS)

Mass Spectrometry is used to measure exact molecular weight in order to determine exact molecular formular and to indicate within a molecule the points at which it prefers to fragment in order to characterize organic molecules (Kalsi, 2012).

2.9.2.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy involves the change of the spin state of a nuclear magnetic moment when the nucleus absorbs electromagnetic radiation in a strong magnetic field; there are two types which are proton NMR and carbon-13 NMR (Kalsi, 2012).

^{1}H – ^{1}H Correlation Spectroscopy (^{1}H – ^{1}H COSY): This spectrum is a two dimensional spectrum of protom NMR (2D) that shows correlation between protons that are coupled to each other once they form a box pattern symmetric to their diagonal in ^{1}H NMR spectrum. Chemical shift axes that are identical are plotted orthogonally and the resolution of the two are different.

Attached Proton Test (APT): It is an experiment of one dimension of carbon - 13 (1D) that records signals for all the carbons where methine (CH), methyl (CH_3) are positive signals and quartenary (C) and methylene (CH_2) are negative signals.

Distortionless Enhancement of Polarization Transfer (DEPT): This is 1D experiment that shows only protonated carbons (CH, CH_2 and CH_3). They are DEPT – 45 that shows all protonated carbons and are all positive signals, DEPT – 135 also shows all protonated carbons, CH and CH_3 are positive signals, CH_2 is a negative signal and DEPT – 90 that shows only CH and as positive signal.

Heteronuclear Single Quantum Coherence Spectroscopy (^{1}H – ^{13}C HSQC): This is two dimentional spectrum (2D) that shows which carbons are directly attached to which protons. Proton spectrum is at horizontal axis while carbon-13 spectrum is at the vertical axis.

Heteronuclear Multiple Bond Correlation Spectroscopy (^{1}H – ^{13}C HMBC): This is 2D that records correlations between carbons and protons that are separated by multiple bonds. Also, horizontal axis is for proton spectrum while vertical axis is carbon -13 spectrum. It is useful in assignment of carbons without attachment of protons.

Nuclear Overhauser Effect Spectroscopy (NOESY): This is 2D that shows all proton – proton Nuclear Overhauser Effects (NOEs) that occurs in a compound of a single experiment. It is similar to spectrum of ^{1}H – ^{1}H COSY, the two orthogonal axis are proton chemical shifts and its spectrum also appears on the diagonal.

2.10 Bioassay techniques

2.10.1 Antimicrobial activity

Microorganisms are living organisms that are microscopic in nature. They may be single celled or multicelled (Madigan and Martinko, 2006). Microorganisms exist everywhere because they can adapt and survive wherever they are found (Choi, 2013). They act as decomposers making them important in ecosystem by recycling nutrients. Some microorganisms are pathogenic and cause disease and even death in plants and animals. Examples of microorganisms are bacteria and fungi. A bacterium is a prokaryote (without nucleus or other organelles) while a fungus is eukaryote (a more complex type of cellular organism, closer in evolutionary terms to a human cell).

Bacteria live in symbiotic and parasitic relationships with plants and animals. These organisms reproduce asexually by binary fission which occurs when a single cell divides to form two new cells called daughter cells. Each daughter cell contains an exact copy of the genetic information contained in the parent cell. The process continues with each daughter cell giving rise to a generation of two new cells. Bacteria inhabit soil, water, acidic hot springs, radioactive waste (Fredrickson *et al*, 2004). Some bacteria are beneficial and immune system makes some harmless while several others are pathogenic and antibiotics are used to treat bacterial infections. Bacteria provide the nutrients needed to sustain life by converting dissolved compounds such as hydrogen sulphide and methane to energy.

Staphylococcus aureus: According to Konrad *et al.,* 2009, about 20–30 % of individuals is persistent carrier of *Staphylococcus aureus* and often found associated with skin, skin glands and mucous membranes. *S. aureus* is one of the main causes of hospital and community-acquired infections which can result in serious consequences (Diekema *et al.,* 2001). It is a gram-positive bacteria, it affects the bloodstream, skin, soft tissues and lower respiratory tracts. *S. aureus* is often responsible for toxin-mediated diseases, such as toxic shock syndrome, scalded skin syndrome, staphylococcal food-borne diseases (SFD) and its remarkable potential to develop antimicrobial resistance (Konrad *et al.,* 2009).

Escherichia coli: It is a specie of the genus Escherichia. It contains mostly motile gram-negative bacilli within the family Enterobacteriaceae and the tribe Escherichia (Nataro and Kaper, 1998). According to Nataro *et al*., 2004, *E. coli* colonize the

gastrointestinal tract of human infants within a few hours after birth and co-exist with human host in good health and with mutual benefit for decades. It rarely causes disease except in immunocompromised hosts or where the normal gastrointestinal barriers are breached. Three general clinical syndromes can result from infection with inherently pathogenic *E. coli* strains which are enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis. *E. coli* can be isolated from the stool and sent to a qualified reference laboratory for definitive identification (Nataro and Kaper, 1998).

Bacillus subtilis: It is a gram-positive, aerobic, spore-forming soil bacterium ubiquitous in the environment and it can divide symmetrically to make two daughter cells that is, binary fission or asymmetrically to produce a single endospore. It is found in soil and the gastrointestinal tract of ruminants and humans, its spores has beneficial effect on the balance of the intestinal microflora which is the rationale for its general use as a probiotic preparation in the treatment or prevention of intestinal disorders, it is used as a pharmaceutical preparation for oral use (Oggioni *et al.,* 1998). It is used as an immunostimulatory aid in the treatment of gut and urinary tract diseases such as Rotavirus and Shigella (Mazza, 1994).

Pseudomonas aeruginosa: According to Gellatly and Hancock, 2013, gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen that normally inhabits the soil and surfaces in aqueous environments, its infections demonstrate high morbidity and mortality, it is one of the most common pathogens causing respiratory infections of hospitalized patients. *Pseudomonas aeruginosa* is common in health care institutions because of the poor health status of the patients, the high carriage rate of multidrug-resistant strains in hospital wards and prior use of broad spectrum antibiotics (Otter *et al.,* 2011). Immune deficiencies that can predispose to *Pseudomonas* infection include old age, neutropenia due to cancer chemotherapy or immunosuppression due to organ transplant (Gellatly and Hancock, 2013). Serious *P. aeruginosa* infections are often nosocomial and nearly all are associated with compromised host defenses such as in neutropenia, severe burns or cystic fibrosis (Lyczak *et al.,* 2000). Some of the infections caused by *P. aeruginosa* are soft tissue, urinary tract, bacteremia, diabetic foot, respiratory/pneumonia, otitis externa (swimmer's ear), keratitis (corneal infection), otitis media folliculitis (hot tub rash) and infections by *P. aeruginosa* are notoriously difficult to treat due to its intrinsic ability

to resist many classes of antibiotics as well as its ability to acquire resistance. (Gellatly and Hancock, 2013).

Salmonellae typhi: *Salmonella enterica* subspecies enterica serovar Typhi (*Salmonella typhi*) is a rod-shaped gram-negative facultative anaerobe bacterium belonging to the Enterobacteriaceae family (Zhang *et al.,* 2008). It causes typhoid fever in humans which is a systemic febrile illness, it is transmitted by the fecal-oral route through contaminated food and water, and it can lead to death (Kidgella *et al.,* 2002). Among more than 2,300 closely-related Salmonella serovars bacteria recognized, *S. typhi* is the only one that is pathogenic exclusively for humans, in whom it causes typhoid or enteric fever (Zhang *et al.,* 2008). It is also shown that multi-drug resistance has occurred to the few available antibiotics (Zhang *et al.,* 2008). Three lineages among multidrug-resistant (MDR) Salmonella enteric serotype Typhi isolates in the Gulf of Guinea region in Africa were identified during the 2000s (Baltazar *et al.,* 2015).

Klebsiella pneumoniae: This organism is a member of the human intestine flora; it is frequently associated with hospital-acquired infection. Diabetes mellitus is known as a major risk factor for *Klebsiella pneumoniae* infection (Sung-Sheng *et al.,* 2010). Metastatic infections such as pyogenic brain abscess, meningitis and endophthalmitis are the most important characteristics of *K. pneumoniae* infections (Sung-Sheng *et al.,* 2010). It is the second most common cause of gram-negative bacteremia after *Escherichia coli* (Yinnon *et al.,* 1996).

Fungi have several unicellular species. They can be reproduced both sexually and asexually by budding or binary fission, as well by producing spores. Of all fungi (611,000), only around 600 species are human pathogens (Brown *et al.,* 2012) and this include fungi that cause relatively mild infections of the skin, for example, Dermatophytes and Malassezia species; fungi that cause severe cutaneous infections, for example, *Sporotrix schenkii* and fungi that have the potential to cause life-threatening systemic infections, for example, *Aspergillus fumigatus, Cryptococcus neoformans, Histoplasma capsulatum* and *Candida albicans* (François *et al.,* 2013). The important characteristics to identify fungi morphologically are the shape, size and colour of spores.

Candida albicans: In most individuals, *Candida albicans* resides as a lifelong, harmless commensal but under some circumstances it can cause infections that range from superficial infections of the skin to life-threatening systemic infections (François *et al.,* 2013). *Candida spp* are the fourth most common cause of hospital-acquired systemic infections in the United States with crude mortality rates of up to 50 % (Pfaller and Diekema, 2007; Pfaller and Diekema, 2010). *C. albicans* can cause two major types of infections in humans which are superficial infections, such as oral or vaginal candidiasis and life-threatening systemic infections (François *et al.,* 2013). In healthy individuals this colonization generally remains benign, mildly immunocompromised individuals can frequently suffer from recalcitrant infections of the oral cavity which are termed oral candidiasis (OC) (Ruhnke, 2002). HIV is a major risk factor for developing OC (François *et al.,* 2013). Further risk factors for developing OC include the wearing of dentures and extremes of age (Pappas *et al.,* 2009). Other risk factors include central venous catheters which allow direct access of the fungus to the bloodstream, the application of broad-spectrum antibacterials which enable fungal over growth and trauma or gastrointestinal surgery which disrupts mucosal barriers (Spellberg, 2012). Such infections are predominantly caused by *C. albicans* and can affect the oropharynx and/or the esophagus of persons with dysfunctions of the adaptive immune system (François *et al.,* 2013). Many women also suffer from vulvovaginal candidiasis (VVC) (Sobel, 2007).

Aspergillus niger: It is a common contaminant of food and causes a disease called black mould on certain fruits and vegetables, this can cause economic losses due to spoilage. *A. niger* causes a common postharvest disease of onions, in which the black conidia can be observed between the scales of the bulb. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation. The spores are widespread and are often associated with organic materials and soil. Though disease of human through A. niger is rare but the most common symptoms of pulmonary aspergillosis are a chronic productive cough and hemoptysis (coughing up blood). Amputation of the right foot had to be performed on a malnourished 70 year old man who presented with a painful black "gangrenous appearing" mass on the right foot and tissue samples showed dark pigmented fungal fruiting heads with double sterigmata in which *Aspergillus niger* was identified (Louthrenoo *et al.,* 1990).

Rhizopus stolonifer: This organism is commonly known as black bread mold causes post-harvest diseases on many fruits and vegetables; it is the causal agent of rhizopus rot disease of organic substrates including fruits, vegetables (Hernández-Lauzardo, 2006), jellies, syrups, leather, bread, peanuts and tobacco. It takes food and nutrients from the bread and causes damage to the surface where it lives. It can cause infection in animals including human (Chinn and Diamond, 1982). They reproduce by forming asexual and sexual spores, asexual spores are formed within pinhead-like sporangia and sexual reproduction happens only when opposite mating types come in contact.

Penicillium notatum: This is sometimes called *Penicillium chrysogenum* is a species of fungus in the family *Trichocomaceae*; species of *Penicillium* are ubiquitous saprobes, whose numerous conidia are easily distributed through the atmosphere and are common in soils (Phuwiwat and Soytong, 2001). *Penicillium* produces asexually; it usually reproduces by forming dry chains of spores or conidia. The name *Penicillium* comes from the word brush which refers to the appearance of spores in *Penicillium*. It is common in temperate and subtropical regions and can be found on salted food products (Samson *et al.,* 2010). They are post-harvest pathogens; these species are one of the most common causes of fungal spoilage in fruits and vegetables.

Antimicrobial Test can be used to examine antimicrobial activity using various methods namely thin-layer chromatography (TLC)–bioautography and this include direct bioautography, agar diffusion and agar overlay bioassay; diffusion method which are agar disk-diffusion method, agar well diffusion method, agar plug diffusion method, antimicrobial gradient method (Etest), poisoned food method and cross streak method; dilution methods which are agar dilution and broth dilution method; flow cytofluorometric method; ATP bioluminescence assay and time-kill test (Mounyr *et al*., 2016).

The Susceptibility Assay Methods that are commonly used for antimicrobial test were described according to Jorgensen and Ferraro, (2009) as follows:

Broth dilution assay: This procedure involved preparing two-fold dilutions of antibiotics in a liquid growth medium dispensed in test tubes (Jorgensen and Turnidge, 2007). The antibiotic-containing tubes were inoculated with a standardized bacterial suspension of $1–5\times10^5$ CFU/ mL. This is incubated overnight at 35 °C; the tubes were then examined for visible bacterial growth which is shown by turbidity. Standard trays

contain 96 wells, each containing a volume of 0.1 mL that allows approximately 12 antibiotics to be tested in a range of 8 two-fold dilutions in a single tray; the minimal inhibitory concentrations (MICs) are known using a manual or determined by automated viewing device for inspecting each of the panel wells for growth of organism after incubation (Jorgensen and Turnidge, 2007). MIC is known by the lowest concentration of antibiotic that prevented growth and this MIC makes it possible to generate quantitative result, only that the preparation of the antibiotic solutions for each test, the relatively large amount of reagents and space required for each test are tedious and there is possibility of errors (Jorgensen and Ferraro, 2009).

Antimicrobial gradient method: This principle of this method is the establishment of antimicrobial concentration gradient that is in an agar medium to determine susceptibility. The method of gradient diffusion has intrinsic flexibility which is able to test the drugs the laboratory chooses. Thin plastic test strips for example, E test strips are impregnated on the underside with a dried antibiotic concentration gradient and are marked on the upper surface with a concentration scale used, these strips may be put in a radial fashion on the surface of an agar plate (150 mm) that has been inoculated with a standardized organism suspension like the one used for a disk diffusion test; the strips are viewed from the top of the plate, after overnight incubation. The MIC is determined by the intersection of the lower part of the ellipse shaped growth inhibition area with the test strip. The strips are expensive and makes this method best suited to situations in which an MIC for only 1 or 2 drugs is needed or when a fastidious organism requiring enriched medium or special incubation atmosphere is to be tested for example, penicillin and ceftriaxone with pneumococci (Citron *et al.*, 1991; Huang *et al*., 1992; Jorgensen *et al*., 1994 and Jorgensen and Ferraro, 2009).

Disk diffusion assay: The disk diffusion susceptibility method (Jorgensen and Turnidge, 2007) is simple and practical and has been well-standardized. The test is performed by applying a bacterial inoculum of approximately $1–2\times10^8$ CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. Up to 12 commercially-prepared, fixed concentrations, paper antibiotic disks are put on the surface of inoculated agar. Plates are incubated for 16–24 hours at 35 °C before the determination of results. Around each of the antibiotic disks, measurement of zones of

growth inhibition is taken. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The results of the disk diffusion test are qualitative because the result is taken as susceptible, intermediate or resistant unlike MIC. Although, an approximate MIC can be calculated with some organisms and antibiotics by comparing zone sizes with standard curves of that species and drug stored in an algorithm (Korgenski and Daly, 1998; Nijs *et al.*, 2003). The disk method has the advantages of the test simplicity that does not require any special equipment, the provision of categorical results easily interpreted by all clinicians, flexibility in selection of disks for testing and it is the cheapest of all susceptibility methods, only that it is limited in the lack of mechanization or automation of the test (Jorgensen and Ferraro, 2009).

Automated instrument systems: Use of instrumentation can standardize the reading of end points and often produce susceptibility test results in a shorter period than manual readings because sensitive optical detection systems allow detection of subtle changes in bacterial growth. According to Richter and Ferraro, (2007), there are four automated instruments presently cleared by the FDA for use in the United States. Three of these can generate rapid susceptibility test results within 3.5-16 hours while the fourth is an overnight system. These four systems are the MicroScan WalkAway, BD Phoenix Automated Microbiology System, Vitek 2 System and Sensititre ARIS 2X which is an overnight system (Jorgensen and Ferraro, 2009).

2.10.2 Antioxidant activity

Antioxidant is any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate (Antolovich *et al.*, 2002). Oxidation is a chemical reaction that can produce free radicals and leads to chain reactions that may result in cells damage. A side effect of the oxidative metabolism for the survival of cells is the production of free radicals and other reactive oxygen species (ROS) that cause oxidative changes (Antolovich *et al.*, 2002). ROS are various forms of activated oxygen which include free radicals such as superoxide anion radicals and hydroxyl radicals, as well as non-free radical like hydrogen peroxide species and the singlet oxygen (Halliwel 1995; Squadriato and Peyor, 1998; Yildrim *et al.*, 2001). Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism and ROS react easily

with free radicals to become radicals themselves (Vishwanath *et al.*, 2012). When antioxidant capacity of the organism is exceeded by excessive generation of ROS which can be induced by various stimuli, it leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer (Kourounakis *et al.*, 1999; Gulcin *et al.*, 2002 and Gulcin *et al.*, 2003); oxidation can cause food spoilage like rancidity, deterioration of the nutritional quality, colour, flavour, texture and safety of foods (Shahidi *et al.*, 1992). Oxidative stress in humans arises from an imbalance in the antioxidant status (Antolovich *et al.*, 2002).

This oxidation activity can be prevented through endogenous defences or consumption of dietary antioxidants. Antioxidants terminate the chain reactions that may result in cells damage. Presence of hydroxyl groups in their chemical structure causes their antioxidant effect (Shahidi, 2000). Antioxidants protect the food against oxidative spoilage. According to Antolovich *et al*., (2002), methods of assessing antioxidant behaviour focus on activity in foods or bioactivity in humans; antioxidant activity can be measured by its ability to scavenge free radicals generated in aqueous and lipophilic phases.

There are different methods to establish antioxidant activity. These are electron transfer methods (ET) and hydrogen atom transfer (HAT). ET are DPPH free radical scavenging, ferric reducing antioxidant power (FRAP), copper(II) reduction capacity, trolox equivalent antioxidant capacity (TEAC) decolourization and total phenols by Folin-Ciocalteu while HAT are lipid peroxidation inhibition capacity (LPIC), oxygen radical absorbance capacity (ORAC), hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline), scavenging of super oxide radical formation by alkaline (SASA), inhibited oxygen uptake (IOC), total radical trapping antioxidant parameter (TRAP), ABTS radical scavenging, crocin bleaching nitric oxide radical inhibition activity and scavenging of H_2O_2 radicals (Moharram and Youssef, 2014).

Electron spin resonance (ESR) spectrometry which is the only analytical technique that can specifically detect the free radicals; 2, 2^1-Azinobis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) assay procedure which is based on inhibition of the production of ABTS radical cation, it does not involve substrate and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical which absorbs at 517 nm and the decrease in absorbance is monitored, it is also substrate-free.

DPPH is a free radical that is stable but a scavenger for other radicals; it is a dark-coloured crystalline powder but deep violet colour in solution, it becomes colourless or pale yellow when neutralized and this makes visual monitoring of the reaction possible and the number of initial radicals can be counted from the change in the optical absorption or in the electron paramagnetic resonance signal of the DPPH (Wang and Zhang, 2023).

Antioxidants have many industrial uses such as dietary supplements, preservatives in food and cosmetics, prevention of degradation of polymers and gasoline, stabilizers in fuels and lubricants to prevent oxidation, prevention of the polymerization that leads to the formation of engine-fouling residues in gasoline (Boozer *et al.*, 1955; Dabelstein *et al.*, 2007). The synthetic antioxidants are widely used in the food industry (Adegoke *et al.*, 1998) which includes butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl galate (PG) and tertiary butyl hydroquinone (TBHQ) but being suspected to cause or promote negative health effects (Barlow, 1990; Namiki, 1990; Pokorny, 1991) and this makes the interest in natural antioxidants important.

2.11 Sapindaceae family

Sapindaceae which is also called soapberry family consists of 150 genera that consist of more than 2000 species including ackee, soapberries, pitomba, pulasan, buckeye, maple, horse chestnut, guarana, rambutan, longan, guinip/mamoncillo, korlan and lychee.

2.11.1 Classification of sapindaceae

Twenty-seven genera of Sapindaceae are native to Africa (Acevedo-Rodríguez, 2011a, Acevedo-Rodríguez, 2011b, Acevedo-Rodríguez, 2012, Buerki *et al.*, 2010, Buerki *et al.*, 2011, Harrington and Gadek, 2010 and Jiménez *et al.*, 2011), their locations and genera are as follows:

i. Tropical Africa - *Blighia, Eriocoelum, Placodiscus, Aporrhiza, Lecaniodiscus, Haplocoelum* and *Lychnodiscus*.
ii. East Tropical Africa - *Stadmania*.
iii. Western Tropical Africa - *Chytranthus* and *Macphersonia*.
iv. West Africa - *Pancovi Blighiaa, Laccodiscus* and *Radlkofera*.
v. West Equatorial Africa - *Pseudopancovia*.
vi. East Africa - *Filicium* and *Camptolepsis*.

vii. Southern Africa - *Pappea, Zanha, Atalaya, Smelophyllum, Erythrophysa* and *Deinbollia.*

viii. Central Africa - *Blighiopsis*.

ix. Central and East Africa - *Haplocoelopsis*.

x. Africa - *Paullinia, Majidea* and *Hippobromus*.

2.11.2 Biological activities of distinct Sapindaceae

Antimicrobial activity: Leaf extract of *Sapindus emarginatus* was reported to possess antimicrobial activity against bacteria (*Escherichia coli, Psuedomonas aeruginosa, Pseudomonas testosteroni, Staphylococcus epidermis, Klebsiella pneumoniae, Bacillus subtilis and Proteus morganii*) and fungus (*Aspergillus niger*) (Deepa *et al*, 2012; Nair et al, 2005). Methanolic and aqueous leaf extract of *Sapindus mukorossi* was reported to have antibacterial potential against *E. coli, P. aeruginosa, P. morganii, P. testosterone, K. pneumoniae, B. subtilis* and antifungal effect against *Aspergillus niger* (Deepa *et al*, 2012; Nair *et al.,* 2005). Ubulom *et al.,* in 2013 reported that the ethanol extract of *Blighia sapida* leaves and stem bark possess antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. Aqueous extracts of *Blighia sapida* husks had antibacterial effect against the growth of *Staphylococcus aureus* and *Escherichia coli* (John-Dewole and Popoola, 2013).

Antioxidant activity: The leaves of *Sapindus emarginatus* were reported to exhibit antioxidant activity (Kumar, 2010). Fruit peels of *Nephelium lappaceum* also possess antioxidant activity (Nont *et al*, 2010). Husks of *Xanthoceras sorbifolia* show antioxidant activity (Chun-Yan *et al*, 2016). Methanol extracts of *Blighia sapida* fruits and leaves were reported to have antioxidant activity (Hamzah *et al.,* 2013; Dossou *et al.,* 2014; Oloyede *et al.,* 2014).

Cytotoxicity screening/ Brine shrimp: *Sapindus trifoliatus* fruit exhibited cytotoxic activity in brine shrimp lethality bioassay (Chaitali *et al.,* 2010).

Antihyperglycemic and antidiabetic activity: Leaves extract of *Sapindus emarginatus* showed antihyperglycemic and antidiabetic activity (Srikanth and Muralidharan 2009).

Anti-insect activity or Larvicidal activity: *Sapindus emarginatus* fruit extract showed the presence of saponins which demonstrated larvicidal activity against the larvae of mosquito *Aedes aegypti* (Koodalingam *et al.,* 2009; Koodalingam *et al.,* 2011; Surendran *et al.,* 2009*).* Ethanolic extract of *Sapindus mukorossi* was reported to have significant mortality on insects *Sitophilus oryzae* and *Pediculus humanus* (Rahman *et*

al., 2007). *Dodonaea viscosa* Jacq. also exhibited anti-insect activities (Martina *et al.*, 2015).

Piscidal activity: *Sapindus emarginatus* pericarps demonstrated good piscidal activity (Sharma, 2011).

Antifertility and antiandrogenic activity: The fruit extract of *Sapindus emarginatus* had antifertility and antiandrogenic activity (Venkatesh, 2002).

Antihyperlipidemic activity: The saponins from *Sapindus emarginatus* fruit extract were found to have significant antihyperlipidemic activity (Srikanth and Muralidharan, 2009).

Molluscicidal activity: Hederegenin isolated from *Sapindus mukorossi* demonstrated molluscicidal activity (Zikova and Krivenchuk, 1970).

CNS activity: Methanolic extract from the fruit of *Sapindus emarginatus* was found to produce CNS depressant activity (Srikanth and Muralidharan, 2009). The alcoholic and aqueous extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* were reported to exhibit the dose dependant sound and touch responses by producing moderate to slight depression relating to awareness and alertness (Chandra and Kuppast, 2014).

2.11.3 Isolated compounds from distinct Sapindaceae

Cardiospermum corindum L.: Friedelin [Fig. 2.1], friedelinol [Fig. 2.2], umuhengerin [Fig. 2.3], umbelliferone [Fig. 2.4], scopoletin [Fig. 2.5], luteolin 3′,4′-dimethyl ether [Fig. 2.6], chrysoeriol [Fig. 2.7], epidermin [Fig. 2.8] and (L)-quebrachitol [Fig. 2.9] were isolated and characterised from the aerial parts of this plant (Silva *et al*., 2014).

Sapindus emarginatus: β- sitosterol [Fig. 2.10] (Kanchanapoom, 2001; Sharma, 2011).

Nephelium lappaceum: Ellagic acid [Fig. 2.11], corilagin [Fig. 2.12] and geraniin [Fig. 2.13] from *Nephelium lappaceum* L. Peels (Nont *et al*, 2010).

Dodonaea viscosa Jacq.: Lupeol [Fig. 2.14], stigmasterol [Fig. 2.15], stigmast-7-en-3-ol [Fig. 2.16] and labdane [Fig. 2.17] from the leaves of *Dodonaea viscosa* Jacq (Martina *et al*, 2015).

Xanthoceras sorbifolia: D-mannitol orsellinate [Fig. 2.18] from the husks of *Xanthoceras sorbifolia* (Chun-Yan *et al*, 2016).

Molinaea retusa: 2″,3″,4″,6′-de-*O*-acetylcupacinoside [Fig. 2.19], cupacinoside [Fig. 2.20] and 6′-de-*O*-acetylcupacinoside [Fig. 2.21] from *Molinaea retusa* root (Alexander *et al*, 2013).

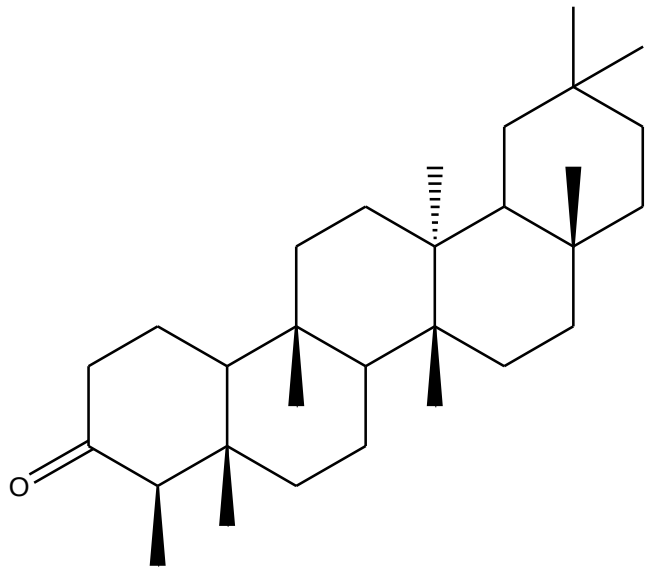


Figure 2.1: Chemical structure of Friedelin (Silva *et al*., 2014)

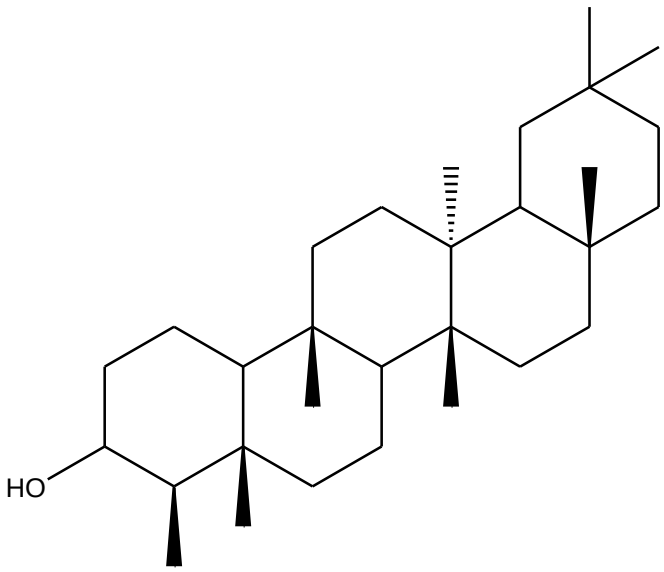


Figure 2.2: Chemical structure of Friedelinol (Silva *et al*., 2014)

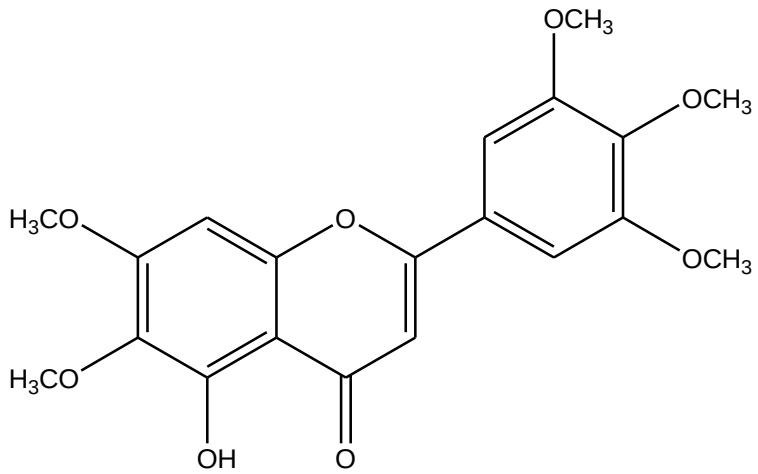


Figure 2.3: Chemical structure of Umuhengerin (Silva *et al*., 2014)

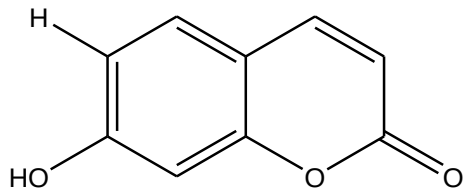


Figure 2.4: Chemical structure of Umbelliferone (Silva *et al*., 2014)

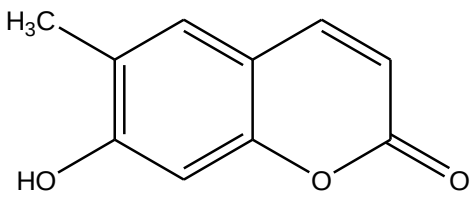


Figure 2.5: Chemical structure of Scopoletin (Silva *et al*., 2014)

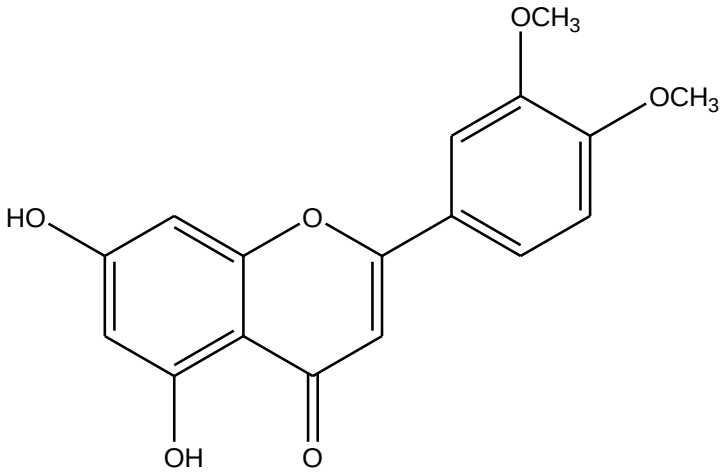


Figure 2.6: Chemical structure of Luteolin 3′,4′-dimethyl ether (Silva *et al*., 2014)

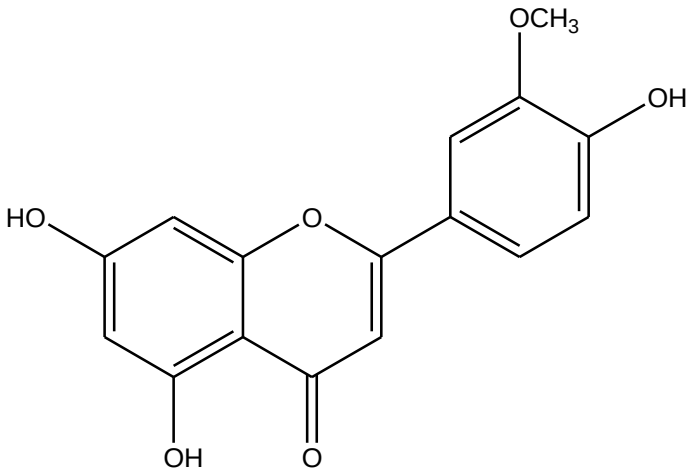


Figure 2.7: Chemical structure of Chrysoeriol (Silva *et al*., 2014)

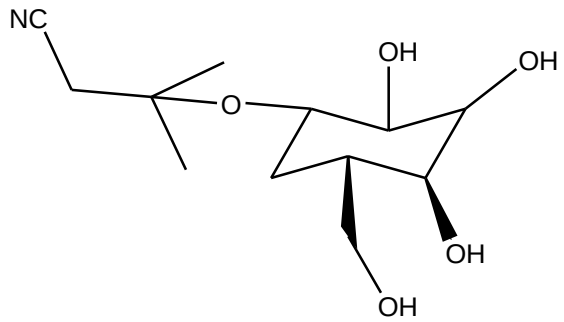


Figure 2.8: Chemical structure of Epidermin (Silva *et al*., 2014)

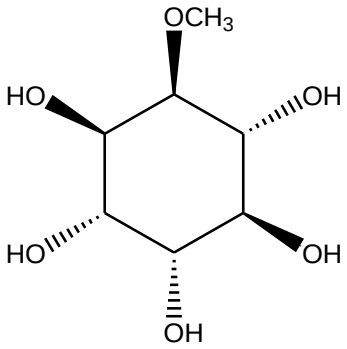


Figure 2.9: Chemical structure of (L)-quebrachitol (Silva *et al*., 2014)

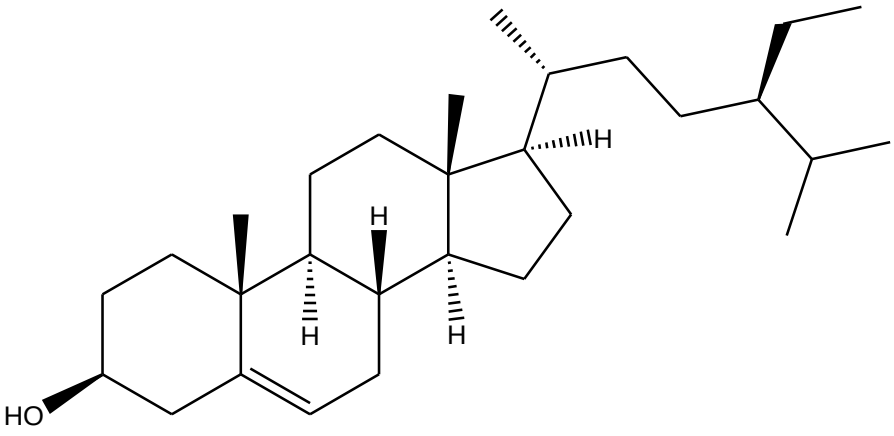


Figure 2.10: Chemical structure of β- sitosterol (Sharma, 2011)

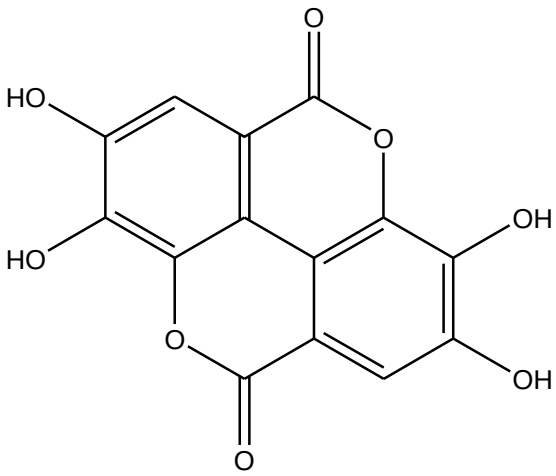


Figure 2.11: Chemical structure of Ellagic acid (Nont *et al*, 2010)

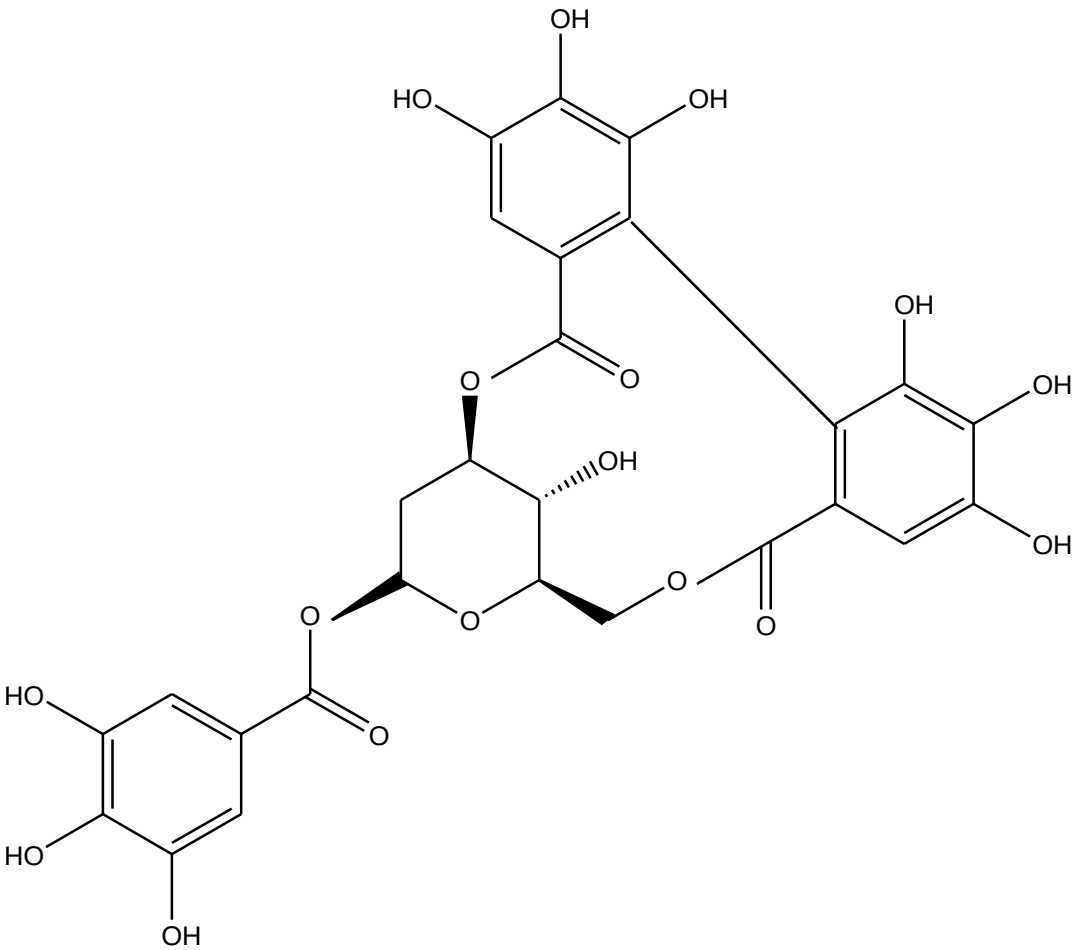


Figure 2.12: Chemical structure of Corilagin (Nont *et al*, 2010)

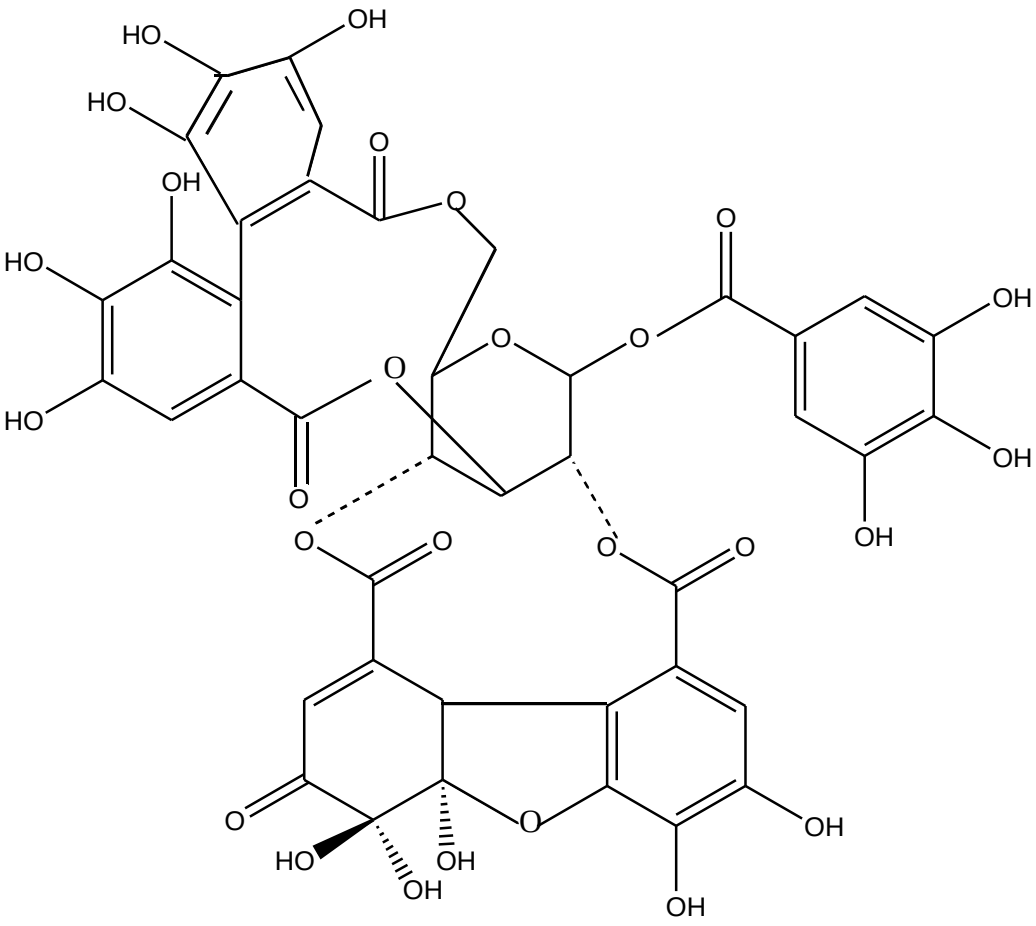


Figure 2.13: Chemical structure of Geraniin (Nont *et al*, 2010)

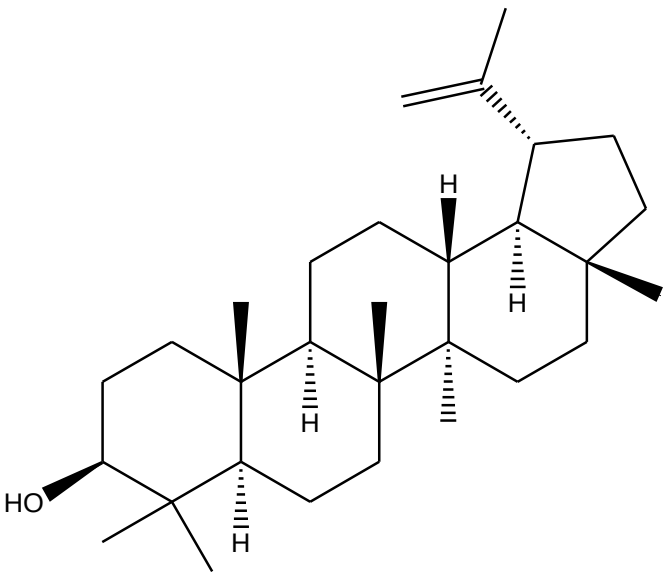


Figure 2.14: Chemical structure of Lupeol (Martina *et al*, 2015)

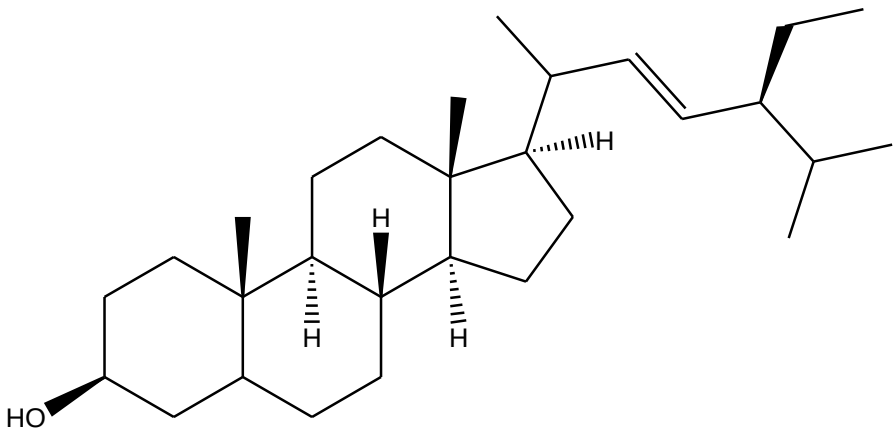


Figure 2.15: Chemical structure of Stigmasterol (Martina *et al*, 2015)

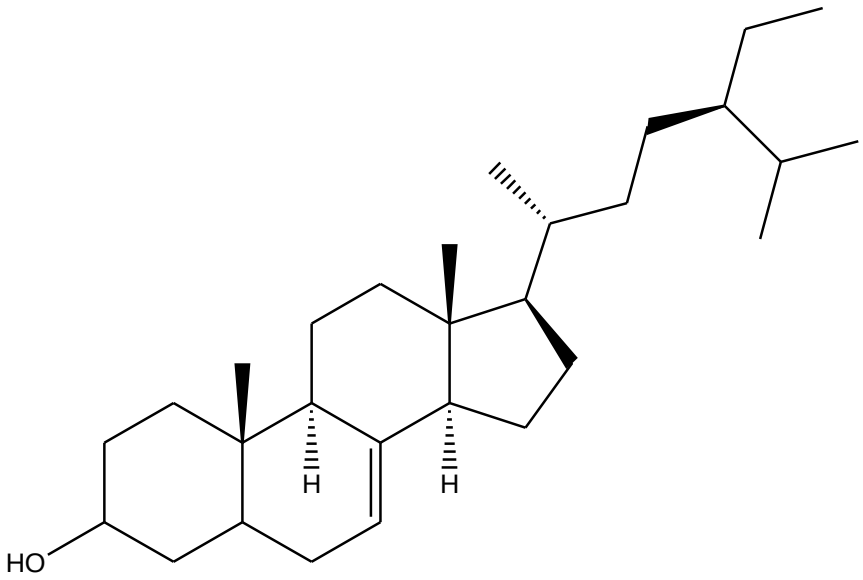


Figure 2.16: Chemical structure of Stigmast-7-en-3-ol (Martina *et al*, 2015)

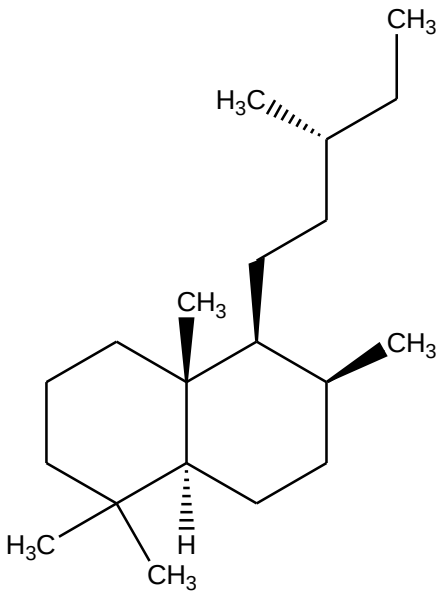


Figure 2.17: Chemical structure of Labdane (Martina *et al*, 2015)

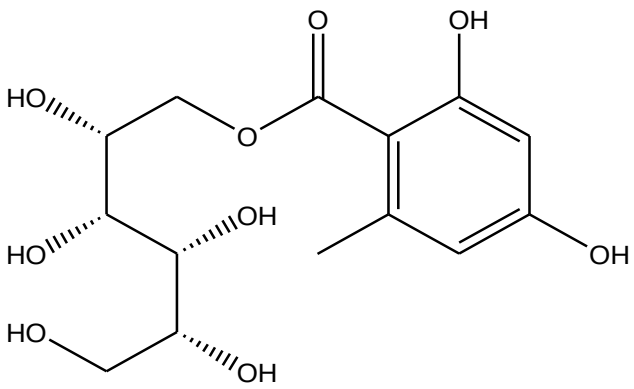


Figure 2.18: Chemical structure of D-mannitol orsellinate (Chun-Yan *et al*, 2016)

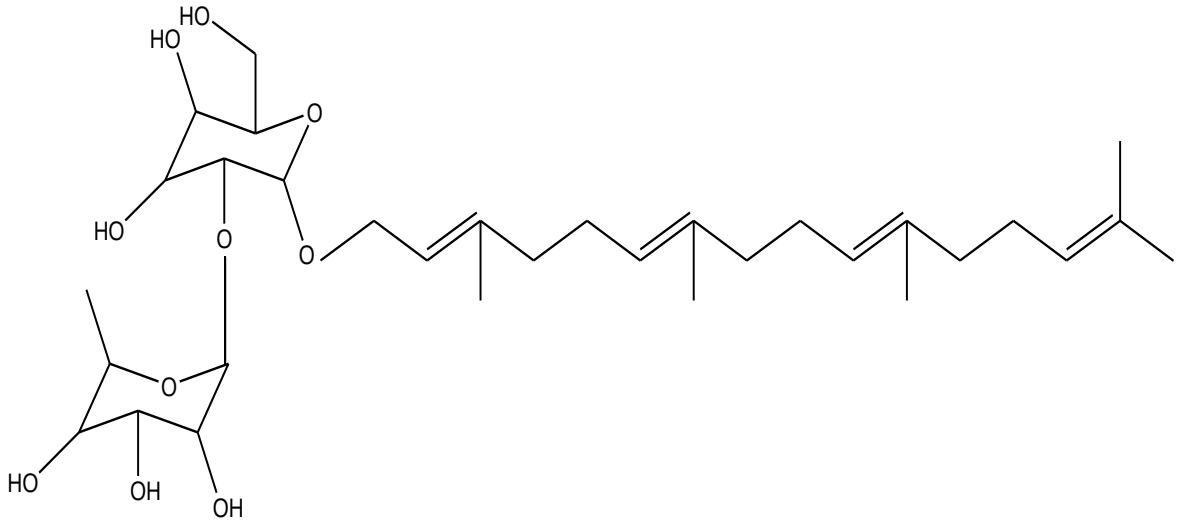


Figure 2.19: Chemical structure of 2″,3″,4″,6′-de-O-acetylcupacinoside (Alexander *et al*, 2013)

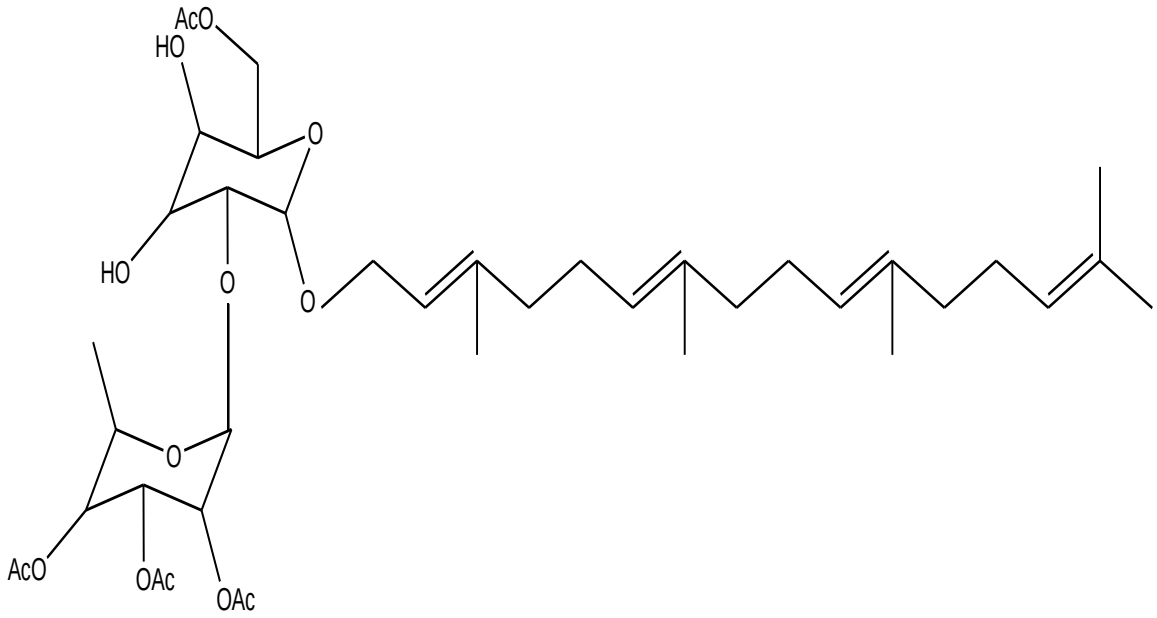


Figure 2.20: Chemical structure of Cupacinoside (Alexander *et al*, 2013)

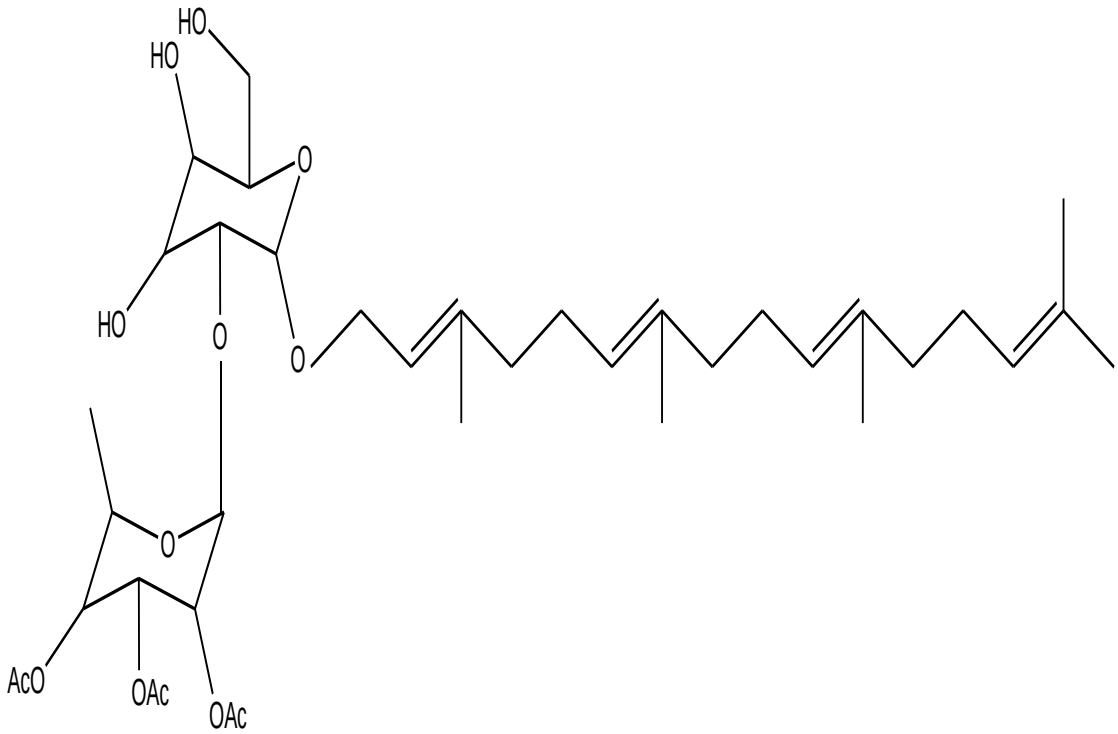


Figure 2.21: Chemical structure of 6′-de-O-acetylcupacinoside (Alexander *et al*, 2013)

2.12 *Blighia sapida*

Blighia is a genus in the Sapindaceae family, named in honour of Captain William Bligh (Lancashire, 2005). There are different species of *Blighia* which include *Blighia sapida, Blighia unijugata* and *Blighia welwitschii*. The trees are evergreen and grow to 10–20 m tall. The fruit usually contains three seeds, edible fleshy cream pulp and a thick, leathery orange or red skin (Aquaisua *et al*., 2011).

Blighia sapida (ackee) belongs to plantae kingdom, Tracheobionta rosidae sub-kingdom, Saphindales as its order, Sapindaceae family, sub-family is Sapindoideae and its genus is *Blighia*. It is a perennial plant that is originated from West Tropical Africa, forest of Gold Coast and Ivory Coast (Atolani *et al.,* 2009). Ghana, Nigeria, Burkina Faso, Guinea, Mali, Togo and Benin are examples of countries of West Africa where this plant is found. *Blighia sapida* has different names depending on the locality or language, examples of the names are ackee in English, arbor del huevo and pera roja in Mexico, aki and arbe fricassee in French, castanna or castanheiro de Africa in Potuguese, aki in Costa Rica (Atolani *et al.,* 2009). In South –western Nigeria where Yoruba is the language, it is called "isin", in Northern-western where Hausa is spoken, it is known as "Gwanja kusa'', ''Okpu'' in Eastern Nigeria whose language is Igbo while "*Yilanchi"* in Nupe. The propagation can be done through cutting, shooting, grafting and use of seeds (Atolani *et al.,* 2009). It was introduced by slave traders in Jamaica during 18th century in 1793 and gained scientific recognition when Captain William Bligh brought *Blighia sapida* from Jamaica to England where the plant was named in honour of him (Atolani *et al.,* 2009).

Blighia sapida was known for food and its poison (Morton, 1987). When the fruit of *Blighia sapida* is ripe, it splits and exposes aril and this is a fleshy creamed coloured pulp attached to a shiny black seed and it is edible and serves as fruit in some part of the world like Jamaica (Moya, 2001). According to Ekué *et al.,* 2010, arils freshly harvest are eaten directly; the oil content is released when grounded into powder and added to the sauce. Aril from an unripe fruit is poisonous when consumed. It also contains hypoglycin A (α-amino–β-(2 methylene cyclopropyl) propionic acid) which can cause vomiting, abdominal pain, cholestatic jaundice, elevated liver function values, convulsions and death (Hill, 1953, Hassall and Hill, 1955, Kean and Hare, 1980; Larson *et al*., 1994).

Neutropenia and thrombocytopenia effects of the aqueous and lipid extracts of the unripe fruit have been reported (Gardiner *et al.,* 1996). According to Barennes *et al.,* (2004), the toxic substance is dispelled by light as the jacket of the ripe fruit opens. The fruit is known to contain saponins which are hemolytic (Aderinola *et al.,* 2007). Jimoh *et al.,* (2012) showed that pod of *Blighia sapida* can be used as adsorbent to remove metallic ions from aqueous solution; husks form lather in water because of its ability to produce saponin while the soap produced is known for its esthetical and medicinal values (Ekué *et al.,* 2010), ashes of husk that is dried and the seed are rich in potash, therefore, they are used to make soap (John-Dewole and Popoola, 2013). Traditionally, immature *Blighia sapida* fruits are cut into pieces and poured inside water to wash clothes (Ekué *et al.,* 2010).

Eye conjunctivitis can be treated with the pulp and leaves (Oladiji *et al.,* 2009); stem bark *w*as reported to have antidiarrheal activity (Antwi *et al*., 2009); leaves, stem bark and root have been reported to have medicinal properties in treating epilepsy, yellow fever, dysentery, eye sore, skin sore, burns, wounds, (Etukudo, 2003; Kean and Hare, 1980) and diabetics (Gbolade, 2009). Stem bark was reported to have antioxidant property and its treatment on diabetes was also proved (Ojo *et al*., 2018). In Benin, many ailments such as malaria, internal haemorrhage, tooth decay, head lice, burns, inflammation, cutaneous infections, yellow fever, constipation and dysentery were treated with different parts of *Blighia sapida* (Ekué *et al.,* 2010). Aloko *et al,* (2019) reported the use of *Blighia sapida* for cosmetics, food and traditional treatment of gonorrhea, dysentery, backache, constipation, fever, cancer, malaria, typhoid, psychosis, hernia, stomach ache, skin infections, bacterial infections and rheumatism.

Blighia sapida possess acaricidal and insecticidal properties (Aloko *et al,* 2019; Mitchell and Ahmed, 2006). Ntiejumokwu and Kolawole (1999) reported the pesticidal activity of the plant. The fruit is used against stored-product insect pests (Khan and Gumbs, 2003). In Colombia and Brazil, there is practice of using extract from the seed to expel parasites; the leaves and bark are used for stomach disorders. In fishing, the seeds, dried stem bark and dried husks are used for easy catch of fish by grinding these plant's parts into powder as fish poison (Ekué *et al.,* 2010). Root of *Blighia sapida* with *Xylopia aethiopica* is applied to terminate unwanted pregnancy (Abolaji *et al.,* 2007). Production of consumable oil and other important industrial

products can be achieved according to Onuekwusi *et al.,* 2014 using the seed. Abolaji *et al.,* 2007 reported that proximate analysis of *Blighia sapida* showed high crude fibre content, phosphorus, magnesium and calcium.

2.12.1 Isolated compounds from *Blighia sapida*

Hypoglycin A [Fig. 2.22] (Atolani *et al*, 2009; Kean and Hare, 1980) and hypoglycin B [Fig. 2.23] (Atolani *et al*, 2009; Bowen-Forbes and Minott, 2009; Kean and Hare, 1980) were found present in the fruits of *Blighia sapida*. Blighinone [Fig. 2.24] was isolated from the aril of the fruit (Sarwar and Botting, 1994; Famuyiwa *et al*., 2018). Three monodesimosidic triterpenoid saponins as Blighoside A [Fig. 2.25], Blighoside B [Fig. 2.26] and Blighoside C [Fig. 2.27] were isolated from the fruit pods (Mazolla *et al*., 2011). Also, vomifoliol [Fig. 2.28] was isolated from the leaves and stems (Staurt *et al.*, 1976, Atolani *et al*., 2009) while phenanthrenol [Fig. 2.29] (Ogunwande and Oladosu, 2011), friedelin [Fig. 2.30] and α-amyrin [Fig. 2.31] (Famuyiwa *et al*., 2018) were isolated from stem bark.

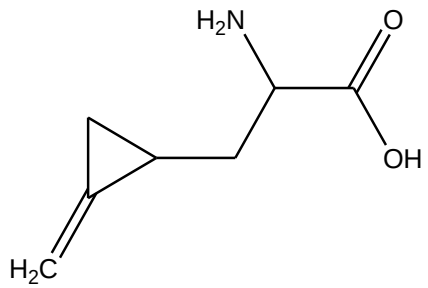


Figure 2.22: Chemical structure of Hypoglycin A (Atolani *et al*, 2009)

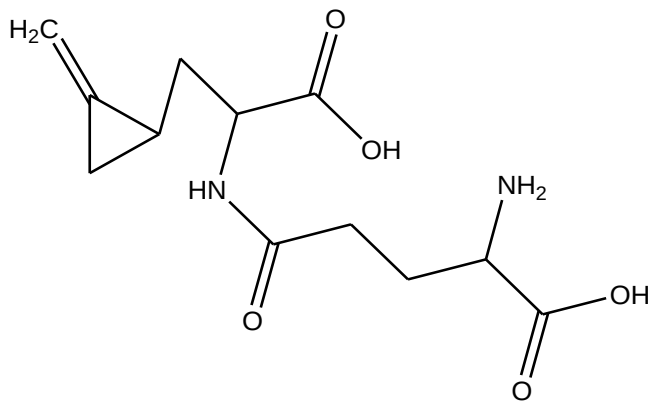


Figure 2.23: Chemical structure of Hypoglycin B (Bowen-Forbes and Minott, 2009)

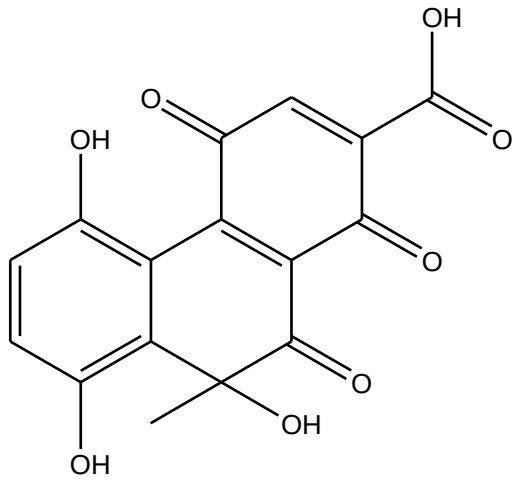


Figure 2.24: Chemical structure of Blighinone (Famuyiwa et al., 2018)

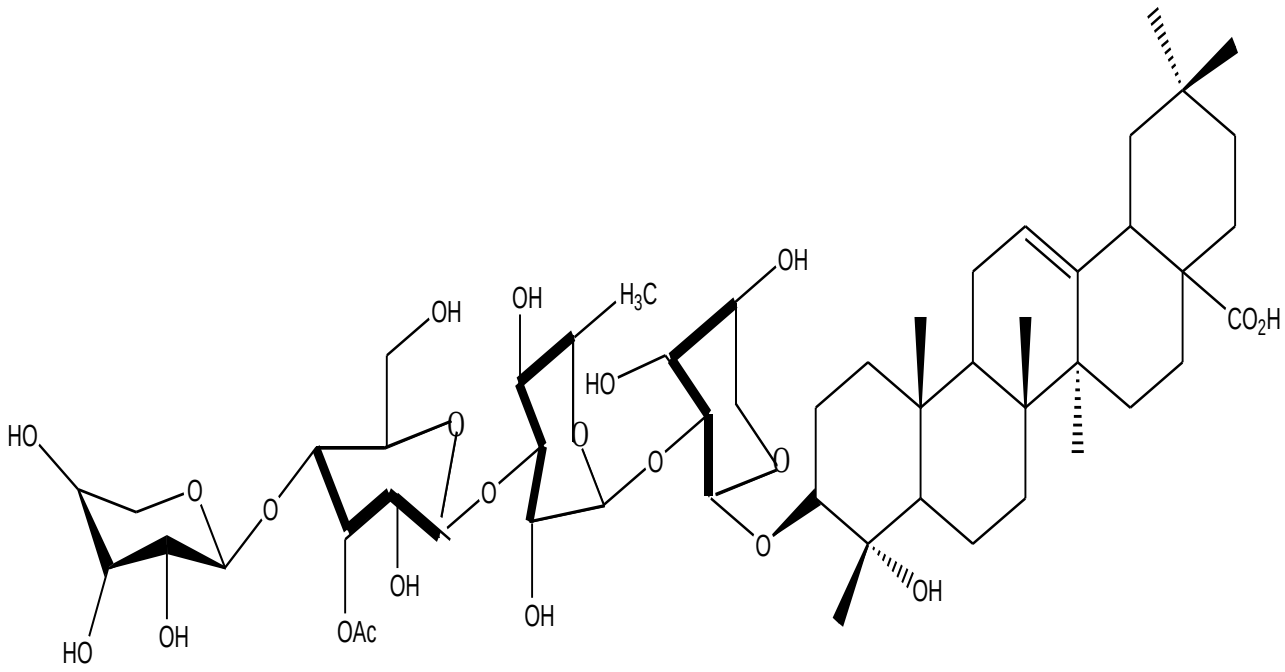


Figure 2.25: Chemical structure of Blighoside A (Mazolla *et al*., 2011)

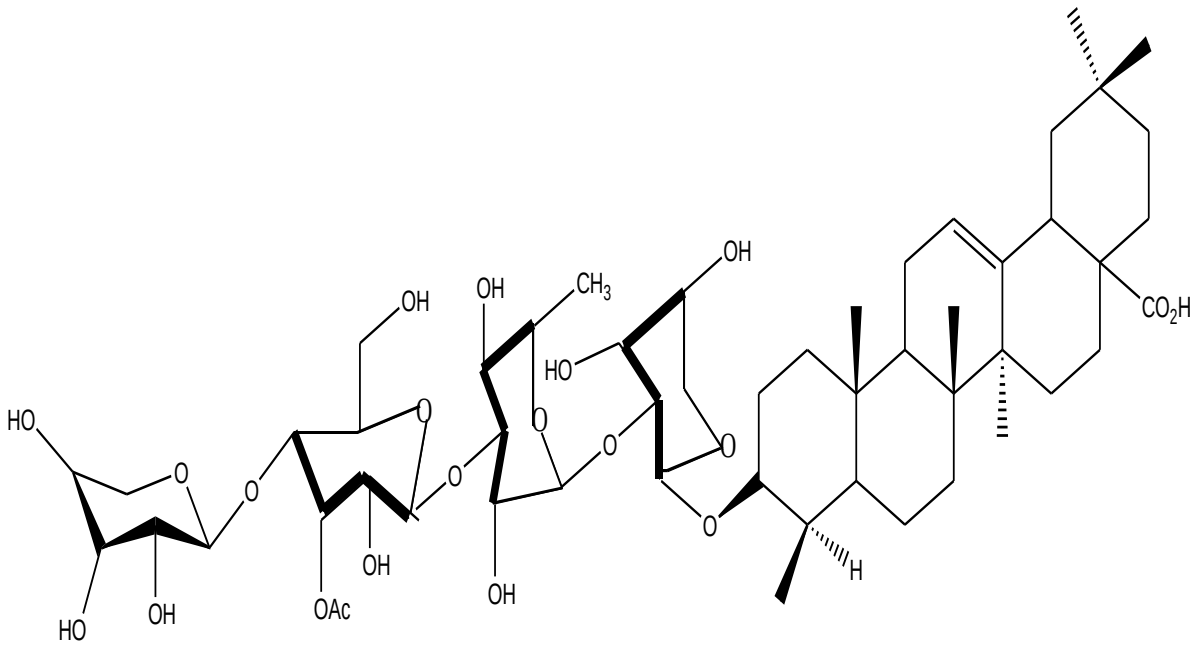


Figure 2.26: Chemical structure of Blighoside B (Mazolla *et al*., 2011)

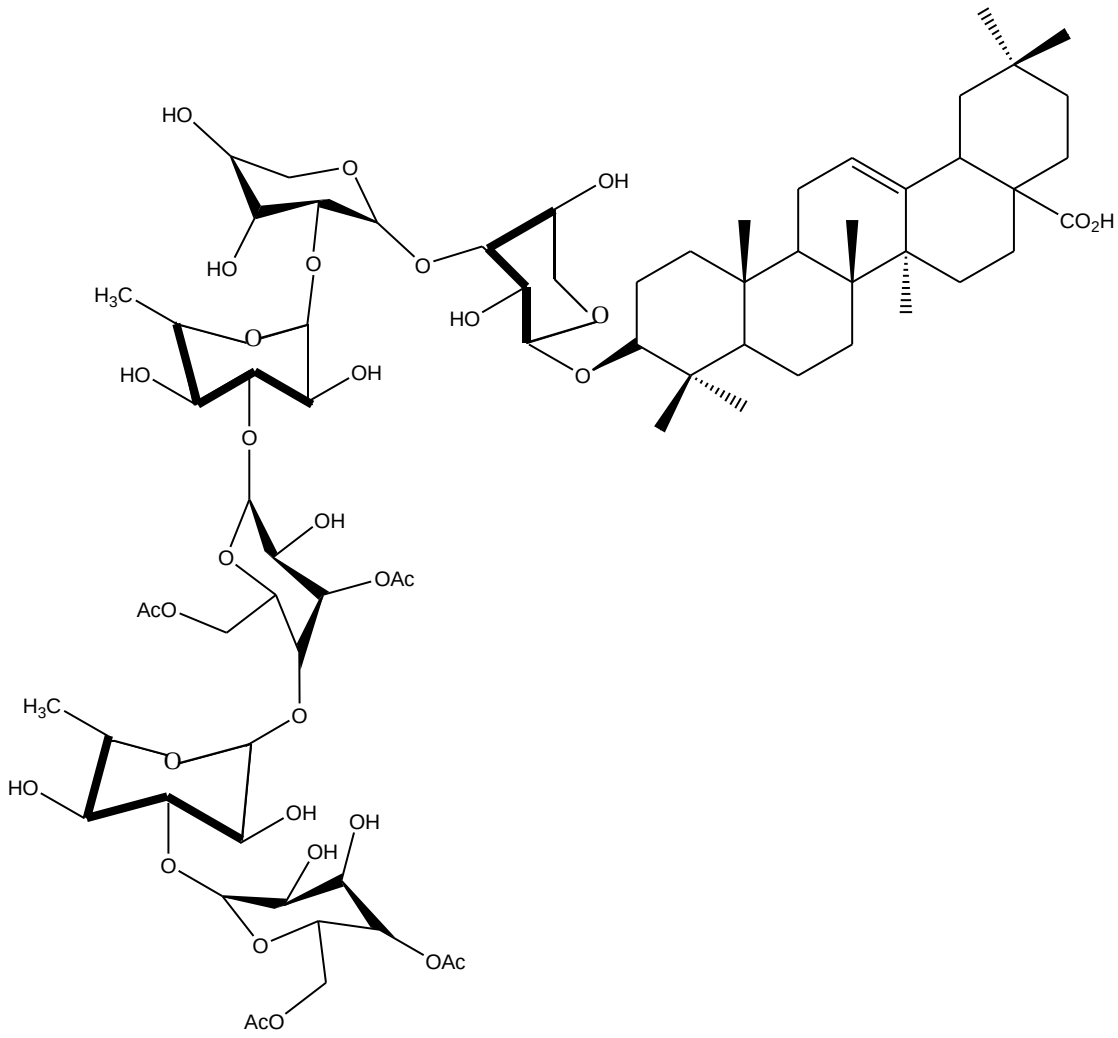


Figure 2.27: Chemical structure of Blighoside C (Mazolla *et al*., 2011)

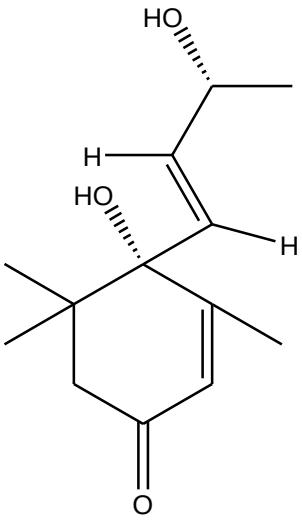


Figure 2.28: Chemical structure of Vomifoliol (Atolani *et al*., 2009)

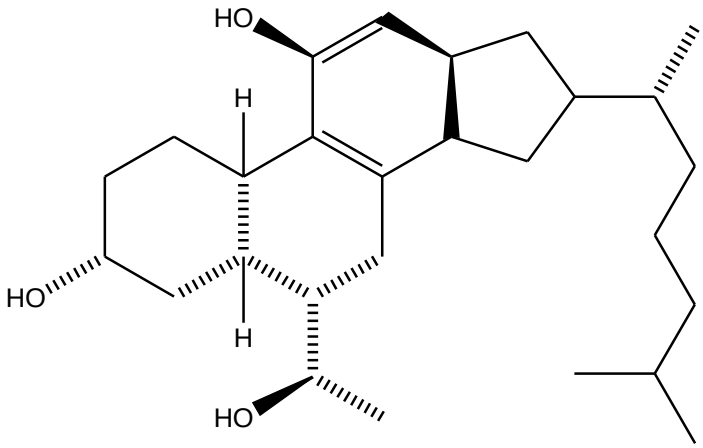


Figure 2.29: Chemical structure of Phenanthrenol (Ogunwande and Oladosu, 2011)

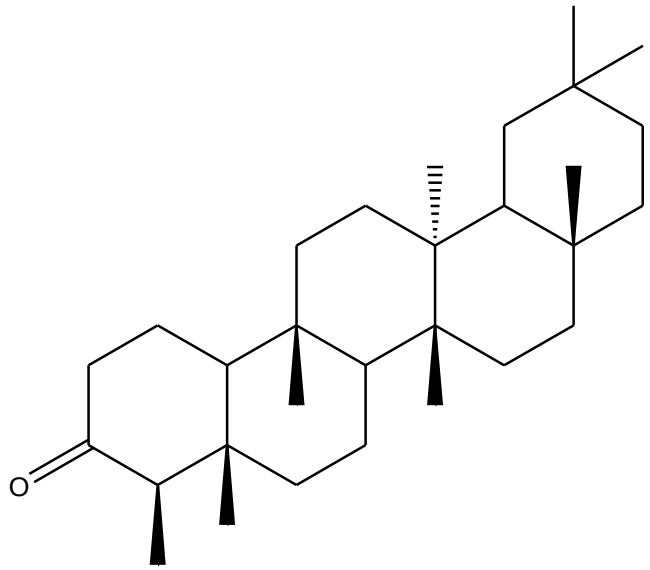


Figure 2.30: Chemical structure of Friedelin (Famuyiwa *et al*., 2018)

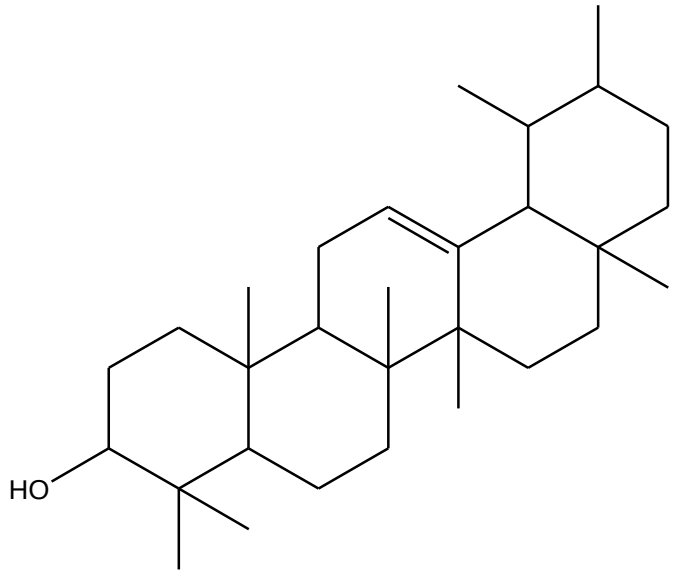


Figure 2.31: Chemical structure of α-amyrin (Famuyiwa *et al*., 2018)

2.12.2 Summary on previous studies on *Blighia sapida*

Ubulom *et al*., 2013 reported that ethanol extract of *Blighia sapida* leaf had activity against *Staphylococcus aureus* and *Bacillus subtilis* only at 200 mg/mL but had no activity against *Escherichia coli* and *Shigella dysenteriae* at the various test concentrations. Ethanol extract of stem bark had antibacterial activity against *Staphylococcus aureus* only at 100 also 200 mg/mL and *Bacillus subtilis* at 60 mg/mL concentration and higher. No inhibition was observed for *Escherichia coli* and *Shigella dysenteriae* at the various test concentrations. The ethanol extract of leaf and stem bark did not inhibit the growth of *Candida albicans, Microsporum canis, Aspergillus niger* and *Aspergillus fumigatus*. John-Dewole and Popoola (2013) reported that aqueous extracts of *Blighia sapida* husks possess antibacterial effect against the growth of *Staphylococcus aureus* and *Escherichia coli* with zone diameters of inhibitions of 14 mm and 20 mm respectively.

Methanol extract of *Blighia sapida* leaves scavenged 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Oloyede *et al*., 2014). *Blighia sapida* fruits methanol extract showed antioxidant activity using DPHH free radical scavenging activity (Hamzah *et al*., 2013; Dossou *et al*., 2014).

Crude fibre, phosphorus, magnesium, calcium, zinc and sodium were reported in *Blighia sapida* root while heavy metals such as lead, chromium and cadmium were not detected (Abolaji *et al*., 2007). The seeds and seed-oils of *Blighia sapida* are rich sources of protein, carbohydrate, fatty acid and amino acid (Esuoso and Odetokun, 2005). Ouattara *et al*., (2010) reported that the proximate nutritional composition of the dried aril and chemical properties of aril oil exposed to the sunlight showed that, it contains a high fat content, high quantity of carbohydrate, lower protein content, low moisture content, low total ash, low iodine value, low acid value, low peroxide value and saponication value which is in agreement with previous work. Essential elements of the dry aril are potassium, magnesium, calcium, sodium, iron and zinc.

Blighia sapida extract produces leucopenia and thrombocytopenia in mice (Bello, 1999). Kela *et al.,* (1999) reported the molluscicidal activity of *Blighia sapida.* Ntiejumokwu and Kolawole (1999) reported the pesticidal activity of *Blighia sapida.*

Structural characterizations of Hypoglycin A [Fig. 2.22] (Atolani *et al*, 2009), Hypoglycin B [Fig. 2.23] (Atolani *et al*, 2009; Bowen-Forbes and Minott, 2009),

Blighinone [Fig. 2.24] (Sarwa and Botting, 1994), Blighoside A [Fig. 2.25], Blighoside B [Fig. 2.26] and Blighoside C [Fig. 2.27] (Mazolla *et al*., 2011) from *Blighia sapida* ackee fruit have been reported. Vomifoliol [Fig. 2.28] was isolated from leaves and stem (Staurt *et al*, 1976). Ogunwande and Oladosu (2011) reported the anticandidal activity of Phenanthrenol [Fig. 2.29] that was isolated from ethyl acetate extract of *Blighia sapida* stem bark and Famuyiwa *et al*. (2018) also isolated Friedelin [Fig. 2.30] and α-amyrin [Fig. 2.31] from the stem bark.

Essential oils of *Blighia sapida* leaf, leaf stalk, stem bark, root, fruit pulp, fruit husk and seed with their chemical characterisation, antibacterial, antifungal and antioxidant activities have been published from this research (Akinpelu *et al*., 2016a; Akinpelu *et al*., 2016b) while essential oils from leaf, stem bark and root chemical constituents and antioxidant were published by Oloyede *et al*., (2022); GC-MS, preparative TLC and FT-IR analysis of methanol extracts of the stem bark and root by Abba *et al*., (2017) but there is need to continue this research work to compare chemical constituents, antimicrobial and antioxidant activities of the oils of *Blighia sapida* with other plants of the same sapindaceae family, then isolate and characterise bioactive compound from *Blighia sapida* root.

2.13 *Lecaniodiscus cupanioides*

Lecaniodiscus cupanioides is a genus in the Sapindaceae family. The flowers have unusual disc which surrounds the stamens, therefore, the name "Lecaniodiscus" means saucershaped disc (Ojo and Ndinteh, 2021). It is evergreen and grow to 6–12 m tall. *Lecaniodiscus cupanioides* (ginger lilly) belongs to plantae kingdom, sub-kingdom is known to be Tracheobionta rosidae, Saphindales order, Sapindaceae as its family while its genus is *Lecaniodiscus*. It is a perennial plant that is widely common in Asia and Africa and has different names depending on the locality: Bulati (Limba) and Babwi (Loko) in Sierra Leone, Bue (Akye) and Klima (Baule) in Ivory Coast. In Nigeria, it is called "Akika" or "Aaka" among Yoruba tribe, "Ukpo" in Igbo language and "Kafi-nama-zaki" among the Hausa tribe (Ojo and Ndinteh, 2021).

Lecaniodiscus cupanioides are useful in many ways as its leaves, stem bark and root are used in treatment of cough, jaundice and typhoid (Soladoye *et al*., 2013; Olusola and Oyeleke, 2015). It was reported that leaves and root are used in the treatment of malaria fever in Benin (Hermans *et al*., 2004). Root was reported to have aphrodisiac

effect in restoration of sexual activity (Chauhan *et al*., 2014; Hermans *et al*., 2004; Ajiboye *et al*., 2014). Bark and root of this plant are used to treat cancer and infection of the scalp (Segun *et al*., 2018; Aworinde and Erinoso, 2015). Yemitan and Adeyemi, 2005 reported that leaves, stem bark and stem are utilized for the treatment of measles, sore, abdominal swelling and wound, also that leaves can be used for burns. Leaves can as well be used on bone fracture (Adeniyi *et al*., 2018). Diabetes and skin infection are also treated with stem bark of *Lecaniodiscus cupanioides* (Borokini *et al*., 2013; Diallo *et al*., 2012).

2.13.1 Isolated compounds from *Lecaniodiscus cupanioides*

Compounds 3-O-[α-L-arabinofuranosyl- (1→3)-α-L-rhamnopyranosyl- (1→2)-α-L-arabinopyranosyl-]-hederagenin [Fig. 2.32] and 3-O- [α-L-arabinopyranosyl- (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl-]-hederagenin [Fig. 2.33] were isolated from *Lecaniodiscus cupanioides* stem (Adesegun *et al*., 2014). Cupanioidesosides A [Fig. 2.34], B [Fig. 2.35] and C [Fig. 2.36] as well as lecanioside A [Fig. 2.37] from *Lecaniodiscus cupanioides* roots (Messi *et al*., 2020).

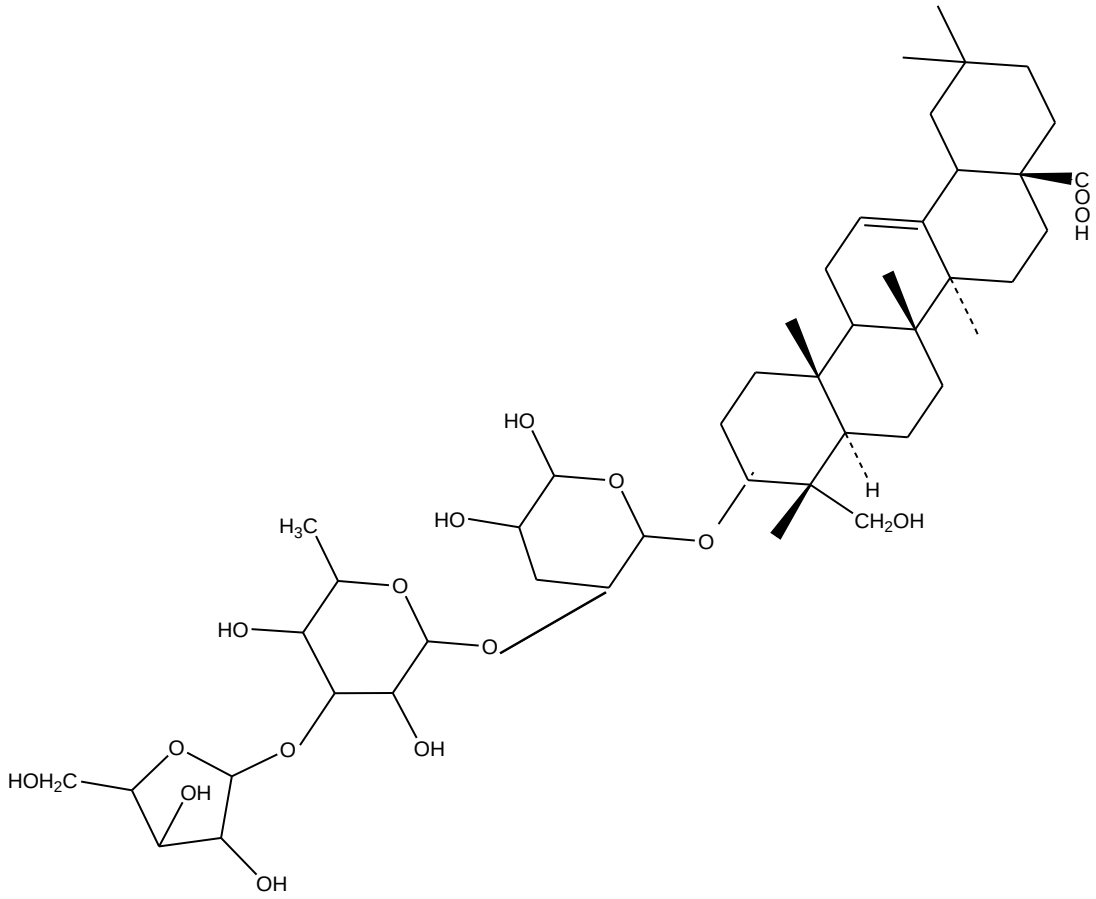


Figure 2.32: Chemical structure of 3-O-[α-L-arabinofuranosyl- (1→3)-α-L-rhamnopyranosyl- (1→2)-α-L-arabinopyranosyl-]-hederagenin (Adesegun *et al*., 2014)

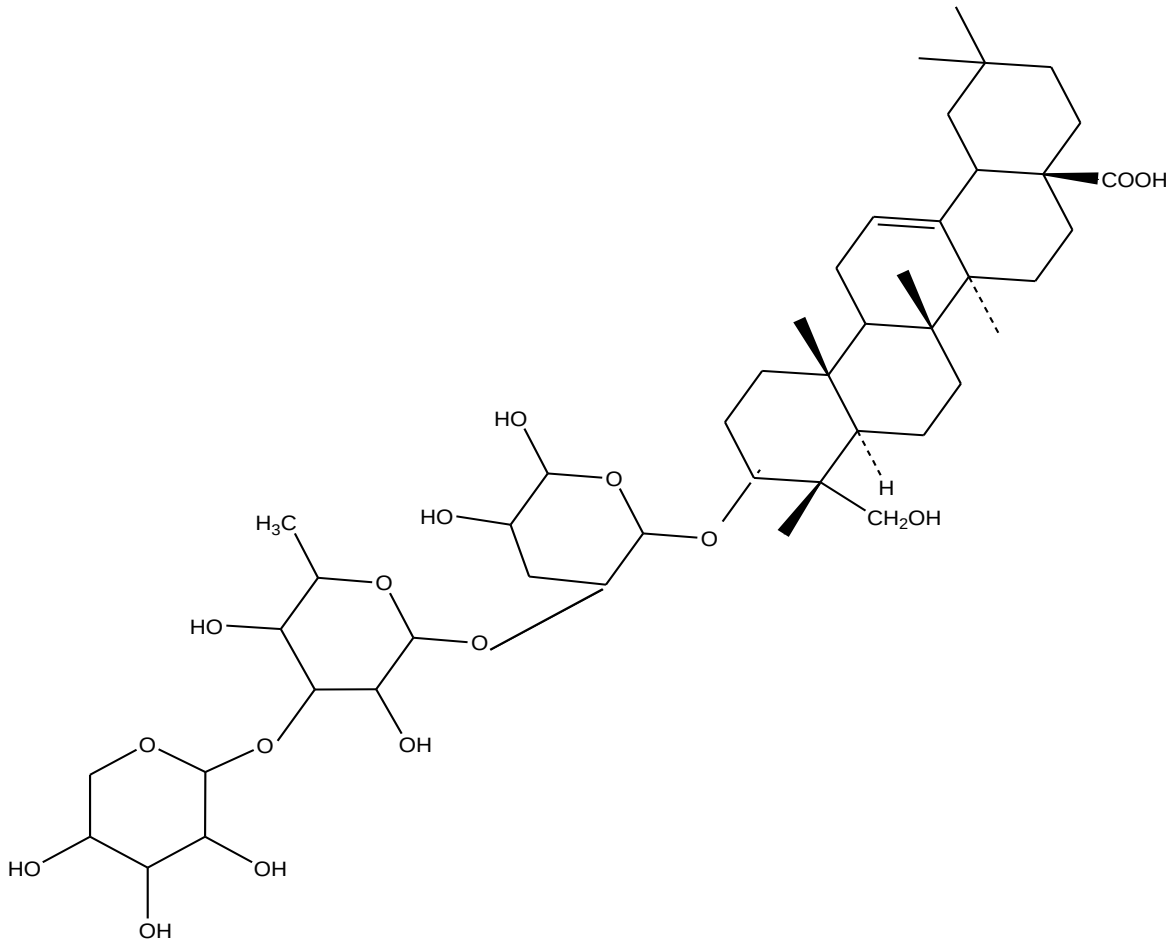


Figure 2.33: Chemical structure of 3-O- [α-L-arabinopyranosyl- (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl-]-hederagenin (Adesegun *et al*., 2014)

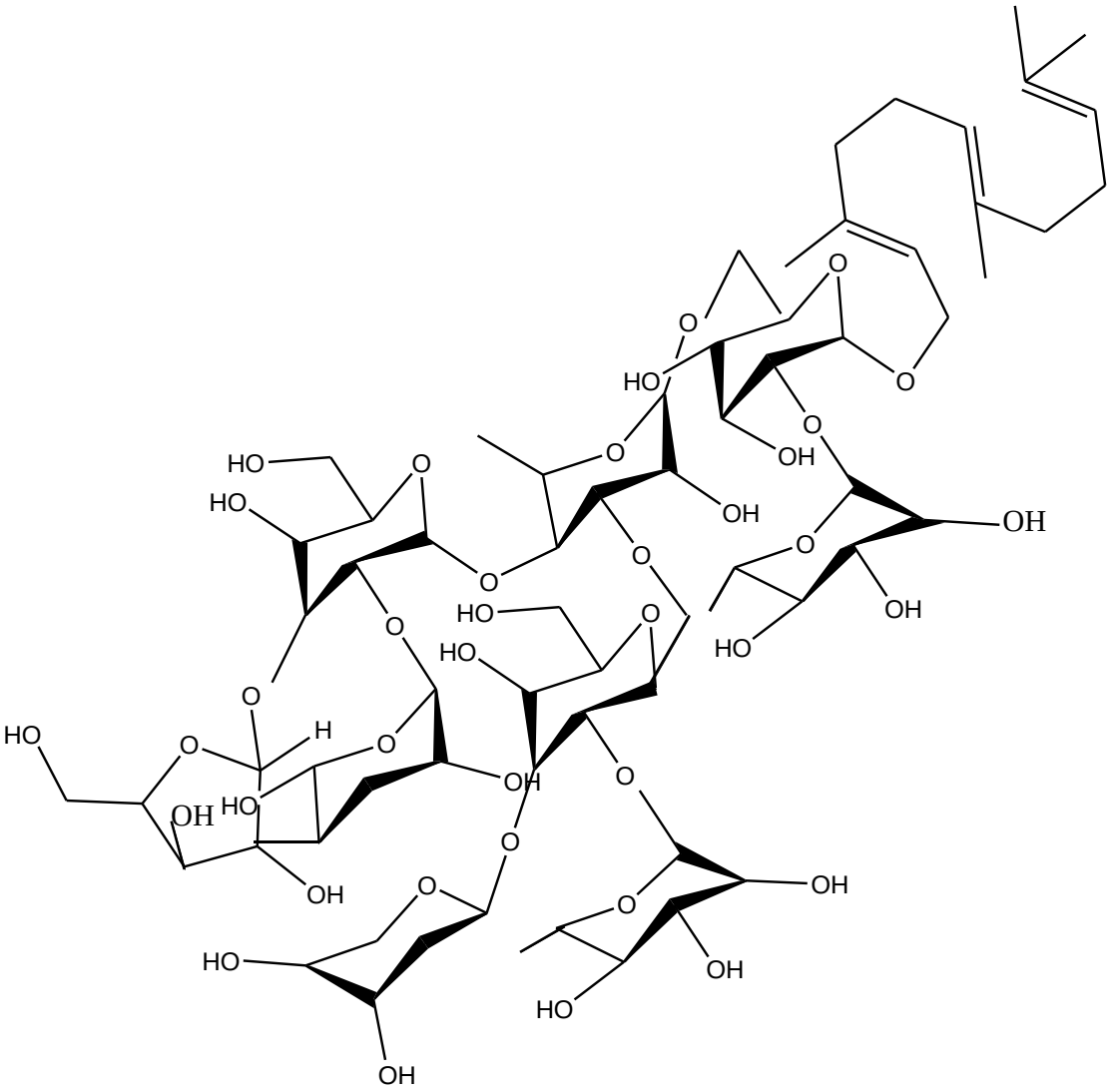


Figure 2.34: Chemical structure of Cupanioidesosides A (Messi *et al*., 2020)

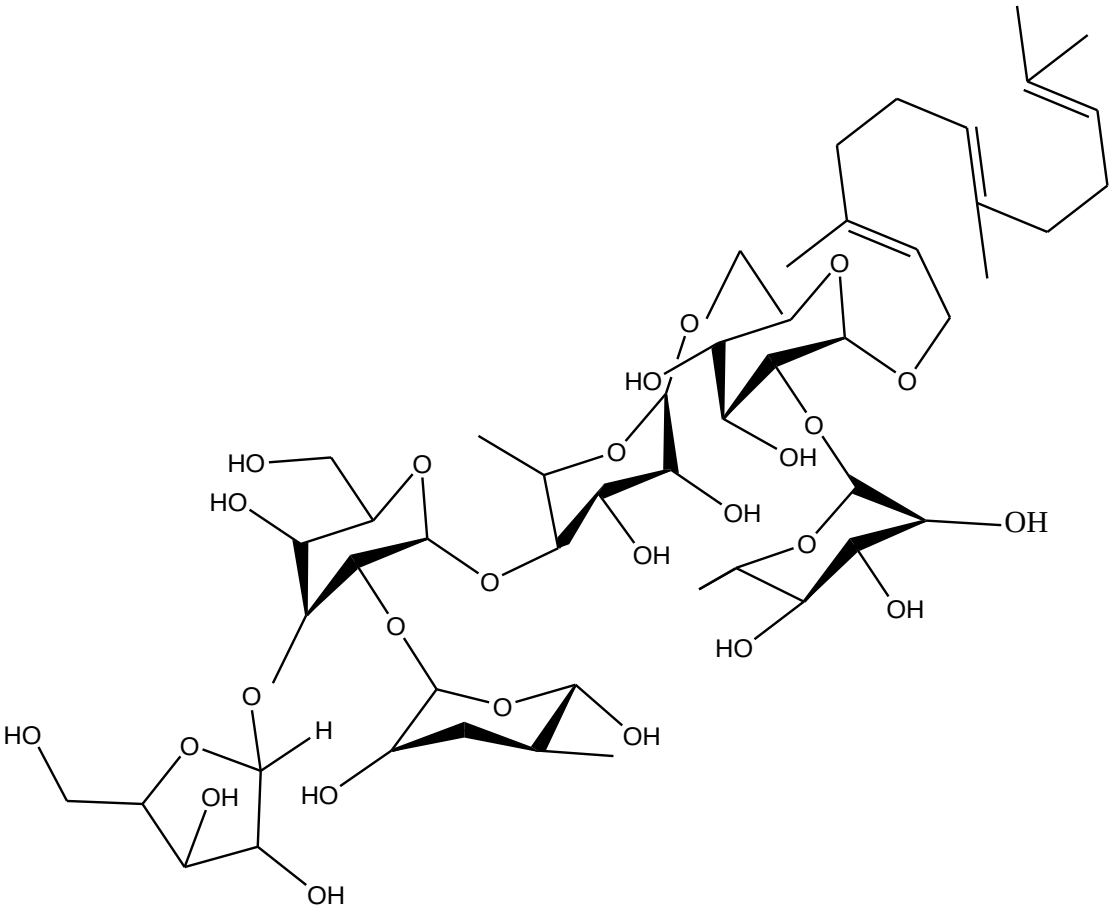


Figure 2.35: Chemical structure of Cupanioidesosides B (Messi *et al*., 2020)

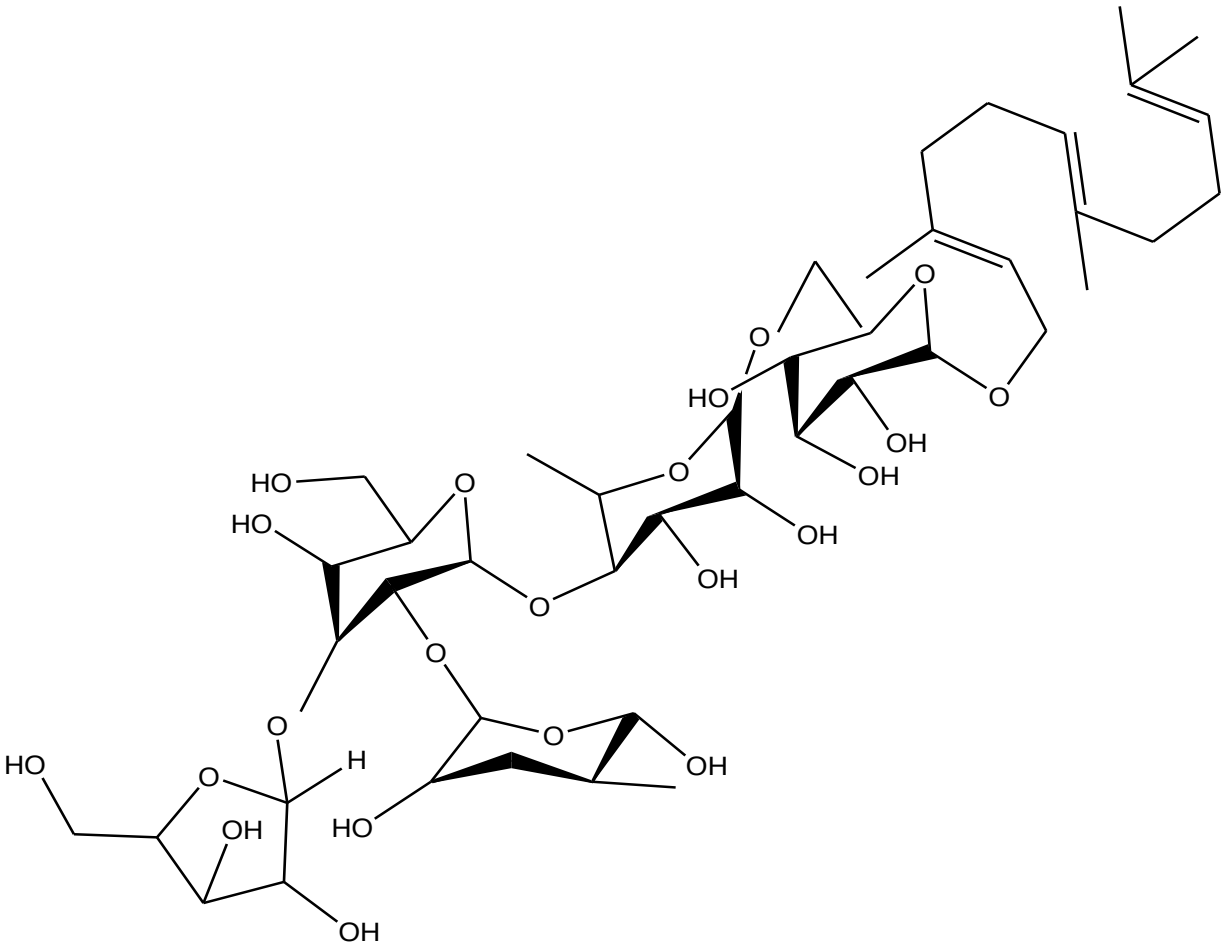


Figure 2.36: Chemical structure of Cupanioidesosides C (Messi *et al*., 2020)

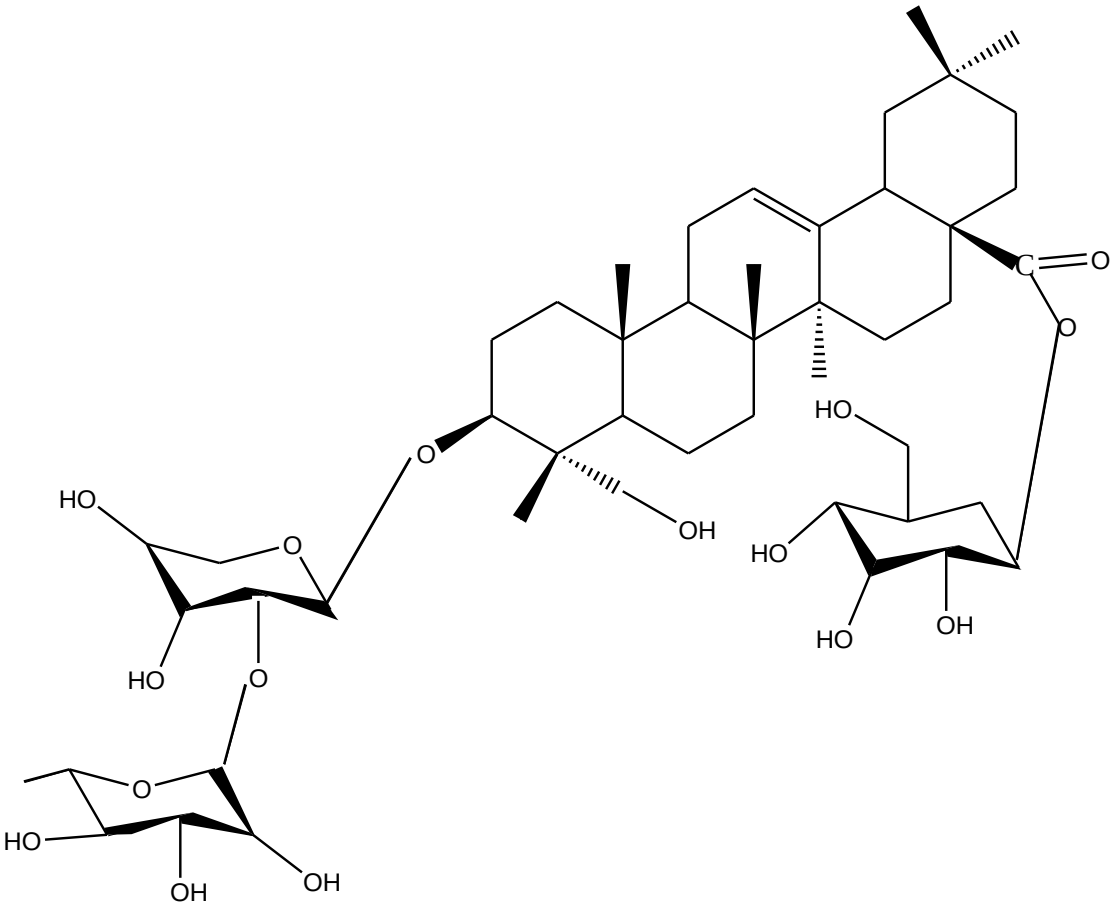


Figure 2.37: Chemical structure of Lecanioside A (Messi *et al*., 2020)

2.13.2 Summary on previous studies on *Lecaniodiscus cupanioides*

Antimicrobial activity of *Lecaniodicus cupanioides* leaf extract was tested against *Shigella sonnei*, *Staphylococcus aureus* and *Candida albicans* and its antioxidant activity was also established (Alayande and Ashafa, 2017; Chanda and Dave, 2009; Sofidiya *et al*., 2008). Kafu and Adebisi, 2015 reported that *Lecaniodiscus cupanioides* root hexane and ethanol extracts were active against *Staphylococus aureus, Streptococcus pneumonia, Pseudomonas aeroginosa, Escherichia coli, Shegella flexmeri and Salmonella typhi*.

Structural characterisations of 3-O-[α-L-arabinofuranosyl- (1→3)-α-L-rhamnopyranosyl- (1→2)-α-L-arabinopyranosyl-]-hederagenin [Fig. 2.32], 3-O- [α-L-arabinopyranosyl- (1→3)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranosyl-]-hederagenin [Fig. 2.33] from *Lecaniodiscus cupanioides* stem, cupanioidesosides A [Fig. 2.34], B [Fig. 2.35], C [Fig. 2.36] and lecanioside A [Fig. 2.37] from *Lecaniodiscus cupanioides* root (Adesegun *et al*., 2014; Messi *et al*., 2020) were established.

2.14 *Paullinia pinnata*

Paullinia pinnata (bread, cheese plant or sweet gum) is a wood or sub-woody climbing shrub and it is of kingdom of plantae, sub-kingdom as Tracheobionta rosidae, Saphindales as the order, family of Sapindaceae and its genus is *Paullinia*. Its origin is from tropical America but now common in the savanna zones of tropical Africa likewise, Madagascar (Adeyemo-Salami, 2020).The plant has different names depending on its locality, among Yoruba, it is called "Kakansenla" or "Ogbe-okuje", Hausa: "Goorondoorinaa", in Edo, it is "Aza", Nupe: Enu Kakanchela", Igala: "Omekpa" or "Egwubi", Liberia (Basa): "Gbe-se", Sierra Leone (Kono): "Kamakagu", Ghana (AdangmeKrobo): "Akplokinakpa" and Togo (AnyiAnifo): "Tolundi". (Adeyemo-Salami, 2020; Ikhane *et al*., 2015).

It is an African tropical plant whose leaves and root are used in traditional medicine for many purposes, for example in erectile dysfunction (Zamble *et al*., 2006). It is used as chewing-sticks, hut building, tying fences and it has many ethnomedicinal applications including treatment of leprosy, rickets, pain, fever, jaundice, eye ailments, cough and infections (Adeyemo-Salami, 2020). *Paullinia pinnata* climbing plant is a multipurpose plant though it was introduced as a fish poison to Africa. In Africa, the

leaf is used to treat dysentery (Akinyemi *et al*., 2005), in West Africa it is used to treat fevers while malaria in Tanzania and colds, bile stimulant and antianaemic tonic in Nigeria likewise in Congo and Senegal (Adeyemo-Salami, 2020). In East Africa it is used to cure gonorrhoea, snake bite and paralysis while eye, rickets and leprosy treatments in Ivory Coast, rickets and tetanus in Ghana (Adeyemo-Salami, 2020). It is used for rheumatism, ulcers and also as purge in Nigeria among Igala tribe and Esan despite its use as anti-diarrhoeatic (Mensah *et al*., 2007; Osarenmwinda *et al*., 2009). It is used for the treatment of haemorrhoids and wounds (Agyare *et al*., 2009). Leaf is also used to prevent miscarriage and use to ease childbirth in Ivory Coast, Tanzania and Gabon while in Gabon and Ghana, it is used to ease menstrual discomfort (Chhabra, 1991). *Paullinia pinnata* root is used in treatment of malaria, dysentery, infections (Annan *et al*., 2005; Jimoh *et al*., 2007; Zamble *et al*., 2006), wounds and haemorrhoids (Agyare *et al*., 2009). The root is used as chewing stick, also in treatment of impotence and rheumatism (Addo-Fordjour *et al*., 2008).

Paullinia pinnata leaf essential oil was characterised with investigation of insecticidal properties (Ogunwande *et al*., 2017). Antimicrobial activities of *Paullinia pinnata* leaf methanol extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ikhane et al., 2015) and ethanol extract against different species of *Salmonella* (Roger *et al*., 2015) were established. Antibacterial activity of aqueous and ethanol extracts of *Paullinia pinnata* leaf, stem, stem bark and root bark against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were also established (Imade *et al*, 2015). Leaf methanol extract showed strong antioxidant activity using DPPH (Jimoh et al., 2007). Zamble *et al*., (2006) also reported that leaf and root methanol extracts showed antioxidant activities using 1, 1-diphenyl 1-2-picryl hydrazyl (DPPH), hydrogen peroxide (H_2O_2), superoxide oxygen (O_2^-) and hypochlorous acid (HOCl).

2.14.1 Isolated compounds from *Paullinia pinnata*

Isolation and characterization of compounds from *Paullinia pinnata* leaves include Methylinositol [Fig. 2.38] (Lunga *et al*., 2014) while 6-O-β-3-methoxy-4-hydroxybenzoyl lupene [Fig. 2.39] (Annan and Houghton, 2010), 3-O-isovaniolloyl lupene [Fig. 2.40] (Lasisi et al., 2015), 6α-(3-methoxy-4-hydroxybenzoyl)-lup-20 (29)-ene-3-one [Fig. 2.41], β-amyrin [Fig. 2.42], paullinomide A [Fig. 2.43], 2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthren-6-one

[Fig. 2.44], ß-sitosterol glucopyranoside [Fig. 2.45], β-sitosterol [Fig. 2.46], l-quebrachitol [Fig. 2.47], 5α- poriferastane- 3ß, 6α-diol [Fig. 2.48] and friedelin [Fig. 2.49] (Jackson *et al*., 2015; Dongo *et al*., 2009; Annan *et al*., 2009) were isolated from *Paullinia pinnata* root.

2.14.2 Summary on previous studies on *Paullinia pinnata*

Antimicrobial activities of *Paullinia pinnata* leaf, stem bark and root extracts (Ikhane et al., 2015; Imade *et al*, 2015; Roger *et al*., 2015) and antioxidant activity assessment of leaf and root extracts (Annan *et al*., 2005; Jimoh et al., 2007; Zamble et al., 2006) were established. Also, there were reports of structural characterizations of Methylinositol [Fig. 2.38] from leaves (Lunga *et al*., 2014), 6-O-β-3-methoxy-4-hydroxybenzoyl lupene [Fig. 2.39], 3-O-isovaniolloyl lupene [Fig. 2.40], 6α-(3-methoxy-4-hydroxybenzoyl)-lup-20(29)-ene-3-one [Fig. 2.41], β-amyrin [Fig. 2.42], paullinomide A [Fig. 2.43], 2-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-rioxaphenanthren-6-one [Fig. 2.44], ß-sitosterol glucopyranoside [Fig. 2.45], β-sitosterol [Fig. 2.46], l-quebrachitol [Fig. 2.47], 5α- poriferastane- 3ß, 6α-diol [Fig. 2.48] and friedelin [Fig. 2.49] from root (Annan and Houghton, 2010; Lasisi *et al*., 2015; Jackson *et al*., 2015; Dongo *et al*., 2009). Although, there is report on chemical constituents and insecticidal properties of *Paullinia pinnata* leaf essential oil but there is need to extract, characterise and investigate the essential oils obtained from *Paullinia pinnata* leaf part, leaf stalk, stem bark and root part of the plant.

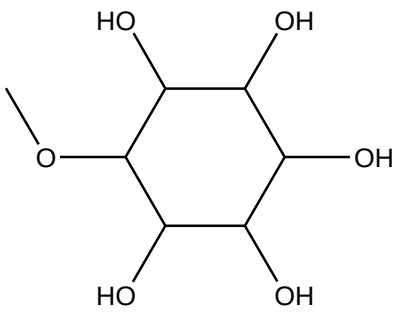


Figure 2.38: Chemical structure of Methylinositol (Lunga *et al*., 2014)

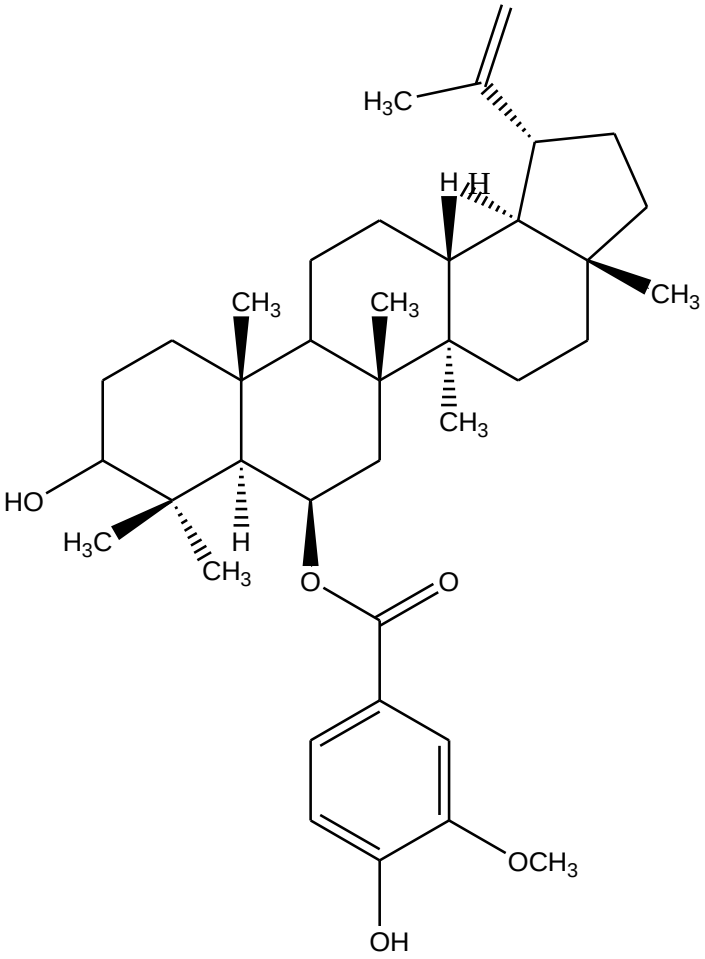


Figure 2.39: Chemical structure of 6-O-β-3-methoxy-4-hydroxybenzoyl lupine
(Annan and Houghton, 2010)

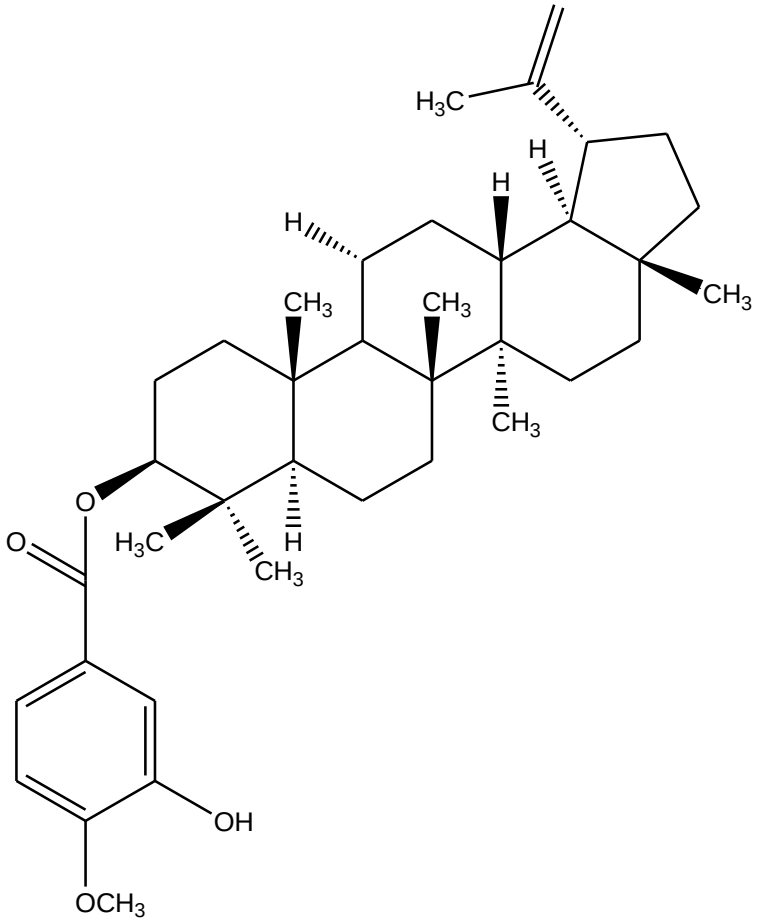


Figure 2.40: Chemical structure of 3-O-isovaniolloyl lupine (Lasisi et al., 2015)

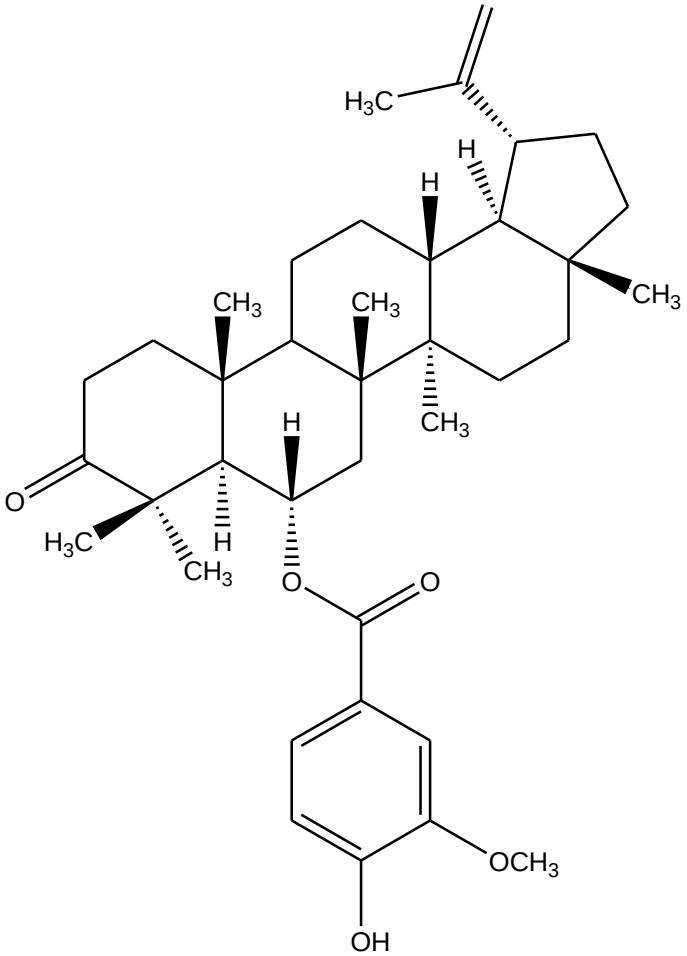


Figure 2.41: Chemical structure of 6*α*-(3-methoxy-4-hydroxybenzoyl)-lup-20 (29)-ene-3-one (Jackson *et al*., 2015)

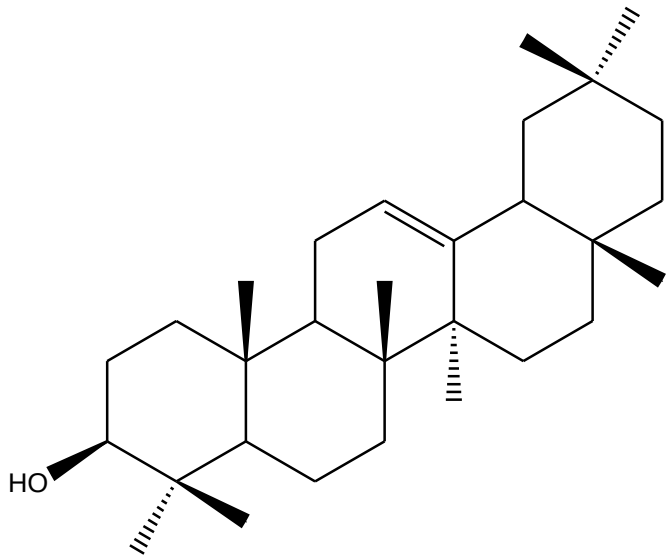


Figure 2.42: Chemical structure of β-amyrin (Jackson *et al*., 2015)

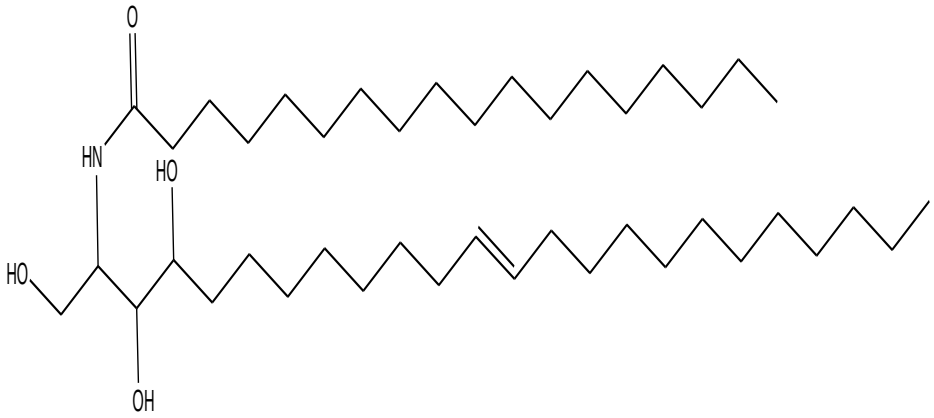


Figure 2.43: Chemical structure of Paullinomide A (Jackson *et al*., 2015)

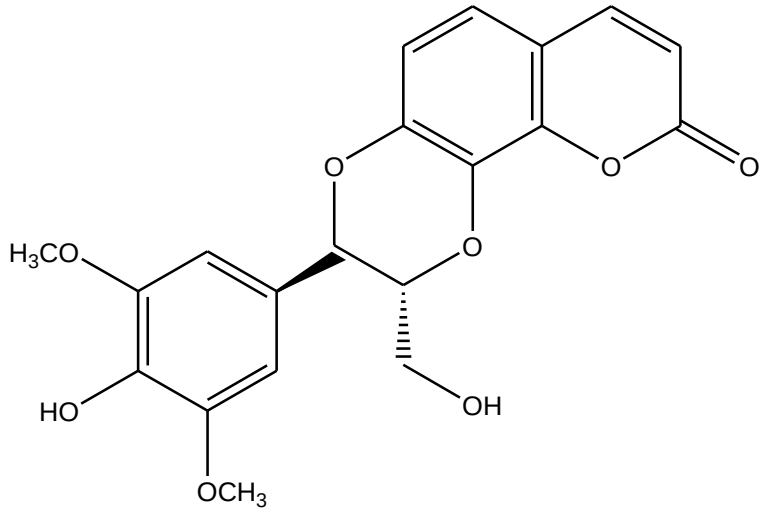


Figure 2.44: Chemical structure of 2-(4-hydroxy-3, 5-dimethoxyphenyl)-3-hydroxymethyl-2, 3-dihydro-1, 4, 5-trioxaphenanthren-6-one (Jackson *et al*., 2015)

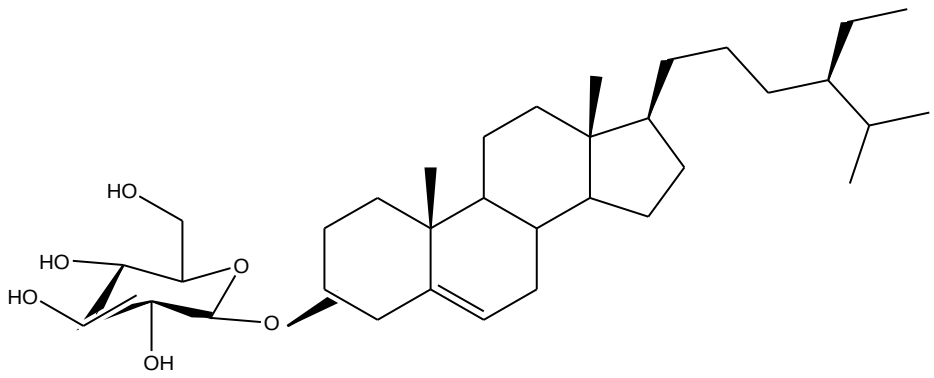


Figure 2.45: Chemical structure of ß-sitosterol glucopyranoside (Jackson *et al*., 2015)

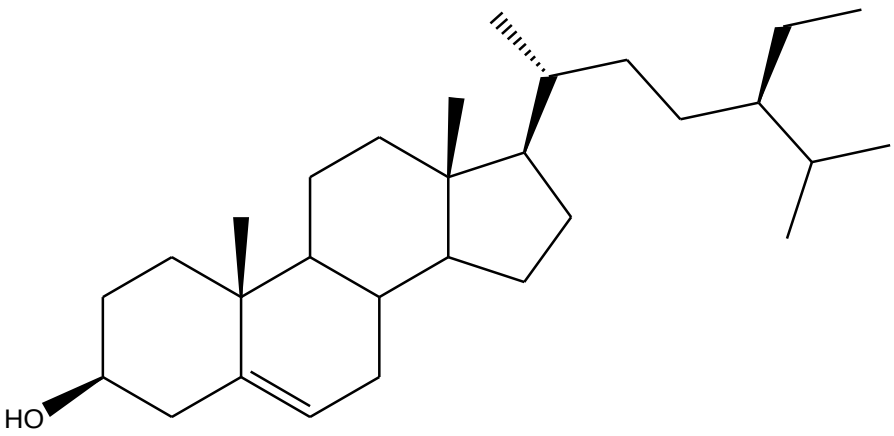


Figure 2.46: Chemical structure of β-sitosterol (Jackson *et al*., 2015)

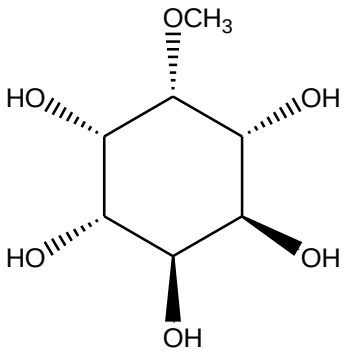


Figure. 2.47: Chemical structure of l-quebrachitol (Jackson *et al*., 2015)

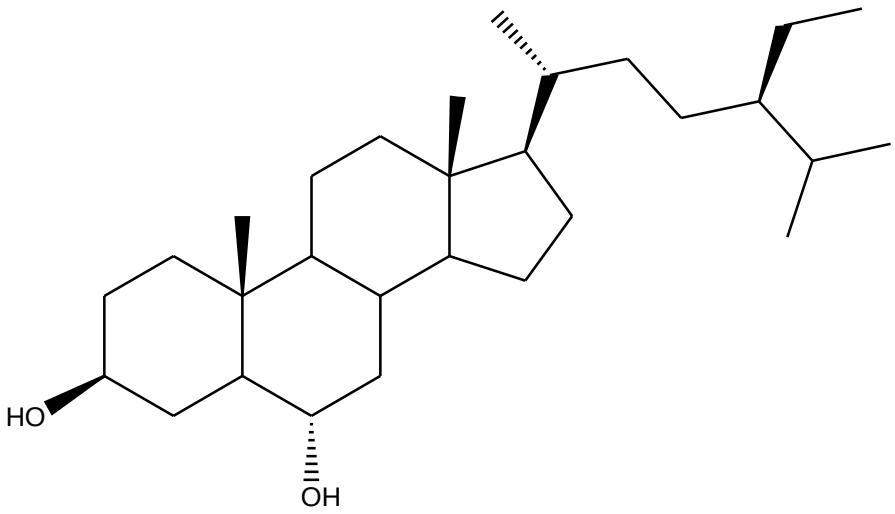


Figure 2.48: Chemical structure of 5α- poriferastane- 3ß, 6α-diol (Jackson *et al*., 2015)

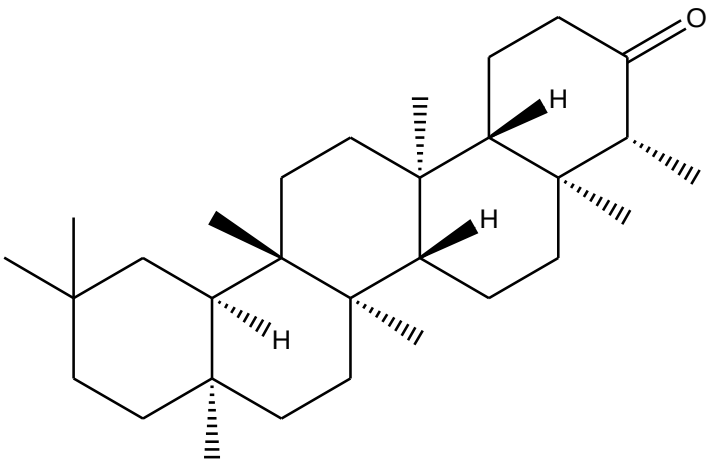


Figure 2.49: Chemical structure of Friedelin (Jackson *et al*., 2015)

CHAPTER THREE

METHODOLOGY

3.1 Plants collection and identification

Leaves, stem-bark, root and fruits of *Blighia sapida* were obtained from Abadina, University of Ibadan on 11th July, 2014 for essential oils as shown in Plate 3.1 - 3.5. *Lecaniodiscus cupanioides* leaves, stem-bark and root as shown in Plate 3.6 - 3.7 from Oloko, Tose, Akinyele Local Government Area, Ibadan on 26th January, 2022. Leaves, stem-bark and root of *Paullinia pinnata* on 28th January, 2022 from Amina Way, University of Ibadan as shown in Plate 3.8 – 3.9. The GPS co-ordinates of sampling locations using GPS MAP 78s (GARMIN) for *Blighia sapida* were N 07^{0}27. 097^{1}, E 003^{0}53. 978^{1}, GPS 4 m, Elevation 230 m; *Lecaniodiscus cupanioides* were N 07^{0}32. 471^{1}, E 003^{0}55. 534^{1}, GPS 4 m, Elevation 273 m and *Paullinia pinnata* were N 07^{0}26. 916^{1}, E 003^{0}54. 110^{1}, GPS 5 m, Elevation 241m; approximately N 7^{0}27 - 7^{0}32, E 3^{0}54 - 3^{0}55 for the three plants. The plants were identified by Mr. D.O. Esimekhuai at University of Ibadan Herbarium with voucher Specimen numbers UIH-22407, UIH-23138 and UIH-23139 respectively.

3.2 Preparation of plant materials for extraction

Leaves (150 g), leaf-stalk (200 g), stem-bark (200 g), root (150 g), fruit-pulp (100 g), fruit-husk (200 g) and seed (200 g) of *Blighia sapida*; leaves (300 g), leaf-stalk (300 g), stem-bark (190 g) and root (300 g) of *Lecaniodiscus cupanioides* and leaves (120 g), leaf-stalk (120 g), stem-bark (35 g) and root (160 g) of *Paullinia pinnata* were dried under shade as shown in Table 3.1 and were separately crushed, ground with Hammer Mill Crusher (15 horse power) stationed at J-laboratory, Chemistry Department, University of Ibadan.

Plate 3.1: *Blighia sapida* tree (UIH - 22407)

Plate 3.2: *Blighia sapida* leaf, leaf stalk and fruit (UIH - 22407)

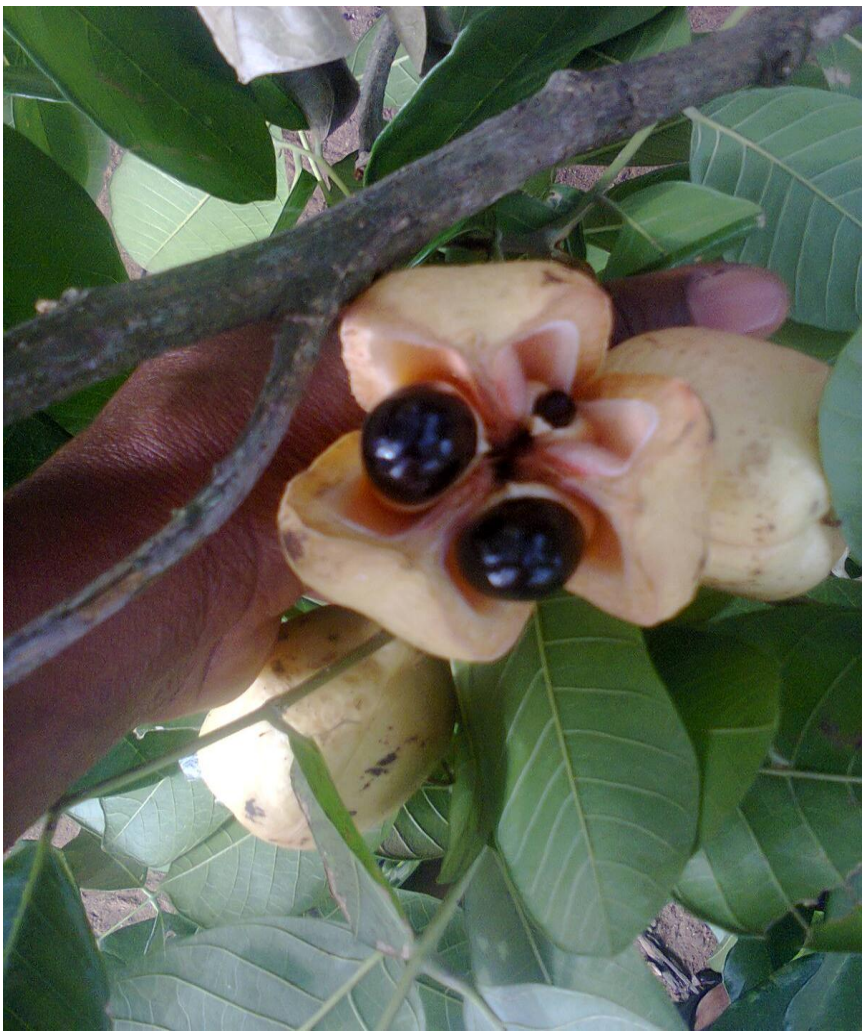


Plate 3.3: *Blighia sapida* opened- fruit showing husks and seeds (UIH - 22407)

Plate 3.4: *Blighia sapida* fruit seed (UIH - 22407)

Plate 3.5: *Blighia sapida* fruit pulp (UIH - 22407)

Plate 3.6a: *Lecaniodiscus cupanioides* tree (UIH - 23138)

Plate 3.6b: *Lecaniodiscus cupanioides* tree (UIH - 23138)

Plate 3.7: *Lecaniodiscus cupanioides* leaf and leaf stalk (UIH - 23138)

Plate 3.8: *Paullinia pinnata* climbing tree (UIH – 23139)

Plate 3.9: *Paullinia pinnata* leaf and leaf stalk (UIH – 23139)

Table 3.1: Drying method and drying time of plant materials

S/N	Sample	Plant's Part	Drying Method	Drying Time
1	*Blighia sapida*	Leaf	Air drying under Shade	7 days
2	*Blighia sapida*	Leaf stalk	Air drying under Shade	7 days
3	*Blighia sapida*	Stem bark	Air drying under Shade	14 days
4	*Blighia sapida*	Root	Air drying under Shade	14 days
5	*Blighia sapida*	Fruit pulp	-	0 day
6	*Blighia sapida*	Fruit husk	-	0 day
7	*Blighia sapida*	Seed	-	0 day
8	*Lecaniodiscus cupanioides*	Leaf	Air drying under Shade	7 days
9	*Lecaniodiscus cupanioides*	Leaf stalk	Air drying under Shade	7 days
10	*Lecaniodiscus cupanioides*	Stem bark	Air drying under Shade	14 days
11	*Lecaniodiscus cupanioides*	Root	Air drying under Shade	14 days
12	*Paullinia pinnata*	Leaf	Air drying under Shade	7 days
13	*Paullinia pinnata*	Leaf stalk	Air drying under Shade	7 days
14	*Paullinia pinnata*	Stem bark	Air drying under Shade	14 days
15	*Paullinia pinnata*	Root	Air drying under Shade	14 days

3.3 Essential oils extraction

Each pulverised plant's part was put in a round bottom flask with water and this was placed on the heating mantle, the flask being coupled with all glass Clevenger-type hydro-distillation apparatus which was designed according to the specifications of British Pharmacopeia, this allowed the essential oil to be trapped through its condenser when content of the flask is boiled for three hours. The extracted oils were preserved in sealed sample vials and kept in a refrigerator until it is ready for analysis.

3.4 Identification of essential oil constituents

3.4.1 Gas Chromatography–Mass Spectrometry analysis

Agilent 5975 C series Gas Chromatograph-Mass Spectrometer (GC-MS) system was used to analyse essential oils extracted from the leaves, leaf stalk, stem bark, root, fruit pulp, fruit husk and seeds of *Blighia sapida*. Productivity Chemstation (Version E.02.01.1177) along with AMDIS (Automated Mass Spectral Deconvolution and Identification System) was used to analyse the data. Equilibration time of column oven program was set at 0.25 min, 70°C as temperature of initial stage for four (4) mins; it was ramped for six (6) mins at 6°C/min to 140°C. Then further ramped for another 4 mins at 15°C/min to 300°C; 36.333 mins was the total run time. Splitless mode was used to inject sample (1 µL), injector temperature was 280°C while column head pressure was 56.756 kPa and this gave 64mL/min as the total flow. Temperature of transfer line was 280°C. The column used was HP-5 MS 5% Phenyl Methyl Silox column having 30 m x 250 µm x 0.25 µm as dimensions and 325°C was used as maximum recommended temperature (MRT). The flow rate of helium as carrier gas was 1 mL/min. In mass spectrometry, acquisition mode of full scan of 4.00 minutes solvent delay was used; m/z 50 – 600 was set as acquisition mass range, voltage of electron multiplier detector was automatically set to 1082 V, temperature of mass spectrometer source was set at 230°C, quadrupole mass analyzer temperature at 150°C and emission current of filament was also automatically set as 34.610 µA.

Essential oils from *Lecaniodiscus cupanioides* and *Paullinia pinnata* leaves, leaf-stalk, stem-bark and root were analyzed with the use of Shimadzu (GC-2030) series GC-MS that was equipped with Headspace (HS-20) and QQQ Mass spectrometer GC-TQ8040NX. Column used was SH-Rxi-5 SILMS with dimensions of 0.25 X 30 X 0.25 while the carrier gas was helium at flow rate of 1.00 mL/min. Initial temperature of the

column was programmed at 50^{o}C held for 4 minutes which was increased to150^{o}C, this was held for 3 minutes then increased to 260^{o}C held for 3 minutes using split ratio (50:50) mode. Each plant sample was diluted with ethylacetate and hexane (10:100 v/v) and sample extract (0.5 µL) was injected using autosampler injector. Temperatures used for injector, ion source and interface were 260^{o}C, 220^{o}C and 240^{o}C respectively. Ionization energy was 70 eV and the mass range of 40–500 AMU.

3.4.2 Compounds identification

Chemical constituents of *Blighia sapida* essential oils were identified using retention indices, this was in reference to a homologous series of the n-alkanes; fragmentation patterns of mass spectral of the compounds were compared with reference compounds in data systems of NIST (National Institute of Standards and Technology) spectra libraries with matching version 8.0. Component spectra were compared with the NIST library spectra by using AMDIS software (version 2.71 build 134.27 (deconvolution)). Deconvolution minimum match factor on AMDIS software was set to 70; all compounds that were identified had match factors which were between 70 and 100. The fragmentation patterns as well as the characteristic retention of some mass spectral were compared with in- built data of Adams (2007). Management of GC-MS system, settings of parameter for GC and mass spectrometry, data receipt and the processing on essential oils of *Lecaniodiscus cupanioides* and *Paullinia pinnata* were performed using Shimadzu Realtime Analysis and the chemical compounds were also identified using NIST library.

3.5 Plant collection for crude extraction

There was limited information on isolation and characterisation from *Blighia sapida* among these three Sapindaceae plants because there was no characterised isolate from *Blighia sapida* root based on the findings therefore, *Blighia sapida* leaves, stem bark and root were collected from University of Ibadan on 25th July, 2016, 14th May, 2019 and 26th February, 2021 for crude extraction at the co-ordinates of N 07^{0}27. 097^{1}, E 003^{0}53. 978^{1}, GPS 4 m, Elevation 230 m

3.6 *Blighia sapida* crude extraction

Leaves (2000 g), stem bark (1800 g) and root (2000 g) of *Blighia sapida* were dried under shade as shown in Table 3.1, crushed separately and ground with 15 horse power Hammer Mill Crusher. Each was soaked in hexane, ethylacetate and methanol

successively for 3 days. Nine crude extracts were obtained and concentrated separately under reduced pressure at 40^{o}C using rotary evaporator. *Blighia sapida* root (2800 g) was also dried under shade, crushed, ground and extracted with soxhlet extractor using hexane, ethylacetate and methanol successively.

3.7 Phytochemical analysis of *Blighia sapida* extracts

Phytochemical screening was carried out on each of the crude extracts to test for the presence of secondary metabolites of these extracts. Qualitative and quantitative phytochemical screenings were carried out on these crude extracts using standard procedure.

3.7.1 Qualitative screening

Blighia sapida Extracts were screened for the presence of reducing sugars, alkaloids, quinones, tannins, phlobatannins, glycosides, saponins, steroids, flavonoids, resins, cardiac glycosides, phenols, anthraquinones, anthocyanins, diterpenes, terpenoids, coumarin, charcones and protein (Manjulika *et al*., 2014; Rimjhim *et al*., 2014; Williams, 2005).

3.7.1.1 Reducing sugars

Each extract of *Blighia sapida* (0.2 g) was dissolved in distilled water. 5 mL of mixture of equal volumes of Fehling's solutions A and B was added to 2 mL of test extract in a test tube. The resultant mixture was boiled for 2 minutes. A brick red precipitate confirmed the presence of reducing sugars.

3.7.1.2 Alkaloids (Hager's Test)

A 0.2 g of each of the extracts was acidified with 1% HCl for 2 minutes and the filtrate was treated with Hager's reagent (saturated picric acid solution). Yellow coloured precipitate confirmed the presence of alkaloids.

3.7.1.3 Quinones (Borntrager's Test)

A 3 mL extract was treated with 3 mL chloroform. 5% potassium hydroxide was added to the separated chloroform layer. Red colour in alkaline phase showed the presence of quinones.

3.7.1.4 Tannins (Braymer's Test)

A 0.2 g *Blighia sapida* extract was stirred with water and filtered. A dirty - green precipitate, blue – black or blue - green precipitate on addition of few drops of 5% ferric chloride to the test extract was taken as an indication of the presence of tannins.

3.7.1.5 Phlobatanins (Precipitate Test)

Extract (2 mL) and 1% HCl (2 mL) were mixed and heated. Red precipitate confirmed the presence phlobatannins.

3.7.1.6 Glycosides (Concentrate H_2SO_4 Test)

A 5 mL H_2SO_4 was added to 0.2 g extract of *Blighia sapida*, the mixture was heated in boiling water for 15 minutes. Fehling's solutions were then added and the resulting mixture was heated to boiling. A brick – red precipitate confirmed the presence of glycosides.

3.7.1.7 Saponins

A 0.2 g extract was dissolved in 5 mL of distilled water. 2 mL of the resulted solution was taken into a test tube and was shaken vigorously for a few minutes. Frothing which persists on warming was taken as an evidence of the presence of saponins.

3.7.1.8 Steroids

A 0.2 g *Blighia sapida* extract was dissolved in 2 mL of chloroform. 0.2 mL of concentrated H_2SO_4 was carefully added to form a lower layer. A reddish - brown colour at the interface between the layers indicated the deoxy – sugar characteristics of cadenolides which confirmed the presence of steroids.

3.7.1.9 Flavonoids

A small quantity of the extract was dissolved in dilute NaOH. A yellow solution that turned colourless on addition of HCl acid confirmed the presence of flavonoids.

3.7.1.10 Resins

A 2.0 g extract was shaken with distilled water and filtered. 1 mL of copper acetate solution was added to 1 mL of the filtrates. The resulting solution was shaken vigorously and allowed to separate. A green - colour solution was an evidence of the presence of resins.

3.7.1.11 Cardiac glycosides

Small quantity of the extract was dissolved in 2 mL of acetic anhydride and cooled well in ice. Concentrated H_2SO_4 was then carefully added. A violet colour which changed to blue and then to green indicated the presence of a steroidal nucleus.

3.7.1.12 Phenols

Test extract was dissolved in ferric chloride solution. Blue - black or brown colouration indicated the presence of phenol.

3.7.1.13 Anthraquinones

A 0.2 g *Blighia sapida* extract was shaken with 4 mL benzene. The mixture was filtered and 2 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of pink red or violet colour in ammoniacal solution (lower phase) indicated the presence of free anthraquinones.

3.7.1.14 Anthocyanins

Extract (2 mL) and 2N HCl (2 mL) were mixed, drops of NH_3 were then added. Colouration of pinkish red to bluish violet showed the presence of anthocyanins.

3.7.1.15 Diterpenes (Copper acetate Test)

Water was used to dissolve the *Blighia sapida* extract, extract solution was treated with 3 - 4 drops of copper acetate solution. Emerald green colouration indicated the presence of diterpenes.

3.7.1.16 Terpenoids

Acetic anhydride (2 mL) was added to 2 mL extract, 2 - 3 drops of concentrated H_2SO_4 was added to the mixture. Formation of deep red colouration showed the presence of terpenoids.

3.7.1.17 Coumarins

Extract (2 mL) was taken and 10% NaOH (3 mL) was added to it. Yellow colouration indicated coumarins.

3.7.1.18 Charcones (Ammonium hydroxide's Test)

Drops of ferric chloride solution (3 – 4 drops) were added to *Blighia sapida* extracts. Colouration of bluish black colour indicated the presence of phenols.

3.7.1.19 Protein (Xanthoproteic Test)

Concentrated H_2SO_4 (1 mL) was mixed with 1mL of extract. White precipitates confirmed the presence of protein.

3.7.2 Quantitative screening

Quantitative Screening of alkaloids, tannins, saponins, flavonoids and total phenolics was determined (Afify *et al*., 2012; Fazel *et al*., 2008; Kaviarasan *et al*., 2007; Manjulika *et al*, 2014; Miean and Mohamed, 2001; Singlet., 1999; Tambe and Bhambar, 2014; Xu and Chang, 2008).

3.7.2.1 Alkaloids

Each *Blighia sapida* extract (1 mg) was dissolved in dimethyl sulphoxide (DMSO), 1 mL of 2 N HCl was added to the extract and filtered. The solution formed was transferred to a separating funnel, 5 mL of phosphate buffer and 5 mL of bromocresol green solution were added to the solution. This solution mixture was shaken with chloroform (1 mL, 2 mL, 3 mL and 4 mL) by vigorous shaking. It was then collected in a 10 mL volumetric flask and diluted to the volume with chloroform. Reference standard solutions of atropine (20, 40, 60, 80 and 100 μg/mL) were prepared with the same method as above. UV/Visible spectrophotometer was used to determine absorbance of test and standard solutions against reagent blank at 470 nm. Total alkaloid content of each sample was expressed as mg of atropine equivalent of extract (mg of AE/g of extract).

3.7.2.2 Tannins

Tannins were determined by Folin - Ciocalteu method. 0.1 mL of extract was added to 10mL volumetric flask containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteuphenol reagent, 1 mL of 35% Na_2CO_3 solution and diluted to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 μg/mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. Tannin content was expressed in terms of mg of GAE/g of extract.

3.7.2.3 % Saponins

Each extract (20 g) was put into a conical flask; 100 mL of 20% aqueous ethanol were added and it was heated over hot water bath for 4 hours at about 55^0C with continuous stirring. This mixture was filtered; the residue was re-extracted with another 200 mL of 20% aqueous ethanol. These extracts were combined and concentrated to 40 mL at about 90^oC over water bath. It was transferred into a 250 mL separating funnel, 20 mL of di-ethyl ether was added and shaken vigorously. Aqueous layer was recovered and the ether layer was discarded. The purification process was repeated. n-butanol (60 mL) was added, n-butanol extract was washed twice with 5% aqueous sodium chloride (10 mL). Remaining solution was heated in a water bath and dried in the oven to a constant weight. Saponin content was calculated as a percentage.

3.7.2.4 Flavonoids

Aluminium chloride colorimetric assay was used to measure total flavonoid content. 1 mL of *Blighia sapida* extract and 4 mL of distilled water were mixed and taken into a 10 mL volumetric flask. 0.30 mL of 5% sodium nitrite was treated and after 5 minutes, 0.3 mL of 10% aluminium chloride was mixed. 2 mL of 1M Sodium hydroxide was treated after 5 minutes and diluted to 10 mL with distilled water. Reference standard solutions of quercetin (20, 40, 60, 80 and 100 μg/mL) were prepared in the same procedure as described earlier. UV/Visible spectrophotometer was used to determine absorbance of test and standard solutions against the reagent blank at 510 nm. Total flavonoid content was expressed as mg of QE/g of extract.

3.7.2.5 Total Phenolics

Spectrophotometric method was used to determine the concentration of phenolics in plant extracts. Determination of total phenol content was done by Folin-Ciocalteu assay method. 1 mL of extract and 9 mL of distilled water were mixed and taken into a 25 mL volumetric flask. Folin-Ciocalteu phenol reagent (1mL) was treated to the mixture and shaken well. 10 mL of 7% Sodium carbonate (Na_2CO_3) solution was treated to the mixture after 5 minutes and the volume was made up to 25 mL. Standard solutions of gallic acid (20, 40, 60, 80 and 100 μg/mL) were prepared in the same manner as described earlier. This was incubated for 90 minutes at room temperature; Ultraviolet (UV)/Visible spectrophotometer was used to determine the absorbance of

test and standard solutions against the reagent blank at 550 nm. Total phenol content was expressed as mg of GAE/g of extract.

3.8 Fractionation of *Blighia sapida* root ethyl acetate extract

The crude extracts were subjected to analytical Thin Layer Chromatography (TLC Silica gel 60 F_{254}, Merck KGaA, 64271 Darmstadt, Germany) and plates were developed in different solvent systems. Spots were visualized under UV Lamp (Ultra-violet radiation, 230V.50Hz, LF-206.LS, 6W-365 nm Tube, 6W-254 nm Tube, Power-12W, Uvitec Lamps, England) with the use of vanillin spray and iodine tank. *Blighia sapida* extracts were pre- adsorbed on white sand, then introduced into a glass column. A 12 g of root ethyl acetate extract of hot extraction was pre-adsorbed on white sand; this was introduced into the column packed with 150 g silica gel (60 - 200 mesh size). The column was eluted using four solvents for gradient elution starting with 100% hexane, then with decreasing concentration of hexane and increasing concentration of dichloromethane till 100% dichloromethane; then with dichloromethane and increasing concentration of ethyl acetate, later with ethyl acetate and methanol. A total of one hundred and fifty 100 mL fractions were collected. Some were also subjected to further purification.

3.9 Isolation and purification of root ethyl acetate fraction (BS6)

Fractions 35-36 eluted with 20% hexane and 80% dichloromethane were greenish yellow liquid, they were pooled together on their similar pattern on TLC plate and later crystallized out as cream powder after evaporation which was further purified by washing with hexane and dichloromethane mixture to remove the yellow supernatant, it was labelled BS6 (71.5 mg). BS6 showed the presence of a fluorescent compound on TLC plate that was visualized under UV light at 254 nm.

3.10 Characterization of BS6

Characterization of BS6 was done using IR, proton and carbon-13 NMR (1H, COSY 1H – 1H Correlation, APT, DEPT 135, HSQC, HMBC and NOESY), MS and melting point. Spectral identification of BS6 was done using IR spectrophotometer (Perkin Elmer Spectrum 100 instrument with an attenuated total reflectance (ATR) attachment), Mass spectrophotometer, Nuclear Magnetic Resonance spectrometer (Bruker AVANCE III 400 MHz), that is, 1H, COSY, HSQC, DEPT 135, APT,

HMBC, NOESY. Melting point (BI Barnstead Electrothermal 9100) of BS6 was also done. These results were also compared with those from literatures.

3.11 Biological assessments of essential oils

3.11.1 Antimicrobial activities of essential oils

3.11.1.1 Microorganisms

In the antimicrobial assay, ten microorganisms were employed which include six bacteria among which are two Gram positives: *Staphylococcus aureus* and *Bacillus subtilis*; four Gram negatives: *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumoniae* and four fungi which are *Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum*. The provision of these test organisms was done by the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan. Identification and characterization of clinical isolates were achieved by standard identification methods.

3.11.1.2 Sample preparation

Stock solution of each essential oil was prepared, serial dilutions were made from the stock solutions to have different six concentrations each of essential oils of *Blighia sapida, Lecaniodiscus cupanioides* and *Paullinia pinnata* as 100, 50, 25, 12.5, 6.25 and 3.125 mg/mL. The 7th test tube was the solvent which was the negative control and the 8th test tube which was the positive control (10 μg/mL) was either the Gentamicin for bacteria or tioconazole for fungi.

3.11.1.3 Antibacterial activity of essential oils

Pour plate method with Mueller Hinton agar was used. There was overnight culture of each organism suspension by taking a loop full of each organism from stock and inoculated it into sterile nutrient broth of 5 mL and incubated for 18-24 hours at 37°C to have broth culture from where 0.1 mL of each organism was taken and inoculated into 9.9 mL of sterile distilled water to have 1:100 dilution. 0.2 mL was taken from this dilution and inoculated into the prepared sterile nutrient agar at 45°C which was poured into sterile petri dishes and allowed to stand for 45-60 minutes to set. Sterile cork borer was used to punch wells of 8 mm diameter into the set agar medium and different concentrations of the essential oils and the controls were introduced into the different wells based on the number of concentration of the essential oils and this was done in duplicates. Pre-diffusion of samples into agar was achieved by allowing the

plates to stay on the bench for 2 hours and these plates were uprightly incubated in the incubator for 18-24 hours at 37°C. Zones of inhibition of the test organism were measured to evaluate antibacterial activity.

3.11.1.4 Antifungal activity of essential oils

Surface spread plate method was utilised of which a sterile Sabouraud Dextrose Agar was prepared according to manufacturer's instruction; this was poured into the sterile plates in duplicates and allowed to set properly. A calibrated micropipette was used to take 0.2 mL from the diluted organism and inoculated onto the surface of the prepared agar. Inoculum was spread to cover the entire surface of the sterile sabouraud dextrose agar using a sterile surface spreader and a sterile cork borer was used to punch wells of 8 mm diameter into the agar medium and different concentrations of essential oils and the control were introduced into the different corresponding wells based on the number of concentration of the samples. It was also done in duplicates. Pre-diffusion of essential oils into agar was achieved by allowing the plates to stay on the bench for 2 hours and these plates were then incubated uprightly in the incubator for 72 hours at 26-28°C. Evaluation of antifungal activity was done by measuring the zone of inhibition of the test organism.

3.11.2 Antioxidant activities of essential oils using DPPH radical scavenging activity

Weight of 3.94 mg from 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was dissolved in 100 mL methanol to give a 10 µM solution. 0.5 mL taken from different concentrations, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL (that is, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL) of each *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paulliana pinnata* essential oils was each added to 3.0 mL methanol solution of DPPH. The mixture was shaken well and then left to stand for 10 minutes, after which the measurement of decrease in absorption of DPPH at 517 nm was done. Known antioxidants that were Butylated Hydroxyl Anisole (BHA), Ascorbic acid (Vitamin C) and α-Tocopherol (Vitamin E) were also evaluated for comparison with the essential oils. All tests were done in triplicates and the average of results was calculated. Actual decrease in absorption induced by test compound was known by subtracting the value of the control. (Mellors and Tappel, 1996; Lugasi *et al.*, 1999; Gow-chin and Hui-Yin, 1995).

The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discolouration using the equation below:

$$\% \text{ RSA or } \% \text{ Inhibition} = \frac{(A_{DPPH} - A_S)}{A_{DPPH}} \times 100 \quad 3.1$$

A_{DPPH} – Absorbance of the DPPH solution

A_S – Absorbance of the solution of essential oils with DPPH

3.12 Biological assessments of *Blighia sapida* crude extracts and isolate

3.12.1 Antimicrobial activity of *Blighia sapida* crude extracts and isolate

3.12.1.1 Microorganisms

Ten microorganisms were used which were six bacteria (two Gram positives: *Staphylococcus aureus* and *Bacillus subtilis*; four Gram negatives: *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumoniae*) and four fungi (*Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum*). These organisms were clinical isolates provided by the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan while their identification and characterization were done by standard identification methods.

3.12.1.2 Sample preparation

Eight test tubes were used for each *Blighia sapida* extract and the isolate, stock solution of each extract and of isolate was prepared by weighing 1.0 g of the *Blighia sapida* extract or the isolate into 5 mL of the solvent of extraction in the first test tube of each sample to make 200 mg/mL concentration and each of the sample was serially diluted by taking 2.5 mL from the stock into 2.5 mL of the solvent in the second test tube to give a concentration of 100 mg/mL, this procedure was repeated until the 6th test tube giving six different concentrations: 200, 100, 50, 25, 12.5 and 6.25 mg/mL while the 7th test tube was the negative control (solvent) and the 8th test tube was the positive control (10 µg/mL) which was either the Gentamicin for bacteria or tioconazole for fungi. Investigations of antibacterial and antifungal activities of these extracts and the isolate were carried out using the methods described by Perez *et al.*, 1990 and Ahmad *et al.*, 1998.

3.12.1.3 Antibacterial activity of *Blighia sapida* crude extracts and isolate

Mueller Hinton agar was utilised following pour plate method as described above in antibacterial activity of essential oils. Different concentrations of each crude extract,

the isolate and the controls were introduced into the different wells based on the number of concentration of the extracts and the isolate, this was done in duplicates. The plates were allowed to stay on the bench for 2 hours for pre-diffusion of samples into agar as described above and then uprightly incubated for 18-24 hours at 37°C. Antibacterial activity was assessed by measuring the zones of inhibition of the test organism.

3.12.1.4 Antifungal activity of *Blighia sapida* crude extracts and isolate

A sterile Sabouraud Dextrose Agar was used following Surface spread plate method as explained above in antifungal activity of essential oil. The incubation was also for 72 hours at 26-28°C. Zone of inhibition was measured to evaluate antifungal activity.

3.12.2 Antioxidant activity of *Blighia sapida* crude extracts and isolate

Weight of 3.94 mg from 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was dissolved in 100 mL methanol to give a 10 µm solution. 0.5 mL taken from different concentrations, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL (that is, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL) of each *Blighia sapida* extracts and isolate was each added to 3.0 mL methanol solution of DPPH. The mixture was shaken well and then left to stand for 10 minutes, after which the measurement of decrease in absorption of DPPH at 517 nm was done. Known antioxidants as described above in antioxidant activity of essential oils were also evaluated for comparison.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Percentage yield and physical characteristics

4.1.1 *Blighia sapida* essential oils

The yield of Essential Oils obtained from leaves, leaves stalk, stem bark, root, fruit pulp, fruit husk and seeds of *Blighia sapida* varied, also with characteristic smell as shown in Table 4.1. Essential oils extracted from these plant parts were colourless and *Blighia sapida* leaf gave the highest percentage oil yield as 0.77% (w/w) among these seven parts as it was also the highest (0.67% (w/w)) among the colourless essential oils extracted from three parts recently reported by Oloyede *et al*., (2022) while the least from this research was obtained from the seed as 0.38% (w/w). Various factors such as weather condition and developmental stage can affect percentage oil yield (Elsharkawy and Nahed, 2018; Usano-Alemany *et al*., 2014). Seventy – eight (78) compounds were detected in leaf which was the highest among *Blighia sapida* essential oils, though seventeen components were reported by Oloyede *et al*., (2022) while the least was from the stem bark as ten (10) which was due to the high fibre content of the stem bark.

4.1.2 *Lecaniodiscus cupanioides* essential oils

Yield of leaves, leaves stalk, stem bark and root essential oils of *Lecaniodiscus cupanioides* varied. *Lecaniodiscus cupanioides* leaf stalk gave the highest percentage oil yield as 0.91% (w/w) while the least was obtained from the leaf as 0.25% (w/w) as shown in Table 4.2. Essential oils obtained from all these plant parts were colourless except for leaf that was greenish yellow. Each of the oils had characteristic smell as shown in Table 4.2. The highest number of compounds were detected also in leaf as ninety-five (95) while the least was from the root as two (2) compounds as a result of high fibre content of this plant's part.

Table 4.1: Yield of essential oils from *Blighia sapida*

S/N	Plant Part	WS (g)	WEOP (g)	% (w/w)	Odour	CI	%C
1	Leaf	150	1.15	0.77	Aromatic and leafy	78	78.77
2	Leaf Stalk	200	0.88	0.44	Woody and leafy	41	70.98
3	Stem Bark	200	0.94	0.47	Slightly choky and nut	10	32.89
4	Root	150	0.91	0.61	Faint woody	14	33.08
5	Fruit Pulp	100	0.71	0.71	Palatable	12	45.25
6	Fruit Husk	200	1.28	0.64	Palatable	11	68.88
7	Seed	200	0.76	0.38	Fibre-like	31	87.37
TOTAL						**197**	

WS- Weight of Sample (g), WEOP- Weight of Essential Oil Procured (g), % (w/w)- Percentage Yield of Essential Oil Procured, CI- Number of Compounds Identified, %C- Percentage Composition.

Table 4.2: Yield of essential oils from *Lecaniodiscus cupanioides*

S/N	Plant Part	WS(g)	WEOP (g)	% (w/w)	Odour	CI	%C
1	Leaf	300	0.76	0.25	Leafy odour	95	96.44
2	Leaf Stalk	300	2.72	0.91	Pleasant odour	42	100
3	Stem Bark	130	0.56	0.43	Woody odour	14	100
4	Root	300	1.14	0.38	Woody odour	02	64.27

WS- Weight of Sample (g), WEOP- Weight of Essential Oil Procured (g), % (w/w) - Percentage Yield of Essential Oil Procured, CI- Number of Compounds Identified, %C- Percentage Composition

4.1.3 *Paullinia pinnata* essential oils

The yield of Essential Oils extracted from *Paullinia pinnata* leaves, leaves stalk, stem bark and root as shown in Table 4.3 also varied. *Paullinia pinnata* stem bark gave the highest percentage oil yield which was 5.55% (w/w) while the least was obtained from the leaf stalk as 0.48% (w/w). Essential oils from these plant parts were all colourless as different from report of Ogunwande *et al*., (2017) that worked on essential oil of *Paullinia pinnata* leaves which was reported as light yellow. *Paullinia pinnata* essential oils had characteristic smell as shown in Table 4.3. Fifty-seven (57) compounds were detected in the leaf stalk and it was the highest among *Paullinia pinnata* while the least was also from the stem bark as nineteen (19) compounds.

4.1.4 Percentage yield of crude extracts of *Blighia sapida*

In summary, the percentage yield of crude extracts of hexane, ethyl acetate and methanol *Blighia sapida* extracts procured from cold extraction of leaf, stem-bark and root of *Blighia sapida* varied and were 1.26%, 2.05%, 0.50%, 0.11%, 0.25%, 1.17%, 0.15%, 0.52%, 2.89% yields respectively. *Blighia sapida* root methanol extract gave the highest percentage yield, followed by *Blighia sapida* leaf ethyl acetate extract, then *Blighia sapida* stem-bark methanol while the least was obtained from the *Blighia sapida* stem-bark hexane extract as shown in Table 4.4. Leaf ethyl acetate extract had the highest yield among leaf extracts, stem-bark methanol among stem-bark extracts and root methanol among root extracts, this follows similar trend to what Abba *et al*., (2017) reported on *Blighia sapida* stem-bark and root. Soxhlet (hot) extraction of hexane, ethyl acetate and methanol extracts of *Blighia sapida* root were 0.28%, 0.57% and 7.18% yields respectively which showed that hot extraction gives higher yield than cold extraction.

Table 4.3: Yield of essential oils from *Paullinia pinnata*

S/N	Plant Part	WS(g)	WEOP (g)	% (w/w)	Odour	CI	%C
1	Leaf	120	0.89	0.74	Leafy odour	37	90.59
2	Leaf Stalk	100	0.48	0.48	Woody odour	57	91.16
3	Stem Bark	30	1.67	5.55	Woody odour	19	100
4	Root	100	1.00	1.00	Pleasant pungent odour	29	97.90

WS- Weight of Sample (g), WEOP- Weight of Essential Oil Procured (g), % (w/w) - Percentage Yield of Essential Oil Procured, CI- Number of Compounds Identified, %C- Percentage Composition

Table 4.4: Yield of *Blighia sapida* extracts

S/N	Plant Part	Code	WS (g)	WCEP (g)	% (w/w)	Physical Properties
1	Bs Leaf Hexane	BsLH	2000	25.24	1.26	Green; Smooth paste
2	Bs Leaf Ethyl acetate	BsLEa	2000	40.91	2.05	Green; Smooth paste
3	Bs Leaf Methanol	BsLM	2000	9.92	0.50	Green; Shining paste
4	Bs Stem-Bark Hexane	BsSbH	1800	2.03	0.11	Yellowish brown; Oily crystal
5	Bs Stem-Bark Ethyl acetate	BsSbEa	1800	4.49	0.25	Green; Smooth paste
6	Bs Stem-Bark Methanol	BsSbM	1800	20.98	1.17	Chocolate brown; Smooth paste
7	Bs Root Hexane	BsRH	2000	2.90	0.15	Yellowish brown; Oily crystal
7b	Bs Root Hexane (Hot)	BRHH	2800	7.70	0.28	Greenish brown; Oily crystal
8	Bs Root Ethyl acetate	BsREa	2000	10.32	0.52	Green; Smooth paste
8b	Bs Root Ethyl acetate (Hot)	BREH	2800	15.89	0.57	Chocolate brown; Smooth paste
9	Bs Root Methanol	BsRM	2000	57.87	2.89	Green; Smooth paste
9b	Bs Root Methanol (Hot)	BRMH	2800	201.16	7.18	Brown; Smooth paste

Bs- *Blighia sapida*, WS- Weight of Sample (g), WEOP- Weight of Crude Extracts Procured (g), % (w/w)- Percentage Yield of Crude Extracts Procured.

4.2 Gas Chromatography-Mass Spectrometry Analysis of the Essential Oils

4.2.1 *Blighia sapida* Essential Oils

The results of *Blighia sapida* leaf, leaf stalk, stem bark, root, fruit pulp, fruit husk and seed essential oils showed that each part had many chemical constituents. Identified compounds from *Blighia sapida* leaf was seventy-eight (78); 41 compounds in *Blighia sapida* leaf stalk; 10 compounds in *Blighia sapida* stem bark; 14 compounds in *Blighia sapida* root; 12 compounds in *Blighia sapida* fruit pulp; 11 compounds in *Blighia sapida* fruit husk and 31 compounds were identified in *Blighia sapida* seed giving total of 197 compounds from *Blighia sapida* while removing compound repeatedly identified in another plant's part made it 142 compounds compared to 17 compounds from *Blighia sapida* leaf; 21 compounds from *Blighia sapida* stem bark and 22 compounds from *Blighia sapida* root reported by Oloyede *et al*., (2022).

There were some identified compounds from this leaf, stem bark and root that are similar with this report. Example of such similar compounds were hexahydrofarnesyl acetone, phytol, α-ionone and β-ionone in leaf; hexahydrofarnesyl acetone in stem bark while geosmin that was reported from *Blighia sapida* leaf was detected in leaf stalk and stem bark of this research but not detected in leaf. There are factors that determine the chemical compositions of essential oils which include genetic factors, seasonal change and plant organs (Moghaddam and Mehdizadeh, 2017).

The significant chemical constituents of *Blighia sapida* leaf essential oil in descending order were α-Ionone (5.17%), β-Ionone (4.14%), phytol (4.03%), (Z)-1,8 (2H,5H)-Hexahydro-8a-methyl-naphthalenedione (3.34%), (Z)-6,10-Dimethyl-5,9-undecadien-2-one (3.25%), Hexahydrofarnesyl acetone (2.79%), Phytanol (2.57%), farnesyl acetone (2.55%), Hexylbenzoate (1.58%), (Z)-3-Hexen-1-ol, benzoate (1.56%), α-Farnesene (1.46%) and (E)-2-Nonen-1-ol (1.39%).

In leaf stalk essential oil, the chemical constituents that were significant in descending order were pentadecanal (5.18%), hexahydrofarnesyl acetone (3.82%), farnesyl acetone (3.02%), $2,2^1,5,5^1$-tetramethyl-$1,1^1$-biphenyl (2.99%), tridecane (2.11%), pyrene (1.95%), isophytol (1.90%), phytol (1.89%), 2,4,6,8-tetramethyl-1-undecene (1.81%), 9-methylene-9H-fluorene (1.74%), heptacosane (1.72%), 1-(1,3α,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5- azulenyl)-ethanone (1.48%), (E)-6,10-dimethyl-5,9-undecadien-2-one (1.47%), 2-phenyl-1H-indene (1.45%), cadalene (1.44%), mono (2-ethylhexyl)-1,2-benzenedicarboxylate (1.36%), tributyl acetylcitrate (1.20%), geosmin (1.16%), 3,3-dimethyl-hexane (1.12%), propanal,(1-methylethy1) hydrazone (1.10%) and 1,3-di-n-propyladamantane (1.07%).

Stem bark essential oil had the following significant chemical compounds in descending order of geosmin (6.31%), pentadecanal (4.83%), farnesol (3.93%), hexahydrofarnesyl acetone (3.61%), 9,9-dimethyl-9-silafluorene (2.68%), 2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane (2.40%) and 6-ethyl-2-methyl-octane (1.14%).

In root essential oil, the chemical constituents of significant quantities in descending order were verticiol (7.92%), 2,3,4,5,6-pentachloroanisole (6.79%), 1,7-dimethyl-4-(1-methylethyl)-spiro(4,5) dec-6-en-8–one (4.49%), α-Elemene (4.40%), α–cadinol (2.01%), $2,2^1,5,5^1$-tetramethyl-$1,1^1$-biphenyl (1.77%) and cadalene (1.45%).

The significant chemical constituents of fruit pulp essential oil in descending order were α–caryophyllene (8.05%), heneicosane (7.03%), cembrene (4.16%), butyldecyls-ulfurate (2.39%), 1-docosene (1.71%), trifluoro-3,7-dimethyloctyl acetate (1.71%), 2,2,3,3-tetramethylpentane (1.43%), tributyl acetyl citrate (1.36%) and $1,1^1$-dimethyl-$1,1^1$-bicyclopropyl (1.13%).

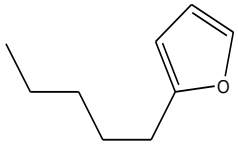
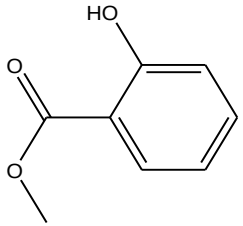
In fruit husk essential oil, the chemical constituents of significant quantities in descending order were verticiol (29.10%), α-terpineol acetate (4.43%), alloaromadendrene oxide (4.39%), tributyl acetylcitrate (2.45%), hexahydrofarnesyl acetone (1.51%) and methyl palmitate (1.06%).

The significant chemical constituents of seed essential oil in descending order were (Z)-11-octadecenoic acid (14.24%), verticiol (9.84%), tributyl acetylcitrate (4.92%), isoterpinolene (3.83%), 2-ethoxy-ethanol (3.20%), methyl palmitate (1.64%), α-caryophyllene (1.64%), (Z)-methyl 9-octadecenoate (1.58%), α-springene (1.22%), hexadecanoic acid (1.22%) and 1,2-diiodo-ethane (1.08%).

The first three dominating identified compounds in each *Blighia sapida* essential oil were α-ionone (5.17%), β-ionone (4.14%) and phytol (4.03%) in leaf; pentadecanal (5.18%), hexahydrofarnesyl acetone (3.82%) and (E), (E)-6,10,14-trimethyl-5,9,13-pentadecatrien-2-one (3.02%) in leaf stalk; geosmin (6.31%), pentadecanal (4.83%) and farnesol (3.93%) in stem bark; verticiol (7.92%), 2,3,4,5,6-pentachloroanisole (6.79%) and 1,7-dimethyl-4-(1-methylethyl)-spiro (4,5) dec-6-en-8-one (4.49%) in root; α-caryophyllene (8.05%), heneicosane (7.03%) and cembrene (4.16%) in fruit pulp; verticiol (29.10%), 3-cyclohexene-1-methanol-α,α,4-trimethyl- acetate (4.43%) and alloaromadendrene oxide (4.39%) in fruit husk and (Z)-11-octadecenoic acid (14.24%), verticiol (9.84%) and tributyl acetylcitrate (4.92%) in fruit seed.

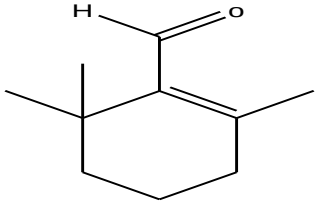
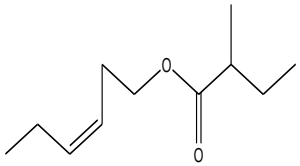
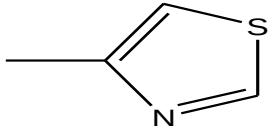
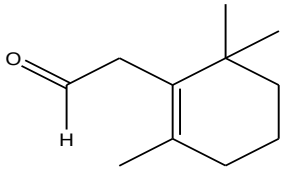
Comparing chemical components of these seven essential oils, 142 different compounds were identified in these seven essential oils of *Blighia sapida* as shown in Table 4.5 because some of the chemical constituents were identified in more than one plant's part of *Blighia sapida* while others were identified in only one part.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications

S/N	Rt (Mins)	Compound	R1	Chemical Structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
1	6.55	2-Pentyl-furan	1273		NT (Furan)	Flavouring agent	n.d.	0.48	n.d.	n.d.	n.d.	n.d.	n.d.
2	9.57	Nonanal	1543		NT (Aldehyde)	Perfumery agent	n.d.	0.57	n.d.	n.d.	n.d.	n.d.	n.d.
3	11.31	(E)-2-Nonen-1-ol	1698		NT (Fatty Alcohols)	Flavouring agent	1.39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	12.03	Methyl salicylate	1765		MTd (Ester)	For joint and muscular pain, flavouring, antiseptic, killing herbivorous insects, as bait for attracting male orchid bees, use for clearing plant or animal tissue samples of colour, in printing work, it is used to restore elastomeric properties of weak rubber rollers	0.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

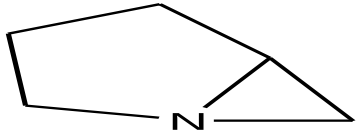
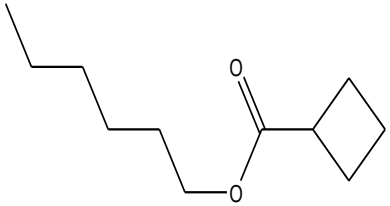
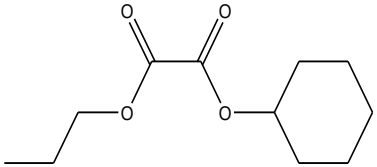
Rt- Retention time (minutes), RI- Retention Index, n.d – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical Structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
5	12.69	β-Cyclocitral	1826		MTd	Flavoring agent, stabilizers, nutrient, emulsifiers and surfactants	0.83	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	12.91	(Z)-3-Hexenyl-α-methylbutyrate	1848		NT (Ester)	Flavouring agent	0.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	13.19	4-Methylthiazole	1875		NT (Thiazole)	Flavouring agent	0.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	13.60	β-Homocyclocitral	1915		MTd (Aldehyde)	Flavouring agent	0.49	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	13.61	(Z)-2-Decenal	1917		NT (Aldehyde)	Flavouring agent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.38

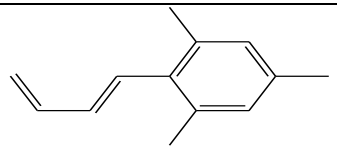
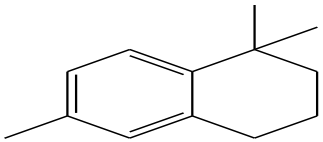
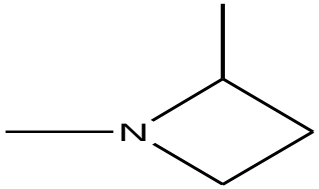
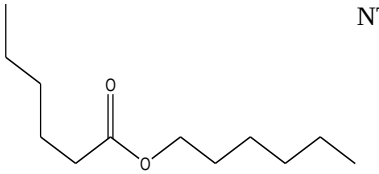
Rt- Retention time (minutes), RI- Retention Index, n.d.– Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical Structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
10	14.92	2,4-Decadienal	2053		NT (Aldehyde)	Flavouring agent	0.26	n.d.	n.d.	n.d.	n.d.	n.d.	0.73
11	15.10	1-Azabicyclo (3,1,0)hexane	2073		NT		0.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12	15.23	Hexyl cyclobutanecarboxylate	2087		NT (Ester)		0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
13	15.39	Cyclohexyl propyloxalate	2105		NT (Oxalate)		0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

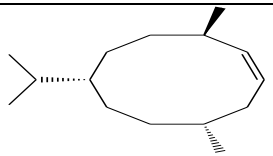
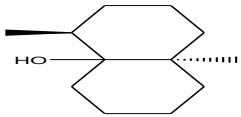
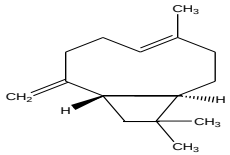
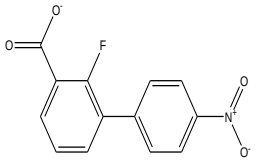
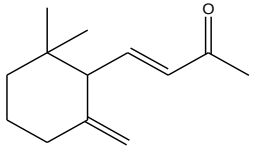
Rt- Retention time (minutes), RI- Retention Index, n.d.– Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical Structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem Bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
14	15.83	1,3,5-Trimethyl-2-(1,3-butadienyl)benzene	2153		MT		0.27	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15	15.91	α-ionene	2161		STd (Tetralin)	Flavouring agent	0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
16	15.98	2-Undecenal	2169		NT (Aldehyde)	Flavouring agent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.38
17	15.99	1,2-DimethylAzetidine	2170		NT (Azetidine)	Drug designs	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
18	16.52	Hexyl hexanoate	2230		NT (Ester)	Fruit flavouring agent	0.18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

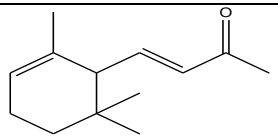
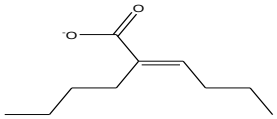
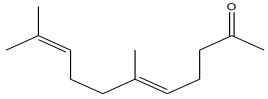
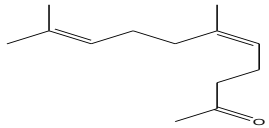
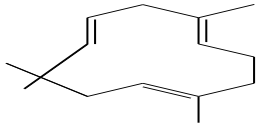
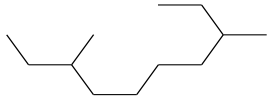
Rt- Retention time (minutes), RI- Retention Index, n.d.– Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical Structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
19	16.78	Germacrene	2261		ST	Antimicrobial and insecticidal properties	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.48
20	16.86	1-Iodononane	2270		NT		n.d.	0.07	n.d.	n.d.	n.d.	n.d.	n.d.
21	17.12	Geosmin	2301		STd	Flavouring agent	n.d.	1.16	6.31	n.d.	n.d.	n.d.	n.d.
22	17.58	β-Caryophyllene	2355		ST	Flavouring agent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.67
23	17.66	4-Nitrophenyl-2-fluorobenzoate	2355		ST	Flavouring agent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.67
24	17.72	ɣ- Ionone	2372		STd	Flavouring agent	n.d.	0.91	n.d.	n.d.	n.d.	n.d.	n.d.

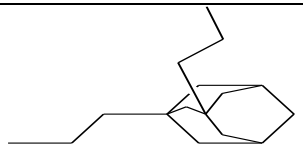
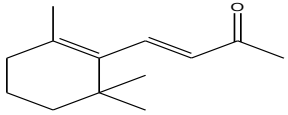
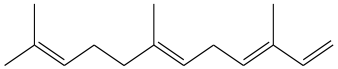
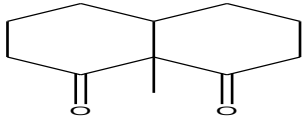
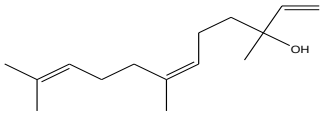
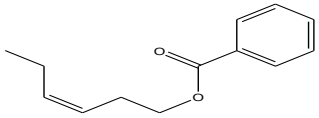
Rt- Retention time (minutes), RI- Retention Index, n.d.– Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
25	17.81	α-Ionone	2383		STd	Flavouring and Perfumery	5.17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
26	17.98	(E)-Butyl-2-hexenoate	2403		NT (Ester)	Flavouring agent	0.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27	18.43	Geranyl acetone	2460		MTd	Flavouring agent	n.d.	1.47	n.d.	n.d.	n.d.	n.d.	n.d.
28	18.52	Neryl acetone	2471		MTd	Flavouring agent	3.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29	18.62	α-caryophyllene	2484		ST	Flavouring agent	n.d.	n.d.	n.d.	n.d.	8.05	n.d.	1.64
30	18.70	3,8-Dimethyldecane	2494		NT		0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

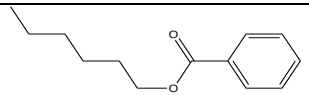
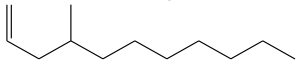
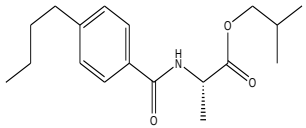
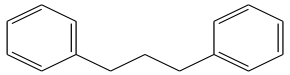
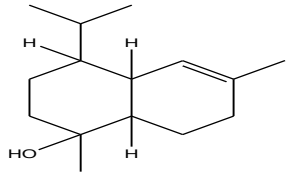
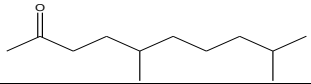
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
31	19.64	1,3-di-n-Propyladamantane	2612		NT (Adamantane)	Drugs, polymeric materials and thermally stable lubricants	n.d.	1.07	n.d.	n.d.	n.d.	n.d.	n.d.
32	19.75	β-Ionone	2626		STd	Flavouring and Perfumery agent. Vitamin A activity	4.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
33	20.48	α-Farnesene	2718		ST	Flavouring agent	1.46	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
34	21.37	(Z)-1,8(2H,5H)-hexahydro-8a-methyl-naphthalenedione	2830		NT	Inflammation and microbial infection	3.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35	22.67	Nerolidol	2994		STd	Flavouring agent	0.31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
36	22.90	(Z)-3-Hexen-1-ol,benzoate	3023		NT (Ester)	Flavouring agent	1.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

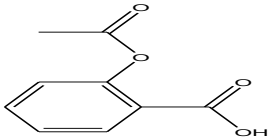
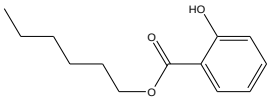
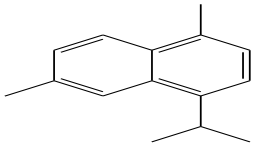
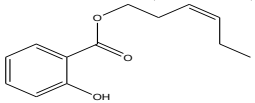
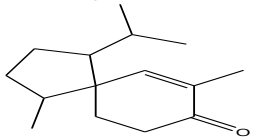
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
37	23.08	Hexyl benzoate	3046		NT (Ester)	Flavouring agent	1.58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
38	23.56	4-Methyl-1-undecene	3106		NT	Flavouring agent	n.d.	0.48	n.d.	n.d.	n.d.	n.d.	n.d.
39	23.85	Myristaldehyde	3142		NT	Surfactant, Emulsifier, Nutrient, Membrane stabilizer, Energy source and Flavouring agent	0.30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
40	24.46	-Alanine,N-(4-butylbenzoyl)-,isobutyl ester	3219		NT		0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
41	24.60	1,3-Diphenylpropane	3237		NT	Impurity in polystyrene container	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
42	24.68	(Z)- 2 (3H)- dihydro-5-(2-Octeny1)-Furanone	3247				0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
43	24.69	α-cadinol	3249		STd	Antimicrobial and flavouring agent	n.d.	n.d.	n.d.	2.01	n.d.	n.d.	n.d.
44	24.95	5,9-Dimethyl-2-decanone	3281		MTd		0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

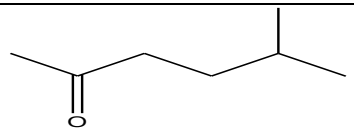
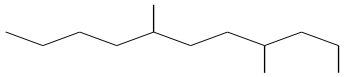
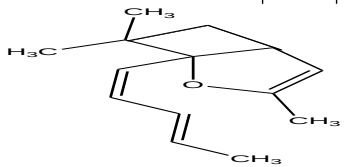
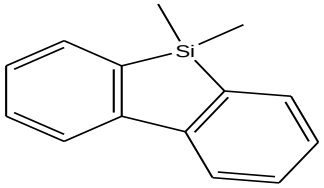
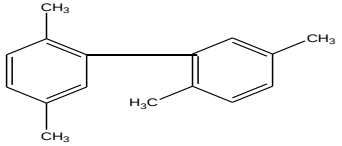
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
45	24.99	1-Dodecanol	3286		NT	Antifungal activity flavouring agent, adhesive, plasticizer, lubricant and paint additives	0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
46	25.01	8-Heptadecene	3289		NT		n.d.	0.24	n.d.	n.d.	n.d.	n.d.	n.d.
47	25.02	Aspirin	3290		NT	Treatment of pain, fever and inflammation	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14
48	25.03	n-Hexyl salicylate	3292		NT (Benzoic acid ester)	Flavouring agent	0.30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
49	25.04	Cadalene	3292		ST	Reduce incidence of adenomas	n.d.	1.44	n.d.	1.45	n.d.	n.d.	n.d.
50	25.13	(Z)-3-Hexenyl Salicylate	3303		NT	Flavouring agent	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
51	25.29	1,7-Dimethyl-4-(1-methylethyl)-spiro(4,5)dec -6-en-8-one	3324		NT		0.14	0.78	n.d.	4.49	n.d.	n.d.	n.d.

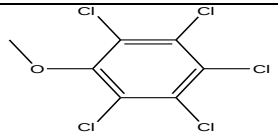
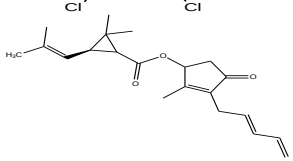
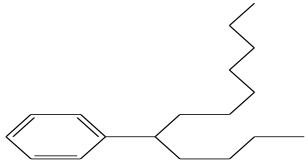
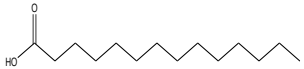
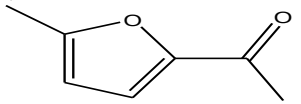
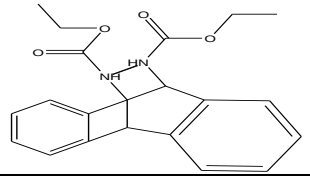
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
52	25.31	5-Methyl-2-hexanone	3327		NT		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.25
53	25.32	4,7-Dimethylundecane	3328		NT		n.d.	0.59	n.d.	n.d.	n.d.	n.d.	n.d.
54	25.37	3,7,7-Trimethyl-1-penta-1,3-dienyl-2-Oxabicyclo(3,2,0)hept-3-ene	3334		NT		0.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
55	25.51	9,9-Dimethyl-9-silafluorene	3352		NT		n.d.	n.d.	2.68	n.d.	n.d.	0.94	n.d.
56	25.52	2, 2^1 $5,5^1$-tetramethyl-$1,1^1$-Biphenyl	3353		NT		0.15	2.99	n.d.	1.77	n.d.	n.d.	0.63
57	25.53	Pentadecanal	3354		NT	Antimicrobial agent	n.d.	5.18	4.83	n.d.	n.d.	0.41	0.15

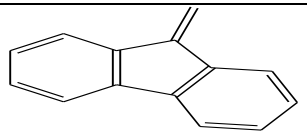
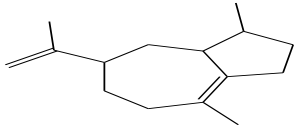
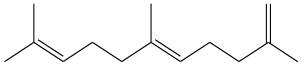
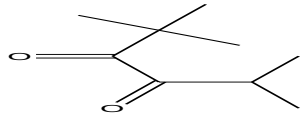
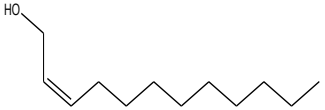
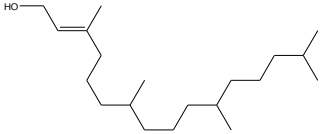
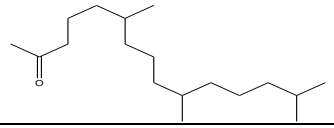
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
58	25.68	2,3,4,5,6-pentachloroAnisole	3373		NT		0.39	0.83	n.d.	6.79	n.d.	n.d.	n.d.
59	25.76	Pyrethrin	3383		NT	Insecticidal	0.54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
60	25.81	1-Butyloctylbenzene	3389		NT		n.d.	0.59	n.d.	n.d.	n.d.	n.d.	n.d.
61	26.16	Myristic acid	3433		NT	Flavouring agent	0.29	n.d.	n.d.	n.d.	n.d.	n.d.	0.37
62	26.27	2-Acetyl-5-methylfuran	3447		NT	Organoleptic and flavouring agent	0.12	0.19	n.d.	0.76	n.d.	n.d.	n.d.
63	26.32	Pentadecanol	3454		NT	Flavouring agent	0.29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
64	26.38	Anthracene-9,10-dihydro-diethyl-9,10-biimine-11,12-dicarboxylate	3462		NT		n.d.	n.d.	n.d.	0.60	n.d.	n.d.	n.d.

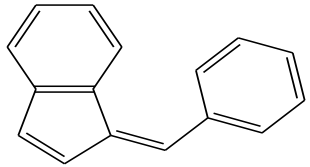
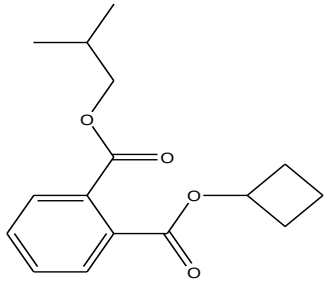
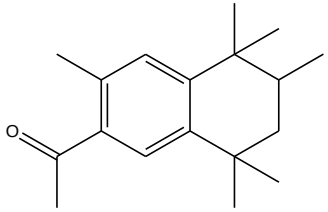
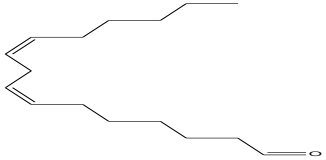
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
65	26.40	9-Methylene-9H-fluorene	3463		NT		0.39	0.43	n.d.	n.d.	n.d.	n.d.	n.d.
66	26.43	1,2,3,4,5,6,7,8-Octahydro-1,4-dimethyl-7-(1-methylethenyl)-,(1s-(lα,4α,7α)]-Azulene	3467		NT		0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
67	26.54	2,6,10-trimethyl-1,5,9-undecatriene	3481		MT		0.40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
68	26.55	2,2,5-Trimethyl-3,4-hexanedione	3482		NT		n.d.	n.d.	0.70	0.49	n.d.	n.d.	n.d.
69	26.74	(Z)-2-Dodecenol	3506		NT	Flavouring agent	n.d.	0.49	n.d.	n.d.	n.d.	n.d.	n.d.
70	26.99	Phytol	3538		DT	Flavouring agent, Precursor of Vitamin E and Vitamin K1	4.03	1.74	n.d.	n.d.	n.d.	n.d.	n.d.
71	27.06	Hexahydrofarnesyl acetone	3542		STd	Flavouring agent	2.79	3.82	3.61	n.d.	n.d.	1.51	0.51

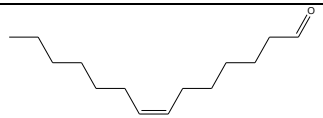
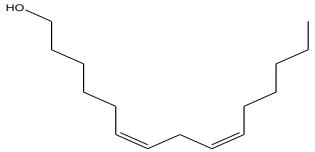
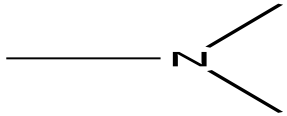
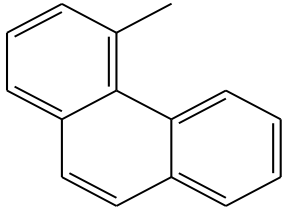
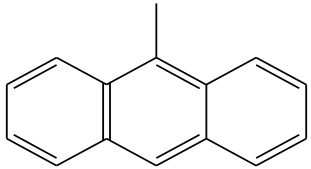
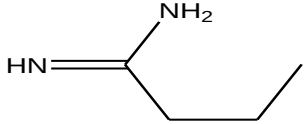
Rt- Retention time (minutes), RI- Retention Index, n.d.– Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
72	27.20	1-Hexadecyne	3565		NT		n.d.	0.78	n.d.	n.d.	n.d.	n.d.	n.d.
73	27.25	1-(Phenylmethylene)-1H-Indene	3571		NT		0.11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
74	27.29	Cyclobutyl isobutyl phthalate	3576		NT		0.34	1.02	n.d.	0.60	n.d.	n.d.	0.43
75	27.31	Tonalid	3578		NT		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.51
76	27.46	(Z),(Z)-7,10-Hexadecadienal	3597		NT		0.38	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

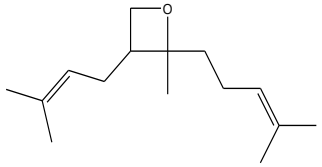
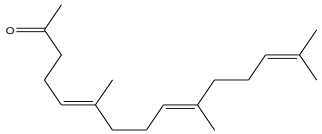
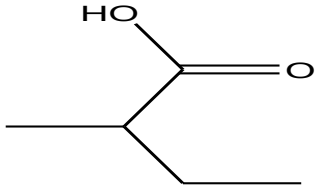
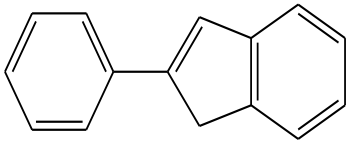
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
77	27.50	(Z)-7-Tetradecenal	3602		NT		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.38
78	27.51	(Z), (Z)-6,9-Pentadecadien-1-ol	3604		NT		0.70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
79	27.56	Trimethylamine	3610		NT	Flavouring Agent	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.40
80	27.60	4-Methyl-phenanthrene	3614		NT		n.d.	0.45	n.d.	n.d.	n.d.	n.d.	n.d.
81	27.65	9-Methyl-anthracene	3621		NT		n.d.	0.49	n.d.	n.d.	n.d.	n.d.	n.d.
82	27.69	Butanimidamide	3627		NT		0.28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

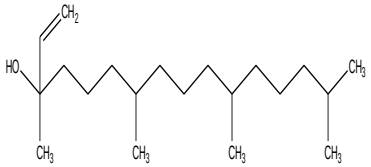
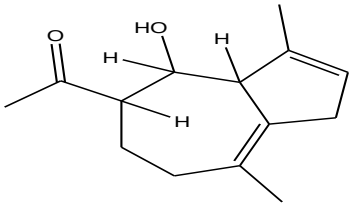
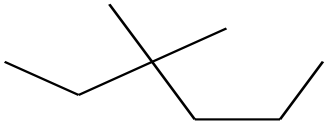
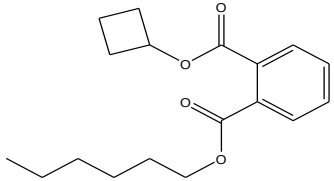
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
83	27.72	2-methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	3630		NT		n.d.	n.d.	2.40	n.d.	n.d.	n.d.	n.d.
84	27.73	Farnesyl acetone	3631		STd	Flavouring agent	2.55	3.02	n.d.	n.d.	n.d.	n.d.	n.d.
85	27.75	2-Methyl-butanoic acid	3634		NT	Flavouring agent	n.d.	1.16	n.d.	n.d.	n.d.	n.d.	n.d.
86	27.76	Methyl palmitate	3634		NT	Flavouring agent	0.27	n.d.	n.d.	n.d.	n.d.	1.06	1.64
87	27.84	2-Phenyl-1H-indene	3645		NT		0.37	1.45	n.d.	n.d.	n.d.	n.d.	n.d.

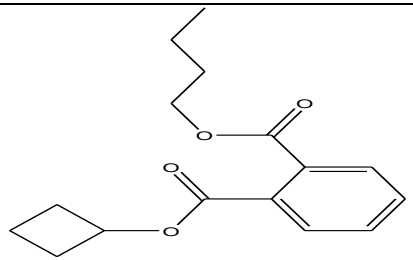
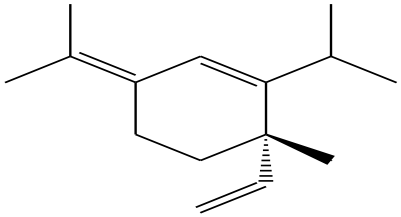
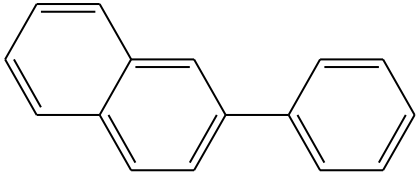
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
88	27.95	Isophytol	3659		STd	Flavouring agent, cosmetics, Precursor of Vitamin E and Vitamin K1	0.58	1.90	n.d.	n.d.	n.d.	n.d.	n.d.
89	27.97	1- (1,3α,4,5,6,7-Hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)-ethanone	3661		STd		n.d.	1.48	n.d.	n.d.	n.d.	n.d.	n.d.
90	28.01	3, 3-Dimethylhexane	3667		NT		0.24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
91	28.10	Cyclobutyl hexyl phthalate	3678		NT		n.d.	n.d.	n.d.	0.73	n.d.	n.d.	n.d.

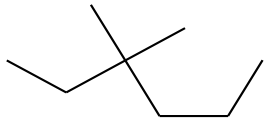
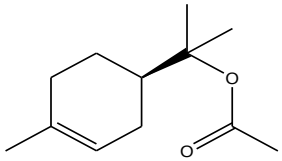
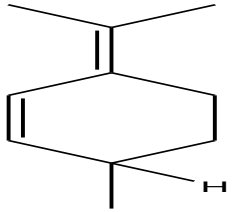
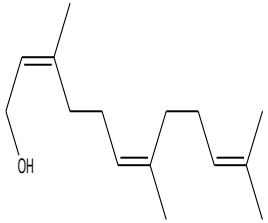
Rt- Retention time (minutes), RI- Retention Index, n.d.– Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
92	28.11	Butyl cyclobutyl phthalate	3679				9.11	5.05	n.d.	n.d.	n.d.	n.d.	n.d.
93	28.20	α – Elemene	3690		ST	Flavouring agent	n.d.	n.d.	n.d.	4.40	n.d.	n.d.	n.d.
94	28.21	Hexadecanoic acid	3691		NT	Thickener, stabalizer, emulsifier and gelling agent	0.37	n.d.	n.d.	n.d.	n.d.	n.d.	1.22
95	28.27	2-Phenyl-naphthalene	3700		NT		0.60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

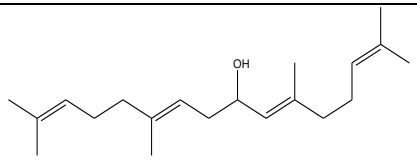
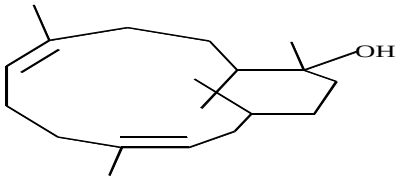
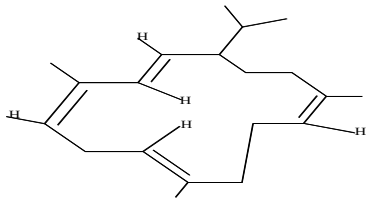
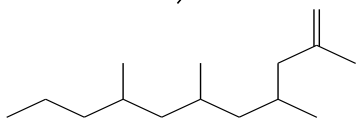
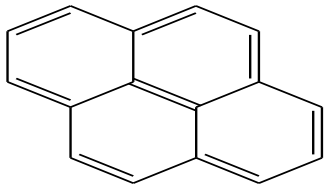
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
96	28.30	Methyl-6-deoxy-6-fluoro-2,3,4-tri-O-ethylàd-galactopyranoside	3703				n.d.	n.d.	n.d.	0.47	n.d.	n.d.	n.d.
97	28.34	3,3-Dimethylhexane	3708		NT		n.d.	1.12	n.d.	n.d.	n.d.	n.d.	n.d.
98	28.54	α-Terpineol acetate	3733		MTd	Flavouring agent	n.d.	n.d.	n.d.	n.d.	n.d.	4.43	n.d.
99	28.57	Isoterpinolene	3736		MTd		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.83
100	28.61	Farnesol	3742		ST	For natural and artificial organic synthesis, use in perfumery and pesticide; chemopreventative and anti-tumor agent; antibacterial agent	n.d.	n.d.	3.93	n.d.	n.d.	n.d.	n.d.

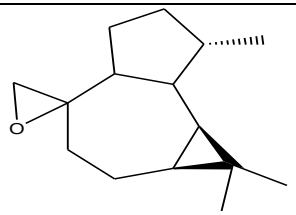
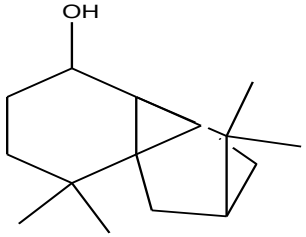
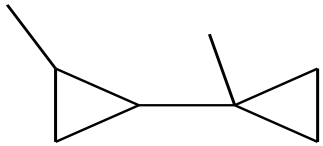
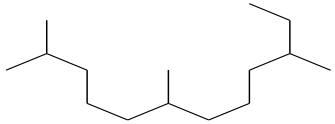
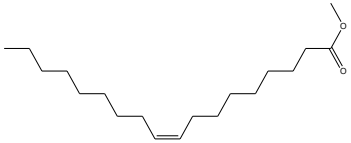
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
101	28.63	Geranylgeraniol	3744		DTd	Antimicrobial agent	0.59	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
102	28.66	Verticiol	3748		DTd		n.d.	n.d.	n.d.	7.92	n.d.	29.1	9.84
103	28.73	Cembrene	3757		DT	Flavouring agent	n.d.	n.d.	n.d.	n.d.	4.16	n.d.	n.d.
104	28.97	2,4,6,8-Tetramethyl-1-undecene	3788		MT		n.d.	1.81	n.d.	n.d.	n.d.	n.d.	n.d.
105	29.01	Pyrene	3792		NT		0.41	1.95	n.d.	n.d.	n.d.	n.d.	n.d.

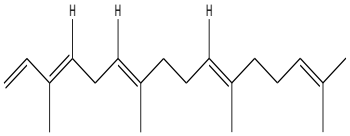
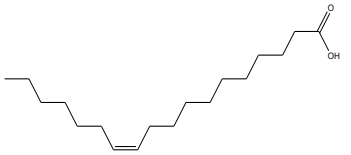
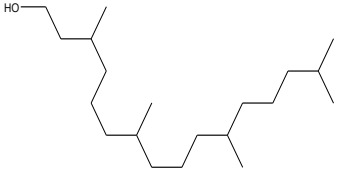
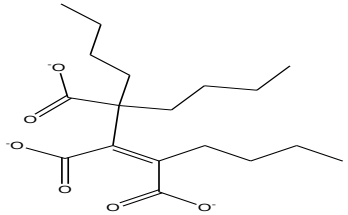
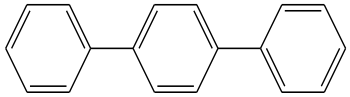
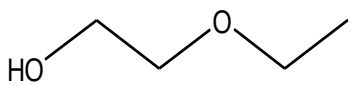
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
106	29.02	Alloaromadendrene oxide	3793		STd		n.d.	n.d.	n.d.	n.d.	n.d.	4.39	n.d.
107	29.06	4,4, 11,11-Tetramethyl-7-tetracyclo(6,2,1,0 (3.8) 0 (3.9)) undecanol	3798		NT		0.88	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
108	29.06	$1,1^1$-Dimethyl-1, 1^1-bicyclopropyl	3799		NT		n.d.	n.d.	n.d.	n.d.	1.13	n.d.	n.d.
109	29.07	2, 6,10-Trimethyldodecane	3800		ST		0.61	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
110	29.10	(Z)-Methyl 9-Octadecenoate	3804		NT		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.58

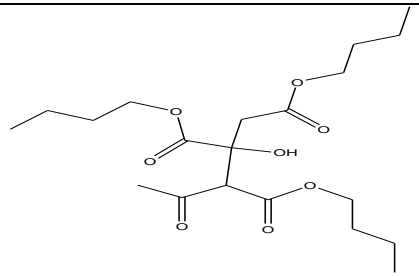
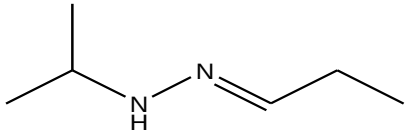
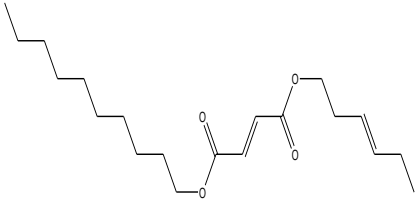
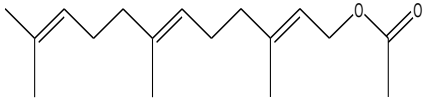
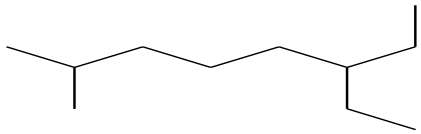
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
111	29.21	α-Springene	3818		DT		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.22
112	29.44	(Z)-11-octadecenoic acid	3841		NT	Anticarcinogenic properties	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	14.24
113	29.46	Phytanol	3815		DT	Precursor of Vitamin E and Vitamin K1 and a flavouring agent	2.57	1.89	n.d.	n.d.	n.d.	n.d.	n.d.
114	29.51	Tributyl-1-propene-1,2,3-tricarboxylate	3856		NT		0.41	1.71	2.21	n.d.	12.69	2.73	1.95
115	29.57	p-Terphenyl	3863		NT	Polymerized with styrene to make plastic phosphor	0.38	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
116	29.69	2-ethoxy-ethanol	3878		NT	Multipurpose cleaner	0.15	n.d.	n.d.	n.d.	n.d.	n.d.	3.20

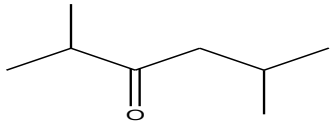
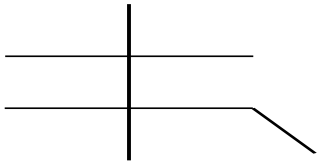
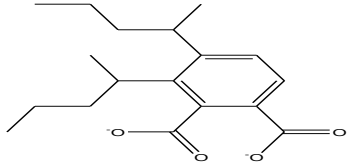
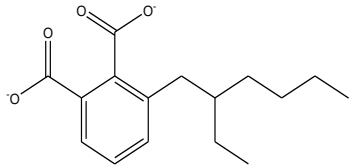
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
117	30.15	Tributyl acetylcitrate	3935		NT	Flavouring agent; Plasticizer	0.71	1.20	n.d.	n.d.	1.36	2.35	4.92
118	30.34	Heptacosane	3960		NT		n.d.	1.72	n.d.	n.d.	n.d.	n.d.	n.d.
119	30.73	Propanal(1-methylethy1)hydrazone	4010		NT		n.d.	1.10	n.d.	n.d.	n.d.	n.d.	n.d.
120	30.83	Decyl trans-hex-3-enyl fumarate	4021		NT		0.51	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
121	30.90	Farnesyl acetate	4030		STd	Antimicrobial agent	0.65	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
122	30.91	6-Ethyl-2-methyl-octane	4032		NT		n.d.	n.d.	1.14	n.d.	n.d.	n.d.	n.d.

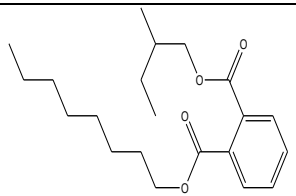
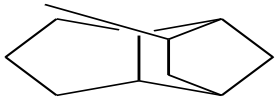
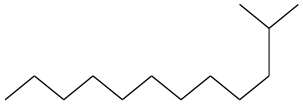
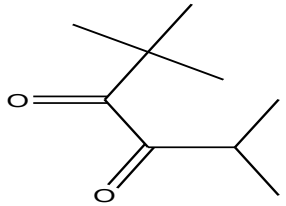
Rt- Retention time (minutes), RI- Retention Index, n.d.– Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
123	30.92	Tridecane	4032		NT	Flavouring agent	n.d.	2.11	n.d.	n.d.	n.d.	n.d.	n.d.
124	31.11	2,5-Dimethyl-3-Hexanone	4057		NT		0.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
125	31.46	2,2,3,3-Tetramethylpentane	4101		NT		n.d.	n.d.	n.d.	n.d.	1.43	n.d.	n.d.
126	31.47	Heptacosane	4102		NT		0.71	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
127	31.79	Bis(2-pentyl_-phthalate	4143		NT		0.44	n.d.	0.98	n.d.	0.52	n.d.	0.95
128	31.79	Mono(2-ethylhexyl)-1,2-benzenedicarboxylate	4142		NT		n.d.	1.36	n.d.	n.d.	n.d.	n.d.	n.d.

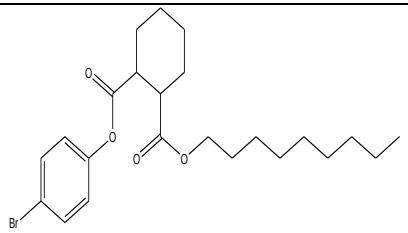
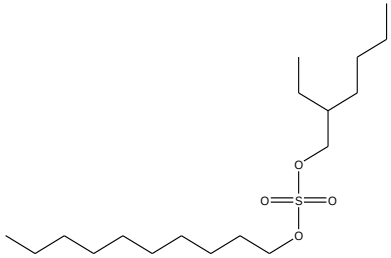
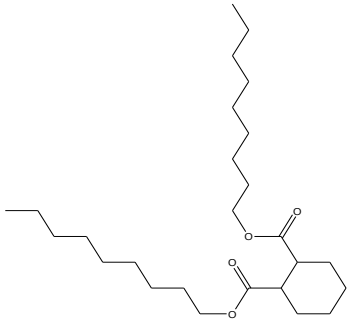
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
129	31.79	2-Methylbutyl octyl phthalate	4142		NT		n.d.	n.d.	n.d.	0.61	n.d.	1.12	n.d.
130	31.82	(Z)-8-methyl-exo-tricyclo(5,2,1,0 (2.6)) decane	4147		NT		0.30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
131	31.98	2-Methyl-dodecane	4167		NT		n.d.	n.d.	n.d.	n.d.	n.d.	0.87	n.d.
132	31.99	2,2,5-Trimethyl-3,4-hexanedione	4167		NT		n.d.	n.d.	n.d.	n.d.	0.52	n.d.	n.d.
133	32.37	1,2Diiodo-ethane	4215		NT		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.08
134	32.38	1-Docosene	4217		NT		n.d.	n.d.	n.d.	n.d.	1.71	n.d.	n.d.
135	32.48	Heneicosane	4231		NT		n.d.	n.d.	n.d.	n.d.	7.03	n.d.	0.72

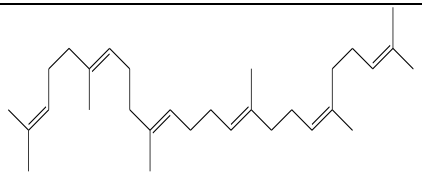
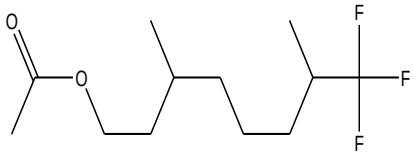
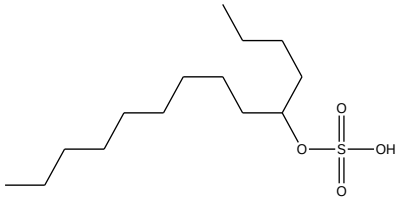
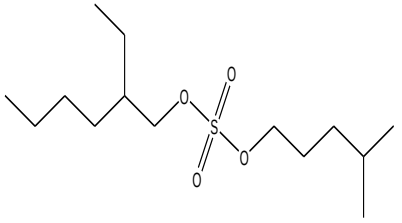
Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
136	32.98	4-Bromophyl nonyl 1,2-cycloheanedicarboxylate	4293		NT		n.d.	n.d.	n.d.	n.d.	n.d.	0.66	n.d.
137	33.02	Decyl 2-ethylhexyl Sulfurate	4297		NT		0.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
138	33.13	Dinonyl 1,2-cyclohexanedicarboxylate	4312		NT	Precursor for synthesis of steroids; skin lubrication and protection in cosmetics; use with surfactants in certain adjuvant formulations	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.39

Rt- Retention time (minutes), RI- Retention Index, n.d.– Not detected.

Table 4.5: Comparison of the compounds of essential oils from *Blighia sapida* and their applications Cont'd

S/N	Rt (Mins)	Compound	R1	Chemical structure	Class of compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
139	33.25		4327		TT		0.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
140	33.48	Trifluoro-3,7-dimethyloctyl acetate	4356		NT		n.d.	n.d.	n.d.	n.d.	1.71	n.d.	n.d.
141	33.60	butyldecylsulfurate	4371		NT		n.d.	n.d.	n.d.	n.d.	2.39	n.d.	n.d.
142	35.02	2-Ethylhexyl isohexyl sulfurate	4550		NT		0.10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total Identified							78	41	10	14	12	11	31

Rt- Retention time (minutes), RI- Retention Index, n.d. – Not detected.

Tributyl-1-propene-1,2,3-tricarboxylate was the most common among the identified compounds of *Blighia sapida* essential oils, it was identified in all the *Blighia sapida* parts except the root essential oil, that is, leaf (0.41%), leaf stalk (1.71%), stem bark (2.21%), fruit pulp (12.69%), fruit husk (2.73%) and fruit seed (1.95%) though regarded as bleed in GC-MS analysis. Tributyl acetyl citrate and Hexahydrofarnesyl acetone were the next, in that they were identified in all the parts except two plant's parts in both cases.

Tributyl acetyl citrate was identified in all the *Blighia sapida* parts except the root and stem bark essential oil, that is, leaf (0.71%), leaf stalk (1.20%), fruit pulp (1.36%), fruit husk (2.35%) and seed (4.92%). Hexahydrofarnesyl acetone was also identified in all the *Blighia sapida* parts except the root and fruit pulp essential oil, that is, leaf (2.79%), leaf stalk (3.82%), stem bark (3.61%), fruit husk (1.51%) and seed (0.51%) similar to hexahydrofarnesyl acetone being detected by Oloyede *et al*., (2022) as 21.44% from leaf, stem bark (2.03%) and absent in root. Sotubo *et al*., 2016 also reported hexahydrofarnesyl acetone as 37.50% from the leaf of *Dehinbollia pinnata* (Sapindaceae). $2,2^{1},5,5^{1}$-tetramethyl-$1,1^{1}$-biphenyl (0.15%, 2.99%, 1.77%, 0.63%) and cyclobutyl isobutyl phthalate (0.34%, 1.02%, 0.60%, 0.43%) were identified in *Blighia sapida* leaf, leaf stalk, root and seed essential oil respectively.

Pentadecanal was identified in *Blighia sapida* leaf stalk (5.18%), stem bark (4.83%), fruit husk (0.41%) and seed (0.15%) essential oil. Bis(2-pentyl)-phthalate was identified in *Blighia sapida* leaf (0.44%), stem bark (0.98%), fruit pulp (0.52%) and seed (0.95%) essential oil. Verticiol was identified in *Blighia sapida* root (7.92%), fruit husk (29.10%) and fruit seed (9.84%) essential oil. 1,7-dimethyl-4-(1-methylethyl)-spiro(4,5)dec-6-en-8-one (0.14%, 0.78%, 4.49%), 2,3,4,5,6-pentachloroanisole (0.39%, 0.83%, 6.79%) and 2-acetyl-5-methylfuran (0.12%, 0.19%, 0.76%) were identified in *Blighia sapida* leaf, leaf stalk and root essential oil respectively.

Methyl palmitate was identified in *Blighia sapida* leaf (0.27%), fruit husk (1.06%) and fruit seed (1.64%) essential oil. 9-methylene-9H-fluorene (0.39%, 0.43%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (4.03%, 1.74%), farnesyl acetone (2.55%, 3.02%), 2-phenyl-1H-indene (0.37%, 1.45%), phytol (2.57%, 1.89%), isophytol (0.58%, 1.90%),

butyl cyclobutyl phthalate (9.11%, 5.05%) and pyrene (0.41%, 1.95%) were identified in *Blighia sapida* leaf and leaf stalk essential oil respectively. Farnesyl acetone from *Blighia sapida* leaf and stem bark essential oil as 7.01% and 1.34% respectively, phytol from leaf (20.45%), isophytol from leaf as 1.21% (Oloyede et al., 2022). Phytol (0.6%) from *Blighia unijugata* leaf (Sapindaceae) Moronkola *et al*., 2017. Sotubo *et al*., 2016 detected farnesyl acetone (17.20%) and phytol (8.1%) from *Dehinbollia pinnata* leaf (Sapindaceae).

Hexadecanoic acid (0.37%, 1.22%) and 2, 4-decadienal (0.26%, 0.73%) were identified in *Blighia sapida* leaf and seed essential oil respectively. Hexadecanoic acid was also reported from *Blighia unijugata* leaf (2.4%), leaf stalk (8.86%), root (9.63) and fruit with seed (2.28%) Moronkola *et al*., 2017. α- caryophyllene (8.05%, 1.64%) and heneicosane (7.03%, 0.72%) were identified in *Blighia sapida* fruit pulp and seed essential oil respectively. Geosmin was identified in *Blighia sapida* leaf stalk (1.16%) and stem bark (6.31%) essential oil. Cadalene was identified in *Blighia sapida* leaf stalk (1.44%) and root (1.45%) essential oil.

Tetradecanoic acid was identified in *Blighia sapida* leaf (0.29%) and fruit seed (0.37%) essential oil. 2,2,5-trimethyl-3,4-hexanedione was identified in *Blighia sapida* stem bark (0.70%) and root (0.49%) essential oil. 2-methylbutyl octyl phthalate was identified in *Blighia sapida* root (0.61%) and fruit husk (1.12%) essential oil.

The class of non-terpene derivatives was the highest among the identified compounds present in the essential oil of *Blighia sapida* leaf, leaf stalk, stems bark, root, fruit pulp and seed with 40.93 %, 42.35 %, 21.15 %, 14.84 %, 29.36 % and 36.80 % respectively as shown in Table 4.6. Likewise ester (non-terpene) dominated *Blighia unijugata* leaf, carboxylic acids (non-terpene) dominated *Blighia unijugata* stem bark and root (Moronkola *et al*., 2017). Hamedeyazdan *et al*., (2013) also reported non-terpene derivatives as highest among the identified compounds from *Marrubium persicum* C. A. Mey. (Lamiaceae). The class of diterpenoid was the highest in fruit husk (29.1 %) as shown in Table 4.6. Most of these non-terpenes detected are known for industrial and medicinal values, for example, esters and aldehydes as perfumes, flavours; terpenes and oxygenated terpenoids are known for free radical scavenging activity which make them important as antioxidant agent, also having antimicrobial and insecticidal properties as shown in Table 4.5.

The classes of identified compounds in *Blighia sapida* leaf in descending order were non-terpene (40.93%), monoterpenoid (10.63%), sesquiterpenoid (7.32%), diterpenoid (4.39%), sesquiterpene (1.46%), diterpene (0.61%) and triterpene (0.33%).

In *Blighia sapida* leaf stalk, the classes of identified compounds present in descending order were non-terpene (41.19%), sesquiterpenoid (9.80%), sesquiterpene (6.24%), diterpenoid (1.89%) and monoterpenoid (0.36%).

There were also non-terpene (14.84%), sesquiterpenoid (13.85%) and monoterpene (1.14%) in *Blighia sapida* stem bark in this descending order. These classes in *Blighia sapida* root in descending order were non-terpene (14.84%), diterpene (8.27%), diterpenoid (7.92%) and sesquiterpenoid (1.45%).

In *Blighia sapida* fruit pulp, non-terpene (29.36%), sesquiterpene (8.05%), diterpene (4.16%) and monoterpenoid (1.71%) were also present in this descending order. In *Blighia sapida* fruit husk, these compounds in descending order were diterpenoid (29.1%), non-terpene (14.07%), sesquiterpene (4.39%) and sesquiterpenoid (1.51%).
In *Blighia sapida* seed, non-terpene (36.80%), sesquiterpene (3.93%), monoterpene (3.83%), diterpene (1.22%) and sesquiterpenoid (0.51%) were also present in this descending order.

Table 4.6: Classes of identified compounds of essential oils from *Blighia sapida*

Class	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)	Fruit pulp (%)	Fruit husk (%)	Seed (%)
Monoterpene	n.d.	n.d.	1.14	n.d.	n.d.	n.d.	3.83
Monoterpenoid	10.63	0.36	n.d.	n.d.	1.71	n.d.	n.d.
Sesquiterpene	1.46	6.24	n.d.	1.45	8.05	4.39	3.93
Sesquiterpenoid	7.32	9.80	13.85	8.27	n.d.	1.51	0.51
Diterpene	0.61	n.d.	n.d.	n.d.	4.16	n.d.	1.22
Diterpenoid	4.39	1.89	n.d.	7.92	n.d.	29.10	9.84
Triterpene	0.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Non-terpene	40.93	41.19	14.84	14.84	29.36	14.07	36.80

n.d. - Not Detected

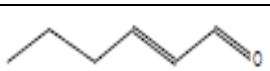
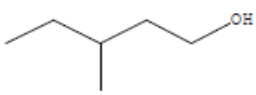
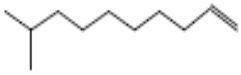
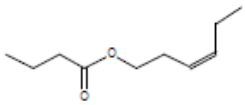
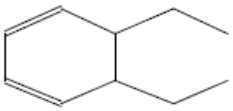
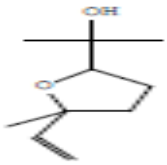
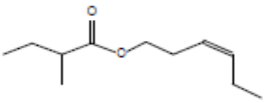
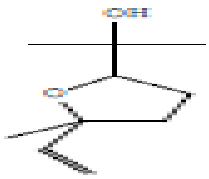
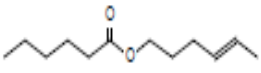
4.2.2 *Lecaniodiscus cupanioides* essential oils

The results of *Lecaniodiscus cupanioides* leaf, leaf stalk, stem bark and root essential oils showed different chemical constituents. There were 95 identified compounds in *Lecaniodiscus cupanioides* leaf; 42 compounds in *Lecaniodiscus cupanioides* leaf stalk; 14 compounds in *Lecaniodiscus cupanioides* stem bark and 2 compounds in *Lecaniodiscus cupanioides* root giving total of 153 compounds from *Lecaniodiscus cupanioides* while removing compound repeatedly identified in another plant's part made it 143 compounds.

The significant chemical constituents of *Lecaniodiscus cupanioides* leaf essential oil in descending order were (E)-2-hexenal (32.68%), 3-methyl-1-pentanol (13.42%), linalool (6.41%), 9-methyl-1-decene (4.87%), (Z)-butanoic acid, 3-hexenyl ester (3.56%), 5,6-diethyl-1,3-cyclohexadiene (2.81%), (Z)-linalool oxide (2.42%), (3Z)-3-hexenyl 2-methylbutanoate (2.28%), (E)-linalool oxide (2.25%), (4E)-4-hexenyl hexanoate (2.15%), (E)-2-hexenoic acid (1.92%), 4-ethylbenzaldehyde (1.52), α-terpineol (1.37), α-ionone (1.16) and (E)-11(12-cyclopropyl)dodecen-1-ol (1.14%) as projected on Table 4.7. α-ionone was also detected in *Blighia sapida* leaf at 5.17%. Table 4.7 is also showing the molecular ion and fragmentation ions of each significant compounds. The bold fragments represent the base peaks, while asteric (*) represents the molecular ion peak.

Fragmentation occurs by passing molecules through the ionization chamber of Mass spectrometer and this will form dissociation of energetically unstable molecular ions; these fragments of molecules give a unique pattern in mass spectrum. Molecules with carbonyl compounds, such as aldehydes or ketones with hydrogen at γ-position to the carbonyl group, undergo McLafferty re-arrangement (Mass spectral cleavage). Fragmentation of radical cation of the carbonyl compound occurs by a rearrangement that causes hydrogen atom to be transferred from a γ-position to the carbonyl group, as observed in hexahydrofarnesyl acetone.

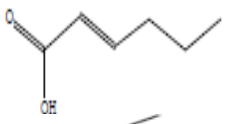
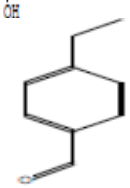
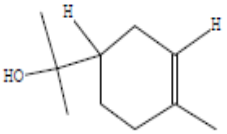
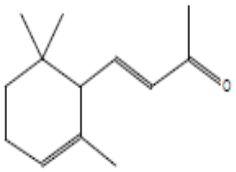
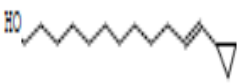
Table 4.7: Significant compounds from *Lecaniodiscus cupanioides* leaf

S/N	Rt (Mins)	Name	Stucture	%C	Fragmentation ions and Molecular ion
1	4.776	(E)-Hexenal		32.68	41,55,**69**,83,98*
2	4.934	3-Methyl-1-Pentanol		13.42	45,55,**56**,69,70,84,102*
3	6.849	Linalool		6.41	55,69,**71**,93,107,121,136, 154*
4	4.117	9-Methyl-1-Decene		4.87	45,55,**56**,69,72,85, 111, 154*
5	7.348	(Z)-Butanoic acid, 3-hexenyl ester		3.56	55,**67**,71,82,88, 95,101, 170*
6	8.408	5,6-Diethyl-1,3-cyclohexadiene		2.81	65,77,**79**,91,107,121,136*
7	6.700	(Z)-Linalool oxide		2.42	45,55,**59**,68,69,79,94,111, 137,155,170*
8	7.618	(3Z)-3-Hexenyl 2-methylbutanoate		2.28	55,57,**67**,82,85,115,184*
9	6.804	(E)-Linalool oxide		2.25	45,55,**59**,68,69,79,94,111, 112,137,155,170*
10	8.527	(4E)-4-Hexenyl hexanoate		2.15	55,**67**,73,82,87,99,157,172,182,198*

Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks,

*- Molecular ion peak

Table 4.7: Significant compounds from *Lecaniodiscus cupanioides* leaf Cont'd

S/N	Rt(Mis)	Name	Structure	%C	Fragmentation ions and Molecular ion
11	6.353	(E)-2-Hexenoic acid		1.92	45,55,60,68,**73**,81,96,99,113,114*
12	7.300	4-Ethylbenzaldehyde		1.52	51,65,77,91,105,119,134*
13	7.456	α-Terpineol		1.37	55,**59**,68,79,81,93,97,110,121,136,139,154*
14	8.997	α-Ionone		1.16	45,55,65,77,91,93,109,**121**,136,159,177,192*
15	10.140	(E)-11(12-Cyclopropyl)dodecen-1-ol		1.14	54,**68**,81,95,98,109,121,149,206,224*

Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks,

*- Molecular ion peak

Molecular ion which is the heaviest ion usually denoted as M^+ and fragmentation ions are used for structural elucidation. Isotopic abundance of select ions could also be used along, which is denoted as $(M+1)^+$, for example, in the presence of carbon as we also have in organic compound; the carbon is dominated by carbon-12 (^{12}C) but there is still small natural abundance of ^{13}C, such leads to $(M+1)^+$ apart from the M^+. In a spectrum, base peak which is the most intensive peak, that is, the tallest peak and its intensity is used as 100 percent is also a tool for elucidation of compound; the base peak is the most abundant in ion source because it is the most stable ion or there are several ways it could be formed during the fragmentations.

The chemical constituents that were significant in leaf stalk essential oil in descending order were 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid (85.55%), 1-octen-3-ol (1.41%),(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-etyhlenetricyclo[4.4.0.02,7]decane-rel (0.94%), caryophyllene (0.83%) and palmitic acid (0.66%) as shown in Table 4.8.

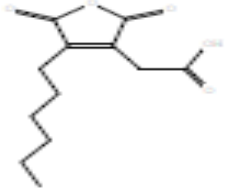
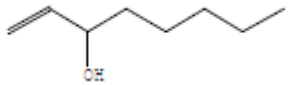
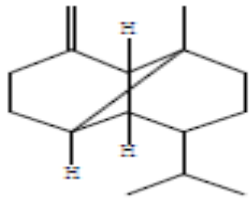
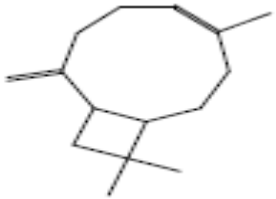
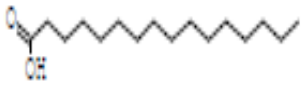
Essential oil from the stem bark had the following significant chemical compounds in descending order of tetrapentacontane (42.01%), nonacosane (12.53%), hexatriacontane (8.77%) and 6,6-diethylhoctadecane (6.88%) as shown in Table 4.9.

Root essential oil displayed a significant chemical constituent of 1,2,3,5,7-pentamethyl-1H-pyrrolo [2,3-f]quinolin-9-ol (30.69%) as shown in Table 4.10 .

The first three dominating identified compounds in *Lecaniodiscus cupanioides* leaf, leaf stalk and stem bark essential oils were (E)-2-hexenal (32.68%), 3-methyl-1-pentanol (13.42%) and linalool (6.41%); 4-hexyl-2,5-dihydro-2,5-dioxo-3-furanacetic acid (85.55%), 1-octen-3-ol (1.41%) and (1R,2S,6S,7S,8S)-8-isopropyl-1-methyl-3-metyhlenetricyclo[4.4.0.02,7]decane-rel (0.94%); tetrapentacontane (42.01%), nonacosane (12.53%) and hexatriacontane (8.77%) respectively while 1,2,3,5,7-pentamethyl-1H-pyrrolo [2,3-f]quinolin-9-ol (30.69%) in root essential oil.

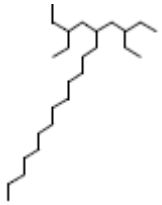
One hundred and forty-three compounds were identified in these four essential oils of *Lecaniodiscus cupanioides*. Some of the chemical constituents were identified in more than one plant's part of *Lecaniodiscus cupanioides* while others were identified in only one part as shown in Table 4.11.

Table 4.8: Significant compounds from *Lecaniodiscus cupanioides* leaf stalk

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
1	9.448	4-hexyl-2,5-dihydro-2,5-dioxo-3-Furanacetic acid		85.55	45,55,67,95,98,125,**126**,140,154,168,223,240*
2	5.982	1-Octen-3-ol		1.41	45,55,**57**,72,85,99,110,128*
3	9.113	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methyhlenetricyclo[4.4.0.02,7] decane-rel		0.94	46,55,69,81,91,105,119,133,**161**,204*
4	9.055	Caryophyllene		0.83	47,55,69,79,**91**,93,105,120,133,147,161,175,189,204*
5	13.723	Hexadecanoic acid		0.66	45,55,60,**73**,85,98,115,129,143,157,171,185,199,213,227,256*

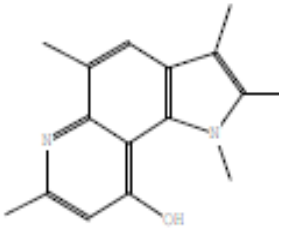
Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks, *- Molecular ion peak

Table 4.9: Significant compounds from *Lecaniodiscus cupanioides* stem bark

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
1	17.679	Tetrapentacontane	$CH_3(CH_2)_{52}CH_3$	42.01	48,55,**57**,71,85,99,113,127,149,169,197,211,239,758*
2	14.384	Nonacosane	$CH_3(CH_2)_{17}CH_3$	12.53	46,55,**57**,71,85,99,113,141,169,197,211,239,267,329,408*
3	17.142	Hexatriacontane	$CH_3(CH_2)_{34}CH_3$	8.77	45,55,**57**,71,85,99,113,127,141,155,239,253,267,281,346,405,506*
4	16.403	6,6-Diethylhoctadecane		6.88	46,55,**57**,71,85,99,127,141,197,281,310*

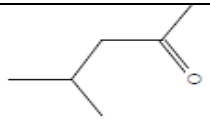
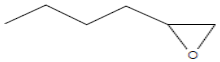
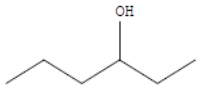
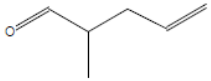
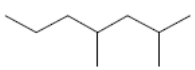
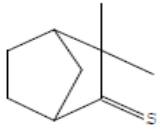
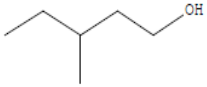
Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks, * - Molecular ion peak

Table 4.10: Significant compound from *Lecaniodiscus cupanioides* root

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
1	20.486	1,2,3,5,7-pentamethyl-1H-Pyrrolo[2,3-f]quinolin-9-ol		30.69	46,55,69,76,91,107,136,148,165,169,195,219,240,**253**,254*

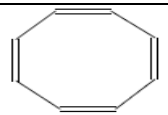
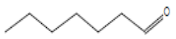
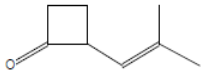
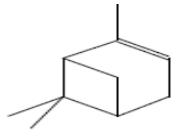
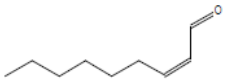
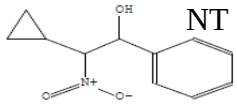
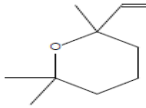
Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks, * - Molecular ion peak

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
1	4.022	Methyl Isobutyl Ketone	690		NT (Ketone)	Solvent	-	0.32	-	-
2	4.023	Butyl oxirane	708		NT (Ether)	Disinfectant	0.09	-	-	-
3	4.075	3-Hexanol	780		NT (Alcohol)	Food additive, Flavouring agent	0.09	-	-	-
4	4.117	9-Methyl-1-Decene	1041		NT		4.87	-	-	-
5	4.121	2,4-Dimethylheptane	788		NT (Hydrocarbons)		-	0.28	-	-
6	4.425	3,3-Dimethylbicyclo[2.2.1]heptane-2-thione	1185		Sesquiterpenoid (Cyclic terpene ketone)		-	-	-	33.57
7	4.776	(E)-2-Hexenal	814		NT (Aldehyde)	Flavouring agent, Antibacterial agent	32.68	-	-	-
8	4.934	3-Methyl-1-Pentanol	796		NT (Alcohol)		13.42	-	-	-

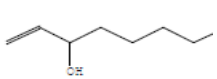
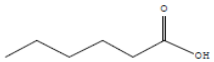
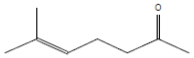
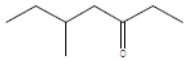
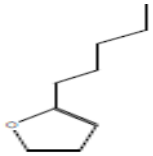
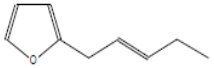
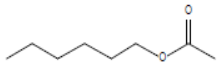
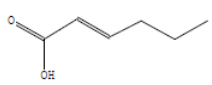
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
9	5.222	(8)-Annulene	888		NT (Non aromatic)	Rubber production	-	0.16	-	-
10	5.275	Heptanal	905		NT (Aldehyde)	Perfumery, Lubricants	0.15	-	-	-
11	5.460	2-(2-Methyl-1-propenyl)Cyclobutanone	995		NT (Ketones)	Alarm pheromone	0.04	-	-	-
12	5.525	n-Hexyl methanoate	981		NT (Ketone)		0.13	-	-	-
13	5.624	Bicyclo[3.1.1]hept-2-ene	948		NT	Insect repellent, Flavouring agent	0.06	-	-	-
14	5.816	(Z)-2-Nonenal	1112		NT		0.07	-	-	-
15	5.899	2-Cyclopropyl-2-nitro-1-phenyl-ethanol	1673		NT		0.24	-	-	-
16	5.940	2,2,6-Trimethyl-6-vinyltetrahydropyran	1067		NT	Flavour and Fragrance	0.16	-	-	-

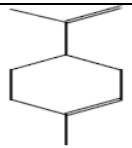
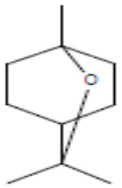
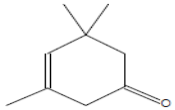
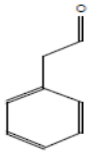
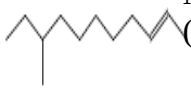
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
17	5.982	1-Octen-3-ol	969		NT (Alcohol)	Attractions of biting insect	-	1.41	-	-
18	5.989	Hexanoic acid	974		NT (Acid)	Flavouring agent	0.34	-	-	-
19	6.040	6-Methyl-5-heptene-2-one	938		NT (Ketones)	Alarm pheromone	0.24	-	-	-
20	6.045	3-Methyl-5-heptanone	888		NT (Ketone)		-	0.28	-	-
21	6.084	2-Pentylfuran	1040		NT (Furan)	Insect repellent, Flavouring agent	0.34	0.27	-	-
22	6.162	(E)-2-(2-Pentenyl)furan	1048		NT (Furan)		0.63	-	-	-
23	6.225	n-Hexyl acetate	984		NT (Ester)	Solvent, Flavouring	0.33	-	-	-
24	6.353	(E)-2-Hexenoic acid	982		NT (Acid)	Flavouring	1.92	-	-	-

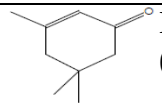
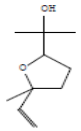
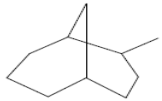
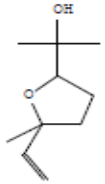
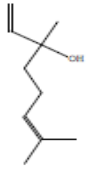
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
25	6.395	D-Limonene	1018		Monoterpene (Cyclic monoterpene)	Clinical solvent, Relief of heartburn and gastroesophageal reflux	0.46	-	-	-
26	6.430	Eucalyptol	1059		Monoterpenoid (Bicyclic ether)	Flavouring, Cosmetics, Fragrance, Insecticidal, repellent, cough suppressant, mouth wash, traditional medicine	0.42	0.12	-	-
27	6.479	3,5,5-Trimethyl-3-cyclohexen-1-one	1097		NT (Ketone)		0.33	-	-	-
28	6.517	Phenalacetaldehyde	1081		NT (Aldehyde)		0.48	-	-	-
29	6.585	(2E)-9-Methyl-2-undecene	1158		NT (Alkene)	Insect and pests attractants, Polymers for packaging and insulation, Flavouring and Fragrance	0.23	-	-	-

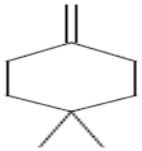
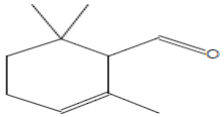
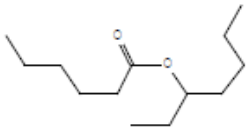
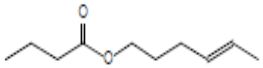
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
30	6.625	3,5,5-Trimethyl-2-cyclohexen-1-one	1097		NT (Cyclohexenones)		0.17	-	-	-
31	6.660	1-Nonanol	1159		NT (Alcohol)	Perfumery and Flavouring, Artificial lemon oil manufacturing	0.17	-	-	-
32	6.700	(Z)- Linalool oxide	1164		Monoterpenoid (Acyclic monoterpene/Tetrahydro furan)		2.42	-	-	-
33	6.765	2-Methylbicyclo[3.3.1]nonane	1042		NT		0.14	-	-	-
34	6.804	(E)-Linalool oxide	1164		Monoterpenoid (Acyclic monoterpene/Tetrahydro furan)		2.25	-	-	-
35	6.849	Linalool	1082		Monoterpenoid (Acyclic monoterpene/Tetrahydro furan)	Antibacterial and antifungal agent, Insecticidal agent, Cleaning agent, Anti-inflammatory, Anti-epileptic, Pain relief, Perfumery agent	6.41	-	-	-

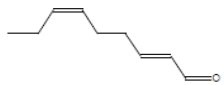
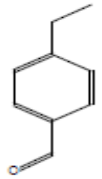
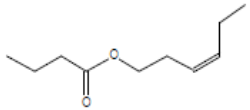
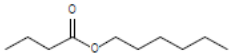
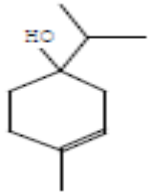
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
36	6.888	Nonanal			NT (Aldehyde)	Cleaning, Disinfectant, Pesticide, Polishes, Waxes, Cosmetics, Perfumery and Fragrance	-	0.41	-	-
37	6.980	1,1-Dimethyl-4-methylenecyclohexane			NT		0.09	-	-	-
38	7.024	2,6,6-Trimethyl-2-cyclohexene-1-carboxaldehyde	1175		Monoterpenoid		0.09	-	-	-
39	7.047	3-Heptyl hexanoate	1417		NT (Ester)		-	0.39	-	-
40	7.100	(4E)-4-Hexenyl butyrate	1191		NT (Ester)		0.17	-	-	-

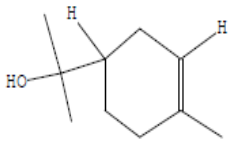
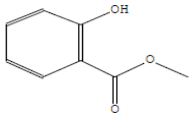
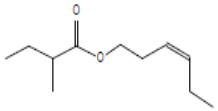
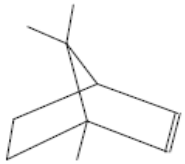
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
41	7.192	(E,Z)-2,6-Nonadienal	1120		NT (Aldehyde)	Flavouring and Perfumery agent	0.40	-	-	-
42	7.224	(E)-2-Nonenal	1112		NT (Aldehyde)	Flavouring and Perfumery	0.30	-	-	-
43	7.300	4-Ethylbenzaldehyde	1195		NT (Aldehyde)		1.52	-	-	-
44	7.348	(Z)-Butanoic acid, 3-hexenyl ester	1191		NT (Ester)	Flavouring	3.56	-	-	-
45	7.378	n-Hexyl butanoate	1183		NT (Ester)	Flavouring	0.81	-	-	-
46	7.390	Terpinen-4-ol	1137		NT (Ester)	Anti-inflammatory, Antioxidant and Anticancer agent	-	0.23	-	-

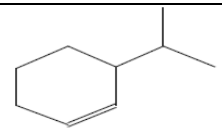
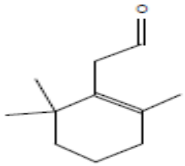
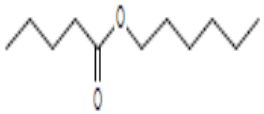
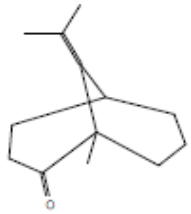
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
47	7.456	α- Terpineol	1143		Monoterpenoid (Monocyclic)	Flavouring and Perfumery agent, Antihypertensive, Anticonvulsant, Antioxidant, Antiulcer, Anti-nociceptive anticancer agent	1.37	-	-	-
48	7.496	Methyl salicylate	1281		NT (Ester)	Flavouring agent Analgesic Antiseptic	0.79	-	-	-
49	7.565	Methyl 6-nonynoate	1200		NT (Ester)		0.10	-	-	-
50	7.618	(3Z)-3-Hexenyl 2-methylbutanoate	1226		NT (Ester)		2.28	-	-	-
51	7.697	1,7,7-Trimethylbicyclo[2.2.1]hept-2-ene	932		NT		0.21	-	-	-

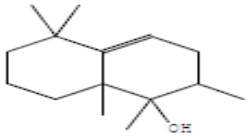
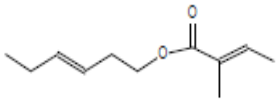
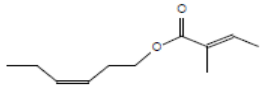
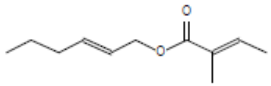
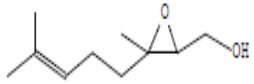
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
52	7.743	3-Isopropyl-1-cyclohexene	897		NT		0.15	-	-	-
53	7.818	(2E)-2-Decenal	1212		Monoterpene	Nematicide	0.49	-	-	-
54	7.874	2,6,6-Trimethyl-1-cyclohexene-1-acetaldehyde	1303		NT (Aldehyde)	Flavour and Fragrance	0.23	-	-	-
55	7.939	Hexyl valerate	1282		NT (Ester)		0.12	-	-	-
56	8.033	1-Methyl-9-(1-methylethylidene)bicyclo[3.3.1]nonan-2-one	1487		NT (Ketone)		0.11	-	-	-

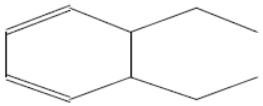
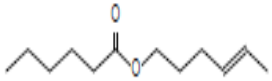
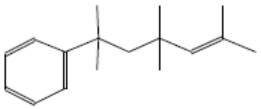
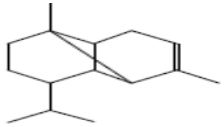
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
57	8.080	Undecanal	1303		NT (Aldehyde)	Antimycobacterial drug	0.06	-	-	-
58	8.100	1,2,5,5,8a-Pentamethyl-1,2,3,5,6,7,8,8a-octahydro-1-naphthalenol	1592		NT		-	-	1.46	-
59	8.115	(E)-Hex-3-enyl (E)-2-methylbut-2-enoate	1275		NT		0.05	-	-	-
60	8.181	(Z)-Hex-3-enyl (E)-2-methylbut-2-enoate	1275		NT		0.26	-	-	-
61	8.250	(E)-2-Hexenyl tiglate	1275		NT		0.08	-	-	-
62	8.300	3-methyl-3-(4-methyl-3-pentenyl)-Oxiranemethanol	1269		NT		0.06	-	-	-

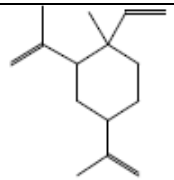
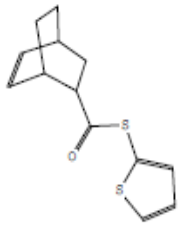
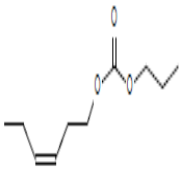
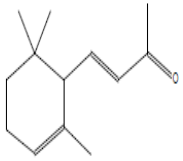
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
63	8.408	5,6-Diethyl-1,3-cyclohexadiene	1005		NT		2.81	-	-	-
64	8.460	2-Undecenal	1311		Monoterpene		0.09	-	-	-
65	8.527	(4E)-4-Hexenyl hexanoate	1389		NT (Ester)		2.15	-	-	-
66	8.645	Chlorooctadecane	2036		NT		0.13	-	-	-
67	8.675	2,4,4,6-Tetramethyl-6-phenyl-2-heptene	1604		NT		0.28	-	-	-
68	8.676	Copaene	1221		Sesquiterpene		-	0.20	-	-

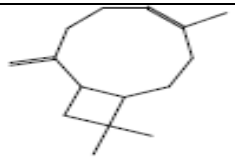
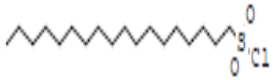
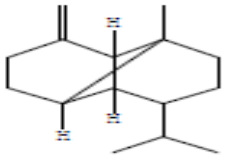
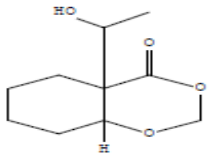
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
69	8.758	1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1.α,2.β,4.β)]-Cyclohexane	1398		NT		-	0.18	-	-
70	8.795	S-(2-Thienyl) bicyclo[2.2.2]oct-5-ene-2-carbothioate	1965		NT (Ester)	Flavour and Fragrance	0.08	-	-	-
71	8.914	(Z)-Hex-3-en-1-yl propyl carbonatess	1266		NT	Flavour and Fragrance	0.29	-	-	-
72	8.997	α-Ionone	1429		Sesquiterpenoid	Flavour and Fragrance	1.16	-	-	-

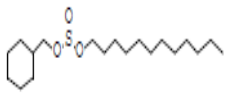
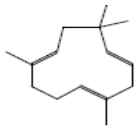
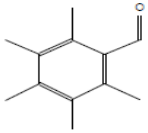
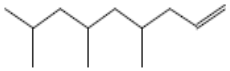
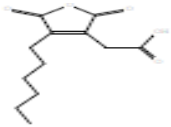
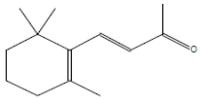
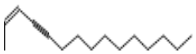
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
73	9.055	Caryophyllene	1494		Sesquiterpene	Antibacterial, Antifungal, Antiseptic, Anti-inflammatory, Antioxidant, Analgestic, Reduces and treats cholesterol, seizure and Osteoporosis	-	0.83	2.79	-
74	9.109	1-Octadecanesulphonyl chloride	2493		NT		0.18	-	-	-
75	9.113	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02.7]decane	1216		NT		-	0.94	-	-
76	9.200	4a-(1-Hydroxy-ethyl)-hexahydrobenzo[1,3]dioxin-4-one	1651		NT		0.05	-	-	-

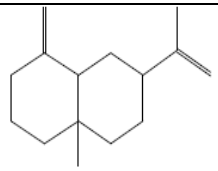
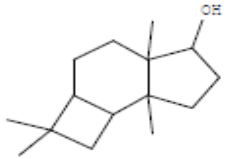
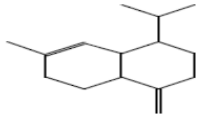
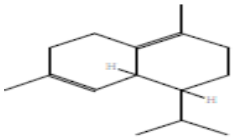
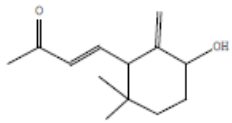
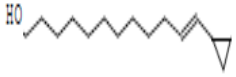
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
77	9.235	Sulfurous acid, cyclohexylmethyl dodecyl ester	2597		NT		0.05	-	-	-
78	9.340	α-Caryophyllene	1579		Sesquiterpene	Food additives, Flavour, Cosmetics	-	0.41	-	-
79	9.382	Pentamethylbenzaldehyde	1548		NT		0.20	-	-	-
80	9.435	4,6,8-Trimethyl-1-nonene	1012		NT		-	0.07	-	-
81	9.448	4-Hexyl-2,5-dihydro-2,5-dioxo-3-Furanacetic acid	2110		NT		-	-	85.55	-
82	9.503	(E)-β-Ionone	1457		Sesquiterpenoid	Fragrance, Antioxidant agent	-	0.67	-	-
83	9.578	(2Z)-2-Pentadecen-4-yne	1538		NT		-	0.30	-	-

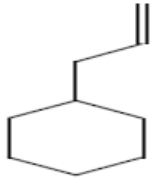
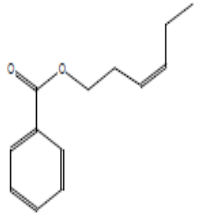
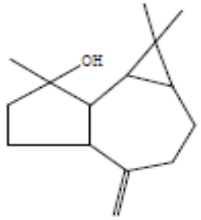
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
84	9.644	decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-,[4aR-(4a.α.,7.α,8a.β.)]-Naphthalene	1469		NT		-	0.16	0.37	-
85	9.655	2,2,4a,7a-Tetramethyldecahydro-1H-cyclobuta[e]inden-5-ol	1593		NT (Alcohol)		-	-	-	1.04
86	9.854	γ-Muurolene	1435		Sesquiterpenoid		-	-	0.39	-
87	9.900	Cadina-1(10),4-diene	1469		Sesquiterpenoid		-	-	0.40	-
88	10.032	(3E)-4-(5-Hydroxyl-2,2-dimethyl-6-methylenecyclohexyl)3-buten-2-one	1612		NT		0.06	-	-	-
89	10.140	E-11(12-Cyclopropyl)dodecen-1-ol	1765		NT		1.14	-	-	-

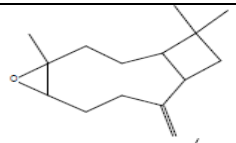
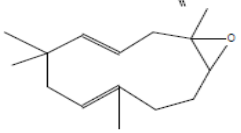
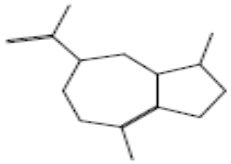
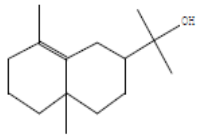
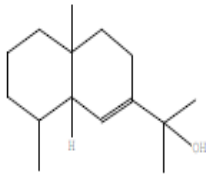
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
90	10.185	(+/-)-(E)-Nerolidol	1564		Sesquiterpenoid	Perfumery, Flavour, Detergent, Cleanser, Antimicrobial	-	-	0.26	-
91	10.221	Allylcyclohexane	969		NT		-	0.15	-	-
92	10.269	(E)-Hex-3-enyl isobutyl carbonate	1302		NT (Ester)		-	0.09	-	-
93	10.365	(Z)-3-Hexenyl benzoate	1565		NT (Ester)		-	0.20	-	-
94	10.619	decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]-1H-Cycloprop[e]azulen-7-ol	1536		NT (Alcohol)		-	0.06	0.10	-

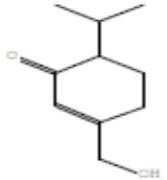
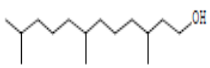
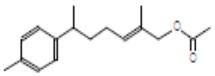
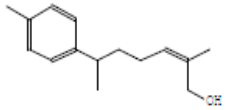
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
95	10.715	Caryophyllene oxide	1507		Sesquiterpenoid	Antifungal	-	0.20	-	-
96	10.992	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	1592		NT		-	0.07	-	-
97	11.056	1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.α.,7.α.,8a.β.)]- □Azulene	1490		NT		-	-	0.30	-
98	11.170	γ-Eudesmol	1626		Sesquiterpenoid		-	-	0.10	-
99	11.397	(Z)-Eudesm-6-en-11-ol	1598		NT (Alcohol0		-	-	0.39	-

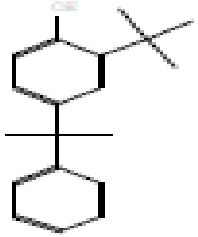
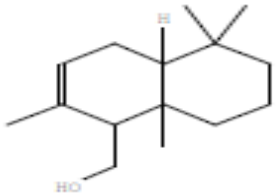
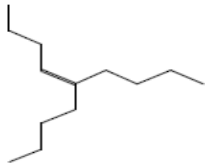
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
100	11.616	3-(Hydroxymethyl)-6-isopropyl-2-cyclohexen-1-one	1401		NT (Ketone)		-	-	0.21	-
101	11.745	Pentadecanal	1701		NT (Aldehyde)		-	0.10	-	-
102	11.869	Hexahydrofarnesol	1563		Sesquiterpenoid	Perfumery Antimicrobial agent	-	0.09	-	-
103	12.049	(E)-2-Hepten-1-ol,2-methyl-6-(4-methylphenyl)-acetate	1890		NT (Ester)		-	-	0.30	-
104	12.204	(2Z, 6S)- 2-methyl-6-(4-methylphenyl)- □2-Hepten-1-ol	1766		NT (Alcohol0		-	-	0.52	-

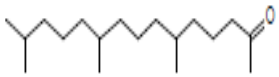
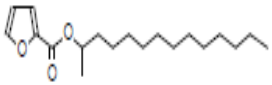
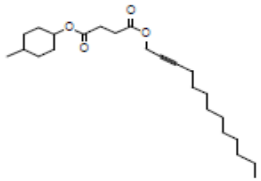
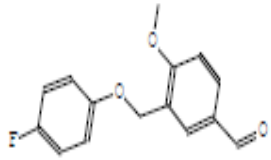
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
105	12.401	2-t-Butyl-4-(dimethylbenzyl)phenol	2128		NT (Alcohol)	-	-	-	1.36	
106	12.475	Carbonic acid, decyl tridecyl ester	2650		NT (Ester)	-	0.04	-	-	
107	12.512	Drimenol	1685		NT	-	-	0.27	-	
108	12.554	5-Butyl-4-nonene	1299		NT	0.58	-	-	-	

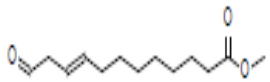
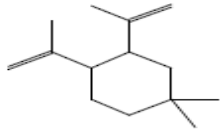
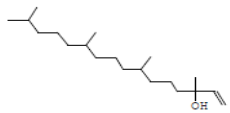
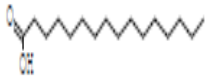
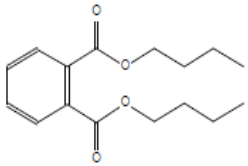
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
109	12.605	2-Hexenal	814		NT (Aldehyde)	Food additive	0.14	-	-	-
110	12.886	Hexahydrofarnesyl acetone	1754		Sesuiterpenoid	Flavouring, Antimicrobial agent	0.94	0.40	-	-
111	12.949	2-Furancarboxylic acid, 2 – tetradecyl ester	2137		NT (Ester)		0.14	-	-	-
112	13.105	Succinic acid, tridec-2-yn-1-yl cis-4-methylcyclohexyl ester	2784		NT (Ester)		0.04	-	-	-
113	13.134	3-[(4-Fluorophenoxy) methyl]-4-methoxybenzaldehyde	2008		NT		-	0.10	-	-

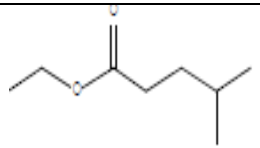
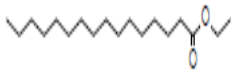
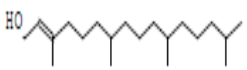
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
114	13.482	Methyl 12-oxo-9-dodecenoate	1677		NT		0.09	-	-	-
115	13.484	3,4-Diisopropenyl-1,1-dimethylcyclohexane	1308		NT		-	0.11	-	-
116	13.653	Isophytol	1899		Diterpenoid	Fragrance, Precursor of Vitamin E and Vitamin k	0.07	-	-	-
117	13.723	Palmitic acid	1968		NT (Acid)	Cleaning agent, Cosmetics to hide blemishes	-	0.66	-	-
118	13.815	Butyl phthalate	2037		NT	Lubricating agent, Perfume fixative, Plasticizer, Adhesives, Printing inks and Glass additives	0.05	0.51	-	-

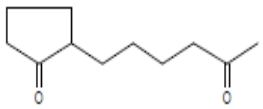
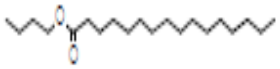
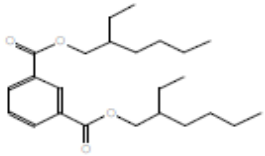
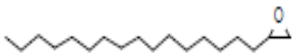
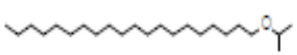
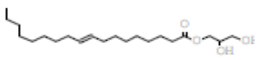
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
119	13.935	Ethyl isohexanoate			NT (Ester)	Perfumery, Flavour	-	0.14	-	-
120	13.943	Ethyl palmitate			NT (Ester)	Flavour, Skin and Hair Conditioning agent	0.08	-	-	-
121	14.384	Nonacosane	2904	$CH_3(CH_2)_{17}CH_3$	NT	Chemical communication of insect	-	-	12.53	-
122	14.734	Phytol	2045		Diterpenoid	Antioxidant, Antinociceptive, Antiallergic and anti-inflammatory agent	0.18	0.29	-	-
123	14.889	(Z,E)-3,7,11-Trimethyl-2,6-Dodecadien-1-ol	1661		Sesquiterpenoid		-	0.15	-	-

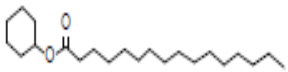
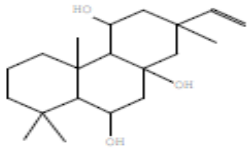
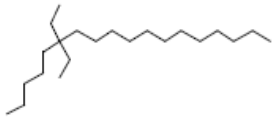
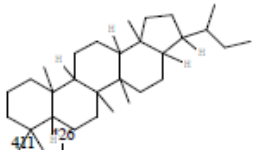
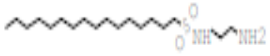
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
124	14.890	2-(5-Oxohexyl)cyclopentanone	1465		NT (Ketone)		0.24	-	-	-
125	15.090	n-Butyl palmitate	2177		NT (Ester)		-	0.46	-	-
126	15.165	2-Methyltetracosane	2442		NT		0.04	-	-	-
127	15.194	Bis(2-ethylhexyl)isophthalate	2704		NT	Plasticizer in Medical tools	-	-	3.58	-
128	15.257	2-Hexadecyloxirane	1901		NT		0.11	-	-	-
129	15.745	Eicosyl isopropyl ether	2319		NT (Ether)	Solvent	-	-	3.38	-
130	16.175	2,3-Dihydroxypropyl (9E)-9-octadecenoate	2689		NT (Ester)		-	0.14	-	-

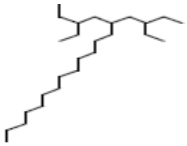
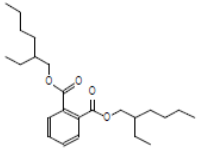
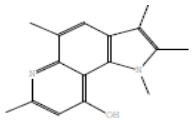
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
131	16.287	Cyclohexyl palmitate	2439		NT (Ester)		0.16	-	-	-
132	16.301	Sandaracopimar-15-ene-6.β.,8.β.,11.α.-triol	2442		NT		-	-	2.69	-
133	16.326	n-Butyl stearate	2375		NT (Ester)	Solvent, Plasticizer	-	0.19	-	-
134	16.403	6,6-Diethylhoctadecane	2124		NT		-	-	6.88	-
135	16.489	17.α(H),21.β.(H)-Homohopane	2728		NT		-	-	5.43	-
136	16.652	N-(3-aminopropyl)hexadecane-1-sulfonamide	2858		NT		-	-	3.30	-

Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.11: Comparison of chemical constituents of essential oils from *Lecaniodiscus cupanioides* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
137	17.142	Hexatriacontane	3600	$CH_3(CH_2)_{34}CH_3$	NT		-	-	8.77	-
138	17.146	Tetracontane	3997	$CH_3(CH_2)_{12}CH_3$	NT		0.07	-	-	-
139	17.146	3-Ethyl-5-(2-ethylbutyl)octadecane	2413		NT		-	0.15	6.88	-
140	17.212	Decanal	1204		NT (Aldehyde)	Fragrance, Flavouring	-	0.14	-	-
141	17.679	Tetrapentacontane	5389	$CH_3(CH_2)_{52}CH_3$	NT		-	-	42.01	-
142	17.686	Bis(2-ethylhexyl)phthalate	2704		NT	Plasticizer in Medical tools	0.08	0.96	-	-
143	20.486	1,2,3,5,7-pentamethyl-1H-Pyrrolo[2,3-f]quinolin-9-ol	2313		NT		-	-	-	30.69
Total Identified							**95**	**42**	**14**	**02**

Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Out of these identified one hundred and forty-three compounds, only ten of them were detected in two plant's parts: 2-pentyl furan in leaf (0.34%) and leaf stalk (0.27%) which was also detected in *Blighia sapida* leaf stalk (0.48%); 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane in leaf (0.42%) and leaf stalk (0.12%); decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-,[4aR-(4a.α.,7.α,8a.β.)]-naphthalene in leaf (0.16%) and leaf stalk (0.37%); decahydro- 1,1,7 - trimethy l-4 –methylene -,[1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]- 1H-cycloprop [e] azulen-7-ol in leaf (0.06%) and leaf stalk (0.10%); hexahydrofarnesyl acetone in leaf (0.94%) and leaf stalk (0.40%) which was detected in *Blighia sapida* leaf (2.79%), leaf stalk (3.82), stem bark (3.61), fruit husk (1.51%) and seed (0.51%); buthyl phthalate in leaf (0.05%) and leaf stalk (0.51%); phytol in leaf (0.18%) and leaf stalk (0.29%), this was also obtained from *Blighia sapida* leaf (4.03%) and leaf stalk (1.74%); caryophyllene in leaf stalk (0.83%) and stem bark (2.79%); 3-ethyl-5-(2-ethylbutyl)octadecane in leaf stalk (0.15%) and stem bark (6.88%); bis(2-ethylhexyl)phthalate in leaf (0.08%) and leaf stalk (0.96%) while others only appeared in one plants's part.

Table 4.12 show that the class of non-terpene derivatives was the highest among the identified compounds present in the essential oil of *Lecaniodiscus cupanioides* leaf, leaf stalk and stem bark with 79.32%, 96.44% and 97.20% respectively likewise non-terpene in *Blighia sapida* leaf (40.93%), leaf stalk (42.35%), stem bark (21.15%) and root (14.84) while the class of sesquiterpenoid was the highest in *Lecaniodiscus cupanioides* root (33.57%). Many detected compounds from these non-terpene and the terpenoids have medicinal and industial applications as reflected in Table 4.11.

The classes of identified compounds in *Lecaniodiscus cupanioides* leaf in descending order were non-terpene (79.32%), monoterpenoid (12.96%), sesquiterpenoid (3.06%) and diterpenoid (0.25%) as the order in *Blighia sapida*. In *Lecaniodiscus cupanioides* leaf stalk, the classes of identified compounds present in descending order were non-terpene (96.44%), sesquiterpenoid (1.70%), sesquiterpene (1.44%) and diterpenoid (0.29%) as in *Blighia sapida*. There were also non-terpene (97.20%) and sesquiterpene (2.79%) in *Lecaniodiscus cupanioides* stem bark while sesquiterpenoid (33.57%) and non-terpene (30.69%) in *Lecaniodiscus cupanioides* root.

Table 4.12: Classes of identified compounds of essential oils from *Lecaniodiscus cupanioides*

Class	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
Monoterpene	1.04	n.d.	n.d.	n.d.
Monoterpenoid	12.96	n.d.	n.d.	n.d.
Sesquiterpene	n.d.	1.44	2.79	n.d.
Sesquiterpenoid	3.06	1.70	n.d.	33.57
Diterpene	n.d.	n.d.	n.d.	n.d.
Diterpenoid	0.25	0.29	n.d.	n.d.
Triterpene	n.d.	n.d.	n.d.	n.d.
Non-terpene	79.32	96.44	97.20	30.69

n.d. - Not Detected

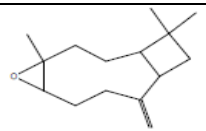
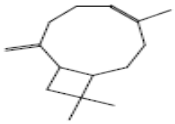
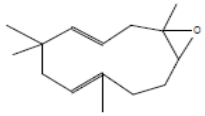
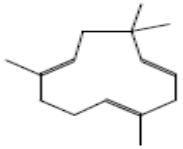
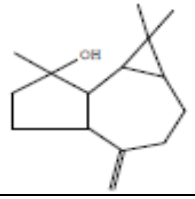
4.2.3 *Paullinia pinnata* essential oils

The results of *Paullinia pinnata* leaf, leaf stalk, stem bark and root essential oils as presented in Table 4.3 showed that *Paullinia pinnata* leaf stalk had the highest number of identified compounds as fifty-seven (57), unlike in *Blighia sapida* and *Lecaniodiscus cupanioides* where the highest number of compounds were from the leaf with seventy-eight (78) and ninety-five (95), respectively. The least number of compounds were detected in *Paullinia pinnata* stem bark (19) and *Blighia sapida* stem bark (10), however, in *Lecaniodiscus cupanioides,* the least was from the root (2).

The results showed 37 compounds in *Paullinia pinnata* leaf as Ogunwande *et al*., (2017) also reported 31 compounds from *Paullinia pinnata* leaf with some similar compounds, such as methyl salicylate which was also detected from this research in the leaf of *Blighia sapida* and *Lecaniodiscus cupanioides*, some other examples of similar compounds of *Paullinia pinnata* leaf with Ogunwande *et al*., (2017) were caryophyllene, α-cubebene, germacrene D, copaene, caryophyllene oxide and α-caryophyllene (α-humulene). The results also showed 57 compounds in *Paullinia pinnata* leaf stalk; 19 compounds from *Paullinia pinnata* stem bark and 29 compounds in *Paullinia pinnata* root resulting in the total of 142 while removing compounds repeatedly detected in other parts gave 96 compounds.

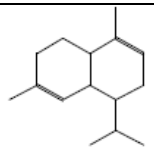
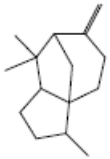
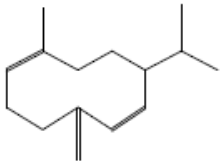
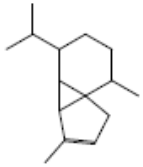
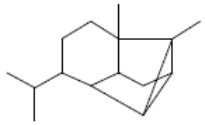
The significant chemical constituents of *Paullinia pinnata* leaf essential oil in descending order were caryophyllene oxide (28.62%), caryophyllene (12.92%), (1R,3E,7E,11R)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (12.18%), α-caryophyllene(6.21%),[1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]-1,1,7-trimethyl-4-ethylene-decahydro-1H-Cycloprop[e]azulen-7-ol (3.95 %), α-muurolene (3.61%), β-cedrene (2.34%),germacreneD(1.91%),αcubebene(1.85%)and[1S(1.α.,2.α.,3a.β.,4.α.,5.α.,7a.β., 8S*)]- octahydro-1,7a-dimethyl-5-(1-methylethyl)- 1,2,4-metheno-1H-indene (1.83%) as in Table 4.13 which also showed the molecular ion and fragmentation ions for structural elucidation.

Table 4.13: Significant compounds of *Paullinia pinnata* leaf

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
1	10.717	Caryophyllene oxide		28.62	55,69,**79**,93,107,121,131,149,161,177,187,205,220*
2	9.057	Caryophyllene		12.92	55,69,79,**93**,105,119,133,147,161,175,189,204*
3	10.992	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene		12.18	55,**67**,81,96,109,123,138,147,205,220*
4	9.342	α-Caryophyllene		6.21	53,67,80,**93**,107,121,136,147,161,175,189,204*
5	10.618	[1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]-1,1,7-trimethyl-4-methylene-decahydro-1H-Cycloprop[e]azulen-7-ol		3.95	45,55,67,79,**91**,105,119,131,145,159,177,187,205,220*

Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks, * - Molecular ion peak

Table 4.13: Significant compounds of *Paullinia pinnata* leaf Cont'd

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
6	9.679	α -Muurolene		3.61	55,69,79,93,**105**,119,133,147,161,175,189,204*
7	9.110	β-Cedrene		2.34	55,69,79,91,105,119,133,**161**,176,189,204*
8	9.572	Germacrene D		1.91	47,55,67,81,91,105,119,133,147,**161**,204*
9	8.453	α-Cubebene		1.85	47,55,69,81,91,**105**,119,133,145,161,189,204*
10	8.645	[1S-(1.α.,2.α.,3a.β.,4.α.,5.α.,7a.β.,8S*)]-octahydro-1,7a-dimethyl-5-(1-methylethyl)- 1,2,4-Metheno-1H-indene		1.83	55,79,94,**105**,120,133,148,161,175,189,204*

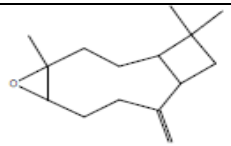
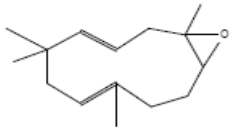
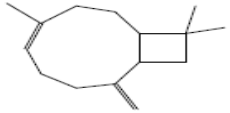
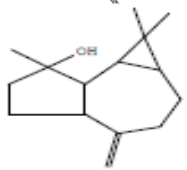
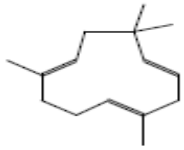
Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks, * - Molecular ion peak

Leaf stalk essential oil showed significant chemical constituents as shown in Table 4.14 in descending order of caryophyllene oxide (37.68%), (1R,3E,7E,11R)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene(13.08%),isocaryophyllene (4.56%), [1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]-decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol (3.61%), α-caryophyllene (2.58%), [1S-(1.α.,2.α.,3a.β.,4.α.,5.α.,7a.β.,8S*)]octahydro-1,7a-dimethyl-5-(1-methylethyl)-1,2,4-metheno-1H-indene(2.40%),(1R,2S,6S,7S,8S)-8-isopropyl-1-methyl-3-methylenetricyclo [4.4.0.02,7]decane-rel- (2.27%), α -muurolene (2.10%), α-cubebene (1.58%) and i-propyl 14-methyl-pentadecanoate (1.41%).

As shown in Table 4.15, essential oil from the stem bark of *Paullinia pinnata* had the following significant chemical compounds in descending order of caryophyllene (39.68%), α-caryophyllene (12.70%), cedrene (7.30%), copaene (6.97%), α-muurolene (6.58%), α-cubebene (4.59%), germacrene D (3.83%), (3R,3aR,7R,8aS)-3,8,8-trimethyl-6-methyleneoctahydro-1H-3a,7-methanoazulene (3.44%), cadina-1(10),4-diene (3.43%) and [1S-(1.α.,2.α.,3a.β.,4.α.,5.α.,7a.β.,8S*)]- octahydro-1,7a-dimethyl-5-(1-methylethyl)- 1,2,4-metheno-1H-indene (2.91%).

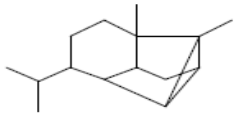
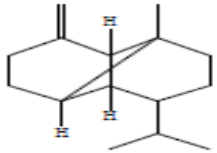
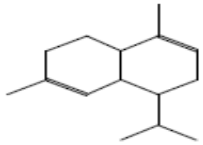
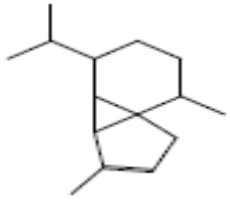
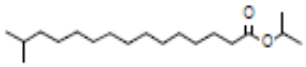
Root essential oil displayed chemical constituents of significant quantities in descending order of caryophyllene oxide (23.79%), caryophyllene (12.65%), (1R,3E,7E,11R)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (7.64%), α-caryophyllene(5.98%),δ-elemene(5.48%),[1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]-decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol (4.34%), hexanal (3.89%), α-muurolene (3.15%) and copaene (2.54%) as shown in Table 4.16.

Table 4.14: Significant compounds of *Paullinia pinnata* leaf stalk

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
1	10.718	Caryophyllene oxide		37.68	45,55,69,**79**,93,107,121,131,149,161,177,187,205,220*
2	10.992	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene		13.08	45,55,**67**,81,96,109,123,138,147,205,220*
3	9.058	Isocaryophyllene		4.56	45,55,69,79,**93**,105,119,133,147,161,175,189,204*
4	10.617	[1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]-decahydro-1,1,7-trimethyl-4-methylene-1H-Cycloprop[e]azulen-7-ol		3.61	45,55,67,79,**91**,105,119,131,145,159,177,187,205,220*
5	9.342	α-Caryophyllene		2.58	55,67,80,**93**,107,121,136,147,161,175,189,204*

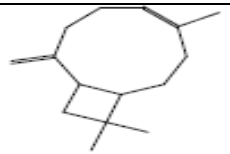
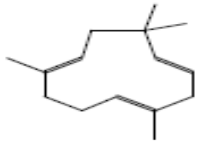
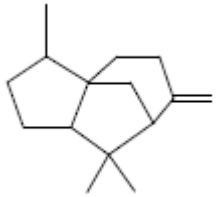
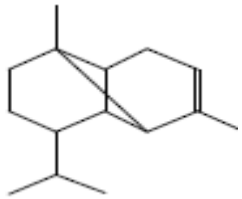
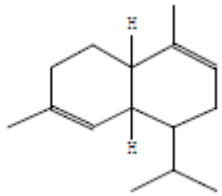
Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks, * - Molecular ion peak

Table 4.14: Significant compounds of *Paullinia pinnata* leaf stalk Cont'd

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
6	8.648	[1S-(1.alpha.,2.alpha.,3a.beta.,4.alpha.,5.alpha.,7a.beta.,8S*)]-octahydro-1,7a-dimethyl-5-(1-methylethyl)-1,2,4-Metheno-1H-indene		2.40	55,67,79,94,**105**,119,133,147,161,175,189,204*
7	8.759	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-		2.27	55,67,81,91,105,119,133,147,**161**,175,189,204*
8	9.681	α -Muurolene		2.10	55,69,79,93,**105**,119,133,147,161,175,189,204*
9	8.453	α-Cubebene		1.58	55,69,81,91,**105**,119,133,147,161,189,204*
10	14.139	i-Propyl 14-methyl-pentadecanoate		1.41	55,**60**,71,85,102,115,129,143,157,171,185,199,213,227,239,256,298*

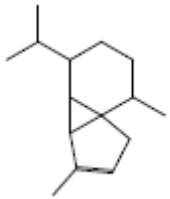
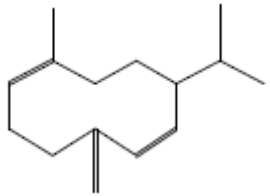
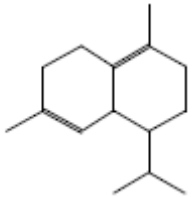
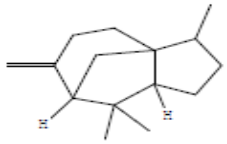
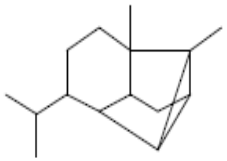
Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks

Table 4.15: Significant compounds of *Paullinia pinnata* stem bark

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
1	14.985	Caryophyllene		39.68	55,69,79,**93**,105,119,133,147,161,175,189,204*
2	15.434	α-Caryophyllene		12.70	55,67,80,**93**,107,121,133,147,161,175,189,204*
3	15.099	Cedrene		7.30	55,69,79,93,105,**119**,133,147,161,175,189,204*
4	14.357	Copaene		6.97	55,69,81,91,**105**,119,133,147,161,175,189,204*
5	15.969	α-Muurolene		6.58	55,65,79,94,**105**,119,133,147,161,175,189,204*

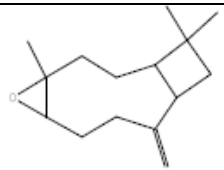
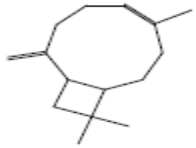
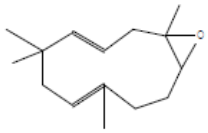
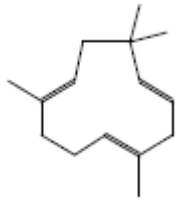
Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks, * - Molecular ion peak

Table 4.15: Significant compounds of *Paullinia pinnata* stem bark Cont'd

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
6	13.958	α-Cubebene		4.59	48,55,69,81,91,**105**,119,133,145,161,189,204*
7	14.536	Germacrene D		3.83	46,55,67,79,91,105,119,133,147,**161**,175,204*
8	16.259	Cadina-1(10),4-diene		3.44	55,69,77,91,105,**119**,134,147,161,176,189,204*
9	15.771	(3R,3aR,7R,8aS)-3,8,8-Trimethyl-6-methyleneoctahydro-1H-3a,7-methanoazulene		3.43	55,69,79,91,105,119,133,148,**161**,177,189,204*
10	14.256	[1S-(1.α.,2.α.,3a.β.,4.α.,5.α.,7a.β.,8S*)]- octahydro-1,7a-dimethyl-5-(1-methylethyl)- 1,2,4-Metheno-1H-indene		2.91	55,67,79,91,**105**,119,133,147,161,175,189,204*

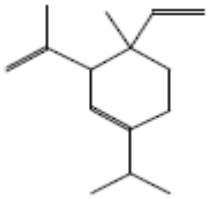
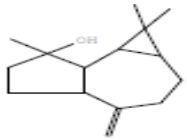
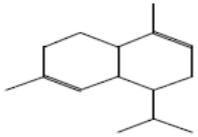
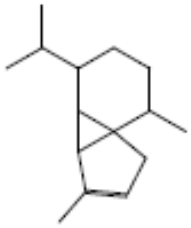
Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks

Table 4.16: Significant compounds of *Paullinia pinnata* root

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
1	10.717	Caryophyllene oxide		23.79	45,55,69,**79**,91,105,121,133,147,161,177,187,205,220*
2	9.057	Caryophyllene		12.65	55,69,79,**93**,105,119,133,147,161,175,189,204*
3	10.991	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene		7.64	45,55,**67**,79,96,109,123,138,147,162,179,205,220*
4	9.343	α-Caryophyllene		5.98	45,53,67,80,**93**,105,121,136,147,161,175,189,204*

Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks, * - Molecular ion peak

Table 4.16: Significant compounds of *Paullinia pinnata* root Cont'd

S/N	Rt (Mins)	Name	Structure	%C	Fragmentation ions and Molecular ion
5	8.366	δ-elemene		5.48	49,67,79,93,105,**121**,136,148,161,189,204*
6	10.620	[1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)] -decahydro-1,1,7-trimethyl-4-methylene-1H-Cycloprop[e]azulen-7-ol		4.34	45,55,69,79,91,105,119,131,145,**159**,177,187,205,220*
7	4.144	Hexanal		3.89	45,55,**56**,72,100*
8	9.680	α-Muurolene		3.15	46,55,69,81,93,**105**,119,133,147,161,175,189,204*
9	8.675	Copaene		2.54	45,55,67,81,91,**105**,119,133,147,161,204*

Rt- Retention time (minutes), %C- Percentage Composition, Bold fragments - the base peaks

The first three dominating identified compounds in each *Paullinia pinnata* essential oil were caryophyllene oxide (28.62%), caryophyllene (12.92%) and (1R,3E,7E,11R)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (12.18%) in leaf while Ogunwande *et al*., (2017) reported pentadecanoic acid (17.9%), isoaromadendrene epoxide (11.5%) and wine lactone (11.2%) as the first three significant compounds; caryophyllene oxide (37.68%), (1R,3E,7E,11R)-1, 5, 5, 8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (13.08%) and isocaryophyllene (4.56%) in leaf stalk; caryophyllene (39.68%), α-caryophyllene (12.70%) and cedrene (7.30%) in stem bark; caryophyllene oxide (23.79%), caryophyllene (12.65%) and (1R, 3E, 7E, 11R)-1, 5, 5, 8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (7.64%) in root.

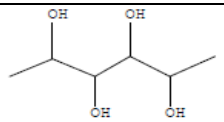
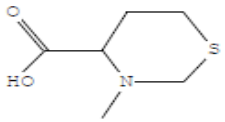
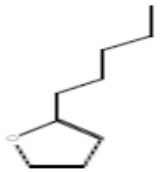
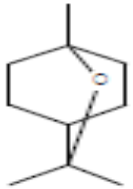
From the four essential oils of *Paullinia pinnata*, 96 compounds were identified. Some of the chemical constituents were identified in more than one plant's part of *Paulinia pinnata* while others were identified in only one part. Out of these identified ninety-six compounds, eight compounds were detected in all these four plants' parts (leaf, leaf stalk, stem bark and root) and these were α-cubebene (1.85%, 1.58%, 4.59%, 1.48%); [1S-(1.α.,2.α.,3a.β.,4.α.,5.α.,7a.β.,8S*)]-octahydro-1,7a-dimethyl-5-(1-methylethyl)-1,2,4-metheno-1H-indene (1.83%, 2.40%, 2.91%, 1.57%); caryophyllene (12.92%, 0.44%, 39.68%, 12.65%), **c**aryophyllene was also detected in *Lecaniodiscus cupanioides* leaf and leaf stalk ; α-caryophyllene (6.21%, 2.58%, 12.70%, 5.98%), α-caryophyllene was also detected in *Blighia sapida* fruit pulp and seed, *Lecaniodiscus cupanioides* leaf stalk; α –muurolene (3.61%, 2.10%, 6.58%, 3.15%); **c**adina-1(10),4-diene (1.40%, 1.14%, 3.44%, 1.36%), **c**adina-1(10),4-diene was detected in *Lecaniodiscus cupanioides* stem bark; **c**aryophyllene oxide (28.62%, 37.68%, 1.68%, 23.79%); (1R,3E,7E,11R)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene (12.18%, 13.08%, 0.47%, 7.64%).

There were six compounds detected in three plant parts, γ-muurolene in leaf (0.72%), stem bark (1.17%) and root (3.29%) which was detected in *Lecaniodiscus cupanioides* stem bark; germacrene D in leaf (1.91%), stem bark (3.83%) and root (1.29%); copaene in leaf stalk (0.18%), stem bark (6.97%) and root (2.54%); [1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]-1,1,7-trimethyl-4-methylene-decahydro-1H-

cycloprop[e]azulen-7-ol in leaf (3.95%), leaf stalk (3.61%) and root (4.34%); hexahydrofarnesyl acetone in leaf (0.45%), leaf stalk (0.41%) and root (1.04%) which was also detected in *Blighia sapida* leaf, leaf stalk, stem bark, fruit husk and seed, *Lecaniodiscus cupanioides* leaf and leaf stalk; (1R,1E,5E,9E)-1,5,9-trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene in leaf (0.39%), leaf stalk (0.33%) and root (0.98%).

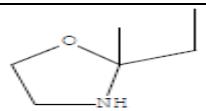
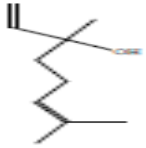
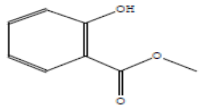
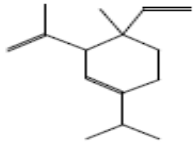
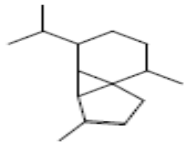
Nine of them were detected in two plant's parts: hexanal in leaf (0.30%) and root (3.89%); 1R,2S,6S,7S,8S)-8-isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7] decane-rel-in leaf stalk (2.27%) and root (1.43%); (1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol in leaf (0.33%) and leaf stalk (0.24%); humulene epoxide in leaf (0.84%) and leaf stalk (1.18%); **(-)-s**pathulenol in leaf (0.41%) and root (1.79%); **y**langenal in leaf (0.30%) and leaf stalk (0.82%); 8-isopropyl-1,5-dimethyltricyclo[4.4.0.02,7]dec-4-en-3-one in leaf (0.22%) and leaf stalk (0.95%); farnesyl acetone in leaf (0.53%) and root (1.12%); dibutyl phthalate in leaf stalk (0.18%) and stem bark (3.96%) while others only appeared in one plants's part as shown in Table 4.17. Some of these compounds that were detected in only one *Paulinia pinnata'*s part were observed in *Blighia sapida and Lecaniodiscus cupanioides*, such as 2-pentyl furan in *Paullinia pinnata* root (*Blighia sapida* leaf stalk and *Lecaniodiscus cupanioides* leaf and leaf stalk), nonanal in *Paullinia pinnata* leaf (*Blighia sapida* leaf stalk and *Lecaniodiscus cupanioides* leaf), methyl salicylate *Paullinia pinnata* leaf (*Blighia sapida* leaf and *Lecaniodiscus cupanioides* leaf), α-ionone in *Paullinia pinnata* leaf (*Blighia sapida* leaf and *Lecaniodiscus cupanioides* leaf), phytol in *Paullinia pinnata* leaf stalk (*Blighia sapida* leaf and leaf stalk, *Lecaniodiscus cupanioides* leaf and leaf stalk), (E)-2-hexenal (*Lecaniodiscus cupanioides* leaf), eucalyptol (*Lecaniodiscus cupanioides* leaf and leaf stalk) and linalool (*Lecaniodiscus cupanioides* leaf).

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
1	4.020	1,6-Dideoxy-l-mannitol	1267		NT (Alcohol)	Antineoplastic drug	-	0.14	-	-
2	4.030	3-methyl-[1,3]Thiazinane-4-carboxylic acid	1391		NT (Acid)		-	-	-	0.94
3	4.140	Hexanal	806		NT (Aldehyde)	Antimicrobial agent It extends shelf life of fruits	0.30	-	-	3.89
4	4.786	(E)-2-Hexenal	814		NT (Aldehyde)	Antibacterial agent Flavouring agent	0.66	-	-	-
5	6.101	2-Pentylfuran	1040		NT (Furan)	Flavouring agent Insect repellent	-	-	-	0.87
6	6.429	Eucalyptol	1059		Monoterpenoid (Bicyclic ether)	Cough suppressant Anti-inflammatory agent Mouth wash ingredients	-	-	-	0.66

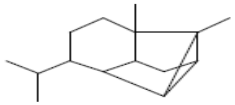
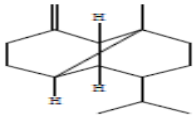
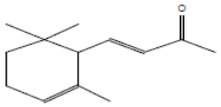
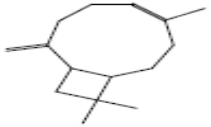
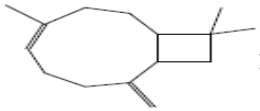
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
7	6.592	2-Ethyl-2-methyl-1,3-oxazolidine	968		NT		-	-	-	0.61
8	6.854	Linalool	1082		Monoterpenoid	Insecticide. Perfumery agent. Flavouring agent as food additive Antimicrobial agent Cleaning agent	0.76	-	-	-
9	6.885	Nonanal	1104		NT (Aldehyde)	PerfumeryFlavouring agent	0.50	-	-	-
10	7.507	Methyl salicylate	1281		NT (Ester)	Analgesic Antiseptic	1.42	-	-	-
11	8.366	δ-elemene	1377		Sesquiterpene		-	-	-	5.48
12	8.453	α-Cubebene	1344		Sesquiterpenoid (Tricyclic)		1.85	1.58	4.59	1.48

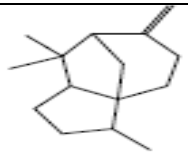
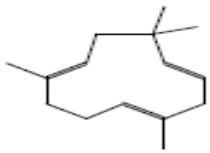
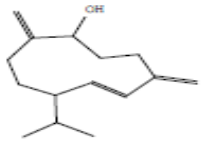
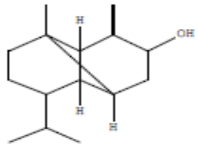
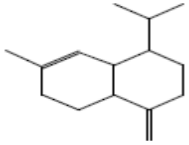
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
13	8.645	[1S-(1.α.,2.α.,3a.β.,4.α.,5.α., 7a.β.,8S*)]-octahydro-1,7a-dimethyl-5-(1-methylethyl)-1,2, 4-Metheno-1H-indene	1125		NT		1.83	2.40	2.91	1.57
14	8.759	1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02, 7]decane-rel-	1216		NT		-	2.27	-	1.43
15	8.935	1,5,9,13-Tetradecatetraene	1409		NT		0.37	-	-	-
16	8.995	α-Ionone	1429		Sesquiterpenoid	Perfumery Flavouring	0.42	-	-	-
17	9.057	Caryophyllene	1494		Sesquiterpene	It treats seizures Prevents Osteoporosis Reduces cholesterol Relieves pain and anxiety	12.92	0.44	39.68	12.65
18	9.058	Isocaryophyllene	1494		Sesquiterpene		-	4.56	-	-

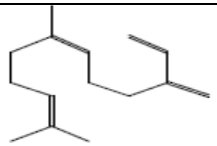
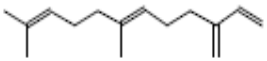
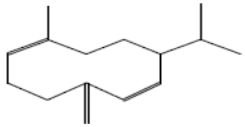
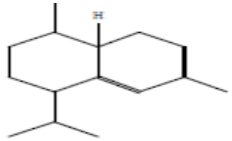
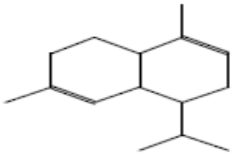
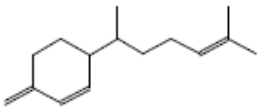
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
19	9.110	β-Cedrene	1398		Sesquiterpene	Perfumery Food additive	2.34	-	-	-
20	9.342	α-Caryophyllene	1579		Sesquiterpene	Food additives	6.21	2.58	12.70	5.98
21	9.405	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	1699		NT		0.33	0.24	-	-
22	9.408	(1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decan-4-ol	1405		NT		-	0.36	-	-
23	9.472	γ-Muurolene	1435		Sesquiterpene		0.72	-	1.17	3.29

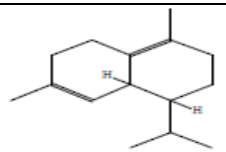
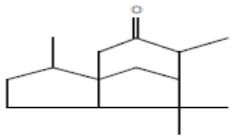
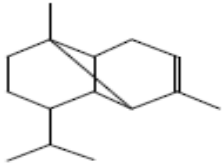
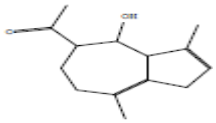
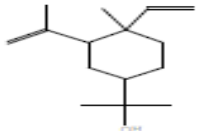
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
24	9.520	(Z)-β-Farnesene	1440		Sesquiterpene		-	0.52	-	-
25	9.522	(E)-.β-Famesene	1440		Sesquiterpene		0.99	-	-	-
26	9.572	Germacrene D	1515		Sesquiterpene	Insecticidal Antimicrobial	1.91	-	3.83	1.29
27	9.580	(Z)-muurola-3,5-diene	1440		Sesquiterpene		-	0.12	-	-
28	9.679	α -Muurolene	1440		Sesquiterpene		3.61	2.10	6.58	3.15
29	9.855	β-Sesquiphellandrene	1446		Sesquiterpene		0.48	-	-	-

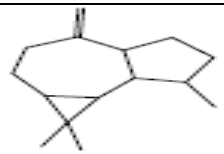
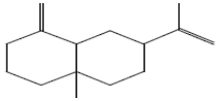
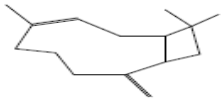
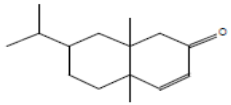
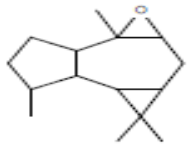
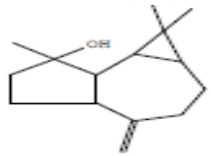
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
30	9.900	Cadina-1(10),4-diene	1469		Sesquiterpene		1.40	1.14	3.44	1.36
31	9.975	9-Cedranone	1564		NT		-	0.20	-	-
32	10.036	Copaene	1221		Sesquiterpene		-	0.18	6.97	2.54
33	10.145	1-(1,3a,4,5,6,7-hexahydro-4-hydroxy-3,8-dimethyl-5-azulenyl)-Ethanone	1758		NT		-	0.69	-	-
34	10.192	[1R-(1.α.,3.α.,4.β.)]-4-ethenyl-.α.,.α.,4-trimethyl-3-(1-methylethenyl)-Cyclohexanemethanol	1522		NT		-	-	-	1.21

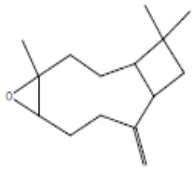
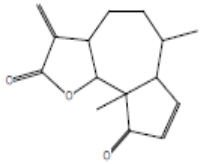
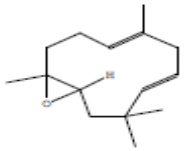
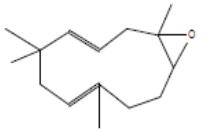
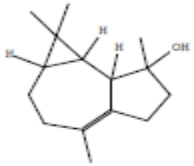
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
35	10.414	1,1,7-trimethyl-4-methylene-decahydro-1H-Cycloprop[e]azulene	1386		NT		1.10	-	-	-
36	10.414	Eudesma-4(14),11-diene	1469		Sesquiterpene		-	0.46	-	-
37	10.414	4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene	1494		NT		-	-	-	1.15
38	10.465	4a,5,6,7,8,8a-hexahydro-7.α.-isopropyl-4a.β.,8a.β.-dimethyl-2(1H)-Naphthalenone	1598		NT		-	0.22	-	-
39	10.519	Isoaromadendrene epoxide	1281		Sesquiterpenoid		-	0.26	-	-
40	10.618	[1ar-(1a.α.,4a.α.,7.β.,7a.β.,7b.α.)]-1,1,7-trimethyl-4-methylene-decahydro-1H-Cycloprop[e]azulen-7-ol	1536		NT		3.95	3.61	-	4.34

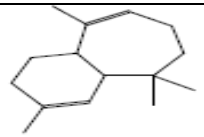
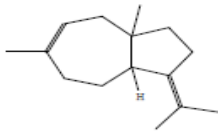
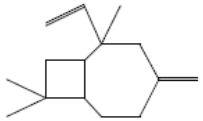
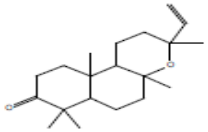
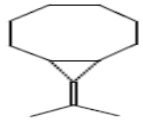
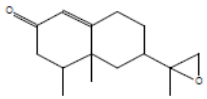
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
41	10.717	Caryophyllene oxide	1507		Sesquiterpenoid	Anti-inflammatory Insect repellent Food additive Local anesthetic	28.62	37.68	1.68	23.79
42	10.795	Ambrosin	1943		Sesquiterpenoid	Mucolytic agent Mucokinetic agent	-	0.64	-	-
43	10.863	Humulene epoxide	1592		Sesquiterpenoid		0.84	1.18	-	-
44	10.992	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0] dodeca-3,7-diene	1592		NT		12.18	13.08	0.47	7.64
45	11.112	Isospathulenol	1569		Sesquiterpenoid		0.41	-	-	-

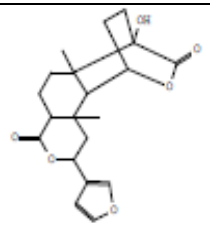
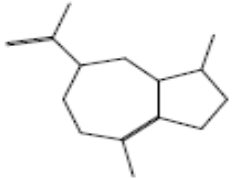
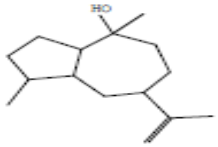
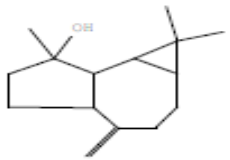
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
46	11.115	γ-Himachalene	1499		Sesquiterpene		-	0.89	-	-
47	11.169	(3aS,8aS)-6,8a-Dimethyl-3-(propan-2-ylidene)-1,2,3,3a, 4,5,8,8a-octahydroazulene	1507		NT		-	0.45	-	-
48	11.215	2,8,8-Trimethyl-4-methylene-2-vinylbicyclo[5.2.0]nonane	1407		NT		0.75	-	-	-
49	11.215	3-ethenyldecahydro-3,4a,7,7,10a-pentamethyl-1H-Naphtho[2,1-b] pyran-8(4aH)-one	2149		NT		-	1.32	-	-
50	11.223	9-(1-Methylethylidene) bicyclo[6.1.0]nonane	1242		NT		-	-	-	1.09
51	11.349	Nootkaton-11,12-epoxide	1742		NT		-	0.56	-	-

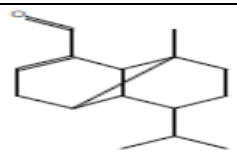
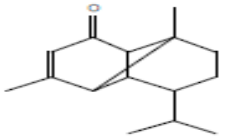
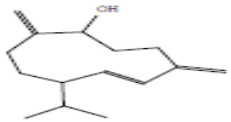
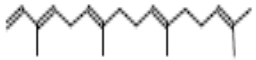
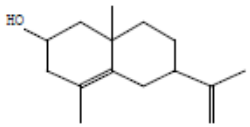
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
52	11.380	17,18-Dinor-8.beta.H,9.beta.H-labda-13(16),14-diene-19,20-dioic acid, 15,16-epoxy-1.beta.,4, 12-trihydroxy-, di-.delta.-lactone	2734		NT		-	0.45	-	-
53	11.434	[1S-(1.α.,7.α.,8a.β.)]-1,2,3,5,6,7, 8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-Azulene	1490		NT		0.34	-	-	-
54	11.435	Pogostole	1601		Sesquiterpenoid		-	0.90	-	-
55	11.450	(-)-Spathulenol	1536		Sesquiterpenoid	Insect repellent Vasodilator agent Anaesthetic agent	0.41	-	-	1.79

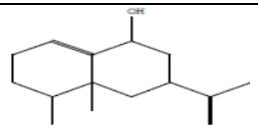
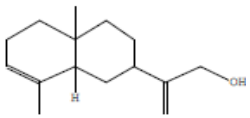
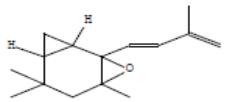
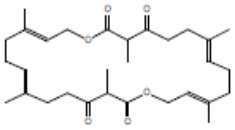
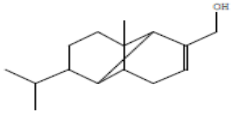
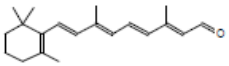
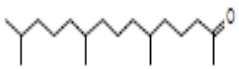
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
56	11.501	Ylangenal	1410		NT		0.30	0.82	-	-
57	11.656	8-Isopropyl-1,5-dimethyltricyclo[4.4.0.02,7] dec-4-en-3-one	1393		NT		0.22	0.95	-	-
58	11.730	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	1699		NT		-	0.37	-	-
59	11.844	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	1940		NT		-	0.24	-	-
60	11.916	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1690		NT		0.10	-	-	-

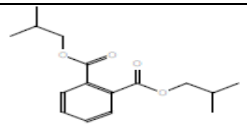
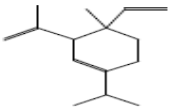
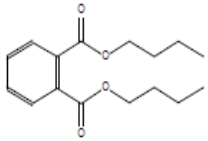
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
61	11.917	4a,5-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalen-1-ol	1662		NT		-	0.25	-	-
62	11.977	2-((2R,4aR,8aR)-4a,8-Dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalen-2-yl)prop-2-en-1-ol	1716		NT		0.15	-	-	-
63	12.054	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo [5.1.0.0(2,4)]octane	1407		NT		-	0.35	-	-
64	12.110	3,7,11,16,20,24-Hexamethyl-1,14-dioxa-cyclohexacosa-7,11,20,24-tetraene-2,4,15,17-tetraone	4320		NT		-	0.14	-	-
65	12.467	1-methyl-8-(1-methylethyl)-Tricyclo[4.4.0.0(2,7)]dec-3-ene-3-methanol	1464		NT		-	0.24	-	-
66	12.501	Retinal	2184		Diterpenoid		-	0.27	-	-
67	12.890	Hexahydrofarnesyl acetone	1754		Sesquiterpenoid	Perfumery Antimicrobial agent	0.45	0.41	-	1.04

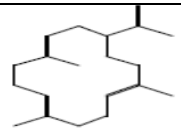
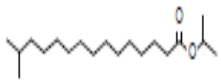
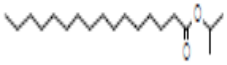
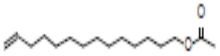
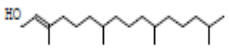
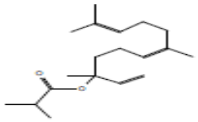
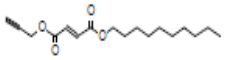
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
68	13.136	Diisobutyl phthalate			NT		-	-	-	1.30
69	13.491	Farnesyl acetone	1902		Diterpenoid	Fragrance	0.53	-	-	1.12
70	13.494	(E) - Geranylgeraniol	2192		Diterpenoid	Synthesis of Vitamins Perfumery	-	0.23	-	-
71	13.785	4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-Cyclohexene	1377		NT		-	-	0.56	-
72	13.820	Dibutyl phthalate	2037		NT	Perfumery Printing ink Glass additive Plasticizer Lubricating agent Adhesives	-	0.18	-	3.96

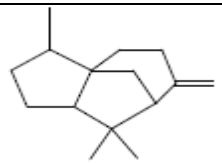
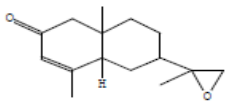
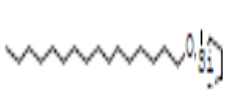
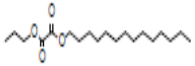
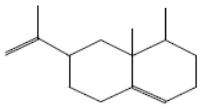
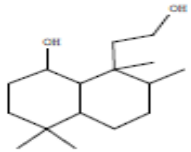
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
73	13.962	(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene	2121		NT		0.39	0.33	-	0.98
74	14.139	i-Propyl 14-methyl-pentadecanoate	1949		NT (Ester)		-	1.41	-	-
75	14.144	Isopropyl palmitate	2013		NT (Ester)	Perfumery Emollient Moisturer	-	-	-	1.30
76	14.387	Hexatriacontane	3202	$CH_3(CH_2)_{34}CH_3$	NT		-	0.91	-	-
77	14.550	13-Tetradecenyl acetate	1769		NT		-	0.15	-	-
78	14.729	Phytol	2045		Diterpenoid	Diluent Fragrance	0.51	-	-	-
79	14.755	Nerolidol isobutyrate	1889		NT (Ester)		-	0.26	-	-
80	14.845	Fumaric acid, decyl propargyl ester	2051		NT (Ester)		-	0.14	-	-

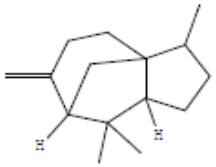
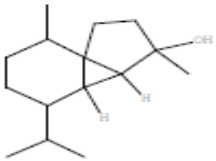
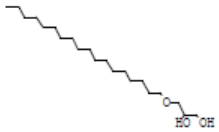
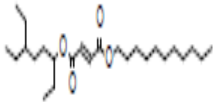
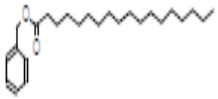
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
81	15.099	Cedrene	1398		Sesquiterpene	Solvent Food Additive Perfumery	-	-	7.30	-
82	15.205	4,8a-Dimethyl-6-(2-methyl-oxiran-2-yl)-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	1742		NT		-	0.07	-	-
83	15.345	1-Methyl-1-n-hexadecyloxy-1-silacyclopentane	2144		NT		-	0.08	-	-
84	15.451	Oxalic acid, propyl tetradecyl ester	2244		NT		-	0.14	-	-
85	15.550	[1R-(1.α.,7.β.,8a.α.)]-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-Naphthalene	1474		NT		-	-	0.32	-
86	15.740	(1R,2R,8S,8Ar)-8-hydroxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-cis-decalin	1962		NT		-	0.07	-	-

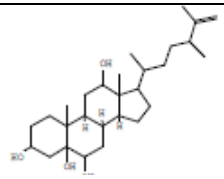
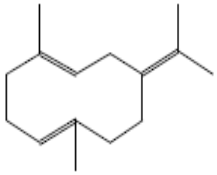
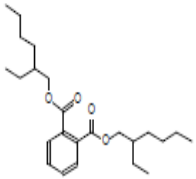
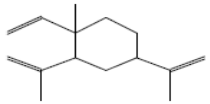
Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index,- – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
87	15.771	(3R,3aR,7R,8aS)-3,8,8-Trimethyl-6-methyleneoctahydro-1H-3a,7-methanoazulene	1398		NT		-	-	3.43	-
88	15.930	Epicubebol	1484		NT		-	-	1.91	-
89	15.887	Batilol	2590		NT	Prevention of radiation sicknesss	-	0.32	-	-
90	15.925	Fumaric acid, 6-ethyloct-3-yl undecyl ester	2721		NT		-	0.25	-	-
91	16.340	Benzyl stearate	2750		NT (Ester)		-	-	0.29	-

Rt- Retention time (minutes), NT- Non-terpene, RI- Retention Index, - – Not detected.

Table 4.17: Comparison of chemical constituents of essential oils from *Paullinia pinnata* and their applications Cont'd

S/N	Rt (Mins)	Compound	RI	Structure	Class of Compound	Applications	Leaf (%)	Leaf Stalk (%)	Stem Bark (%)	Root (%)
92	16.510	Ergost-25(27)-ene-3,5,6,12-tetrol	3179		NT		-	0.17	-	-
93	16.819	Germacrene B	1603		Sesquiterpene	Insecticidal Antimicrobial	-	-	1.72	-
94	17.681	Dotriacontane	3202	$CH_3(CH_2)_{30}CH_3$	NT	Polystyrene synthesis Formation of organogels Solvent	-	1.19	-	-
95	17.689	Bis(2-ethylhexyl) phthalate	2704		NT	Plasticizer of medical tools	0.34	-	-	-
96	23.341	2,4-Diisopropenyl-1-methyl-1-vinylcyclohexane	1398		NT		-	-	0.44	-
Total Identified							**37**	**57**	**19**	**29**

Class of oxygenated terpenoids (sesquiterpenoid) as shown in Table 4.18 were the highest among the identified compounds present in the essential oil of *Paullinia pinnata* leaf (33.00%) and leaf stalk (42.65%) while the sesquiterpenes were the highest in the stem bark (83.39%) and root (35.74%). The classes of identified compounds in *Paullinia pinnata* leaf in descending order were sesquiterpenoids (33.00%), sesquiterpenes (30.58%), non-terpenes (25.23%), diterpenoids (1.04%) and monoterpenoids (0.76%). This is similar to the result obtained in *Paullinia pinnata* leaf essential oil reported by Ogunwande *et al*., (2017), as sesquiterpenoids being the highest followed by sesquiterpene and absence of monoterpene hydrocarbon reported.

In *Paullinia pinnata* leaf stalk, the classes of identified compounds present in descending order were sesquiterpenoids (42.65%), non-terpenes (35.02%), sesquiterpenes (12.99%) and diterpenoids (0.23%). There were sesquiterpenes (83.39%), non-terpenes (10.33%) and sesquiterpenoids (6.27%) in *Paullinia pinnata* stem bark while sesquiterpenes (35.74%), non-terpenes (32.28%) sesquiterpenoids (28.10%), diterpenoids (1.12%) and monoterpenoids (0.66%) were identified in *Paullinia pinnata* root oil.

Over all, the identified compounds from the essential oils from different parts of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata* were classified into monoterpenes, monoterpenoids, sesquiterpenes, sesquiterpenoids, diterpenes, diterpenoids, triterpenes and non-terpenes as shown in Fig. 4.1. The oils of *Blighia sapida* and *Lecaniodiscus cupanioides* were dominated by non-terpenes except for fruit husk of *Blighia sapida* dominated by diterpenoids and root of *Lecaniodiscus cupanioides* by sesquiterpenoids. Leaf and leaf stalk of *Paullinia pinnata* were dominated by sesquiterpenoids, stem bark and root of *Paullinia pinnata* dominated by sesquiterpenes.

Blighia sapida seed had the highest monoterpenes, leaf of *Blighia sapida* and *Lecaniodiscus cupanioides* had the highest content of monoterpenoids, *Paullinia pinnata* stem bark was very rich in sesquiterpenes, *Lecaniodiscus cupanioides* root was the highest in sesquiterpenoids, *Blighia sapida* fruit husk had highest content of

Table 4.18: Classes of identified compounds of essential oils from *Paullinia pinnata*

Class	Leaf (%)	Leaf stalk (%)	Stem bark (%)	Root (%)
Monoterpene	n.d.	n.d.	n.d.	n.d.
Monoterpenoid	0.76	n.d.	n.d.	0.66
Sesquiterpene	30.58	12.99	83.39	35.74
Sesquiterpenoid	33.00	42.65	6.27	28.10
Diterpene	n.d.	n.d.	n.d.	n.d.
Diterpenoid	1.04	0.23	n.d.	1.12
Triterpene	n.d.	n.d.	n.d.	n.d.
Non-terpene	25.23	35.02	10.33	32.28

n.d. – Not Detected

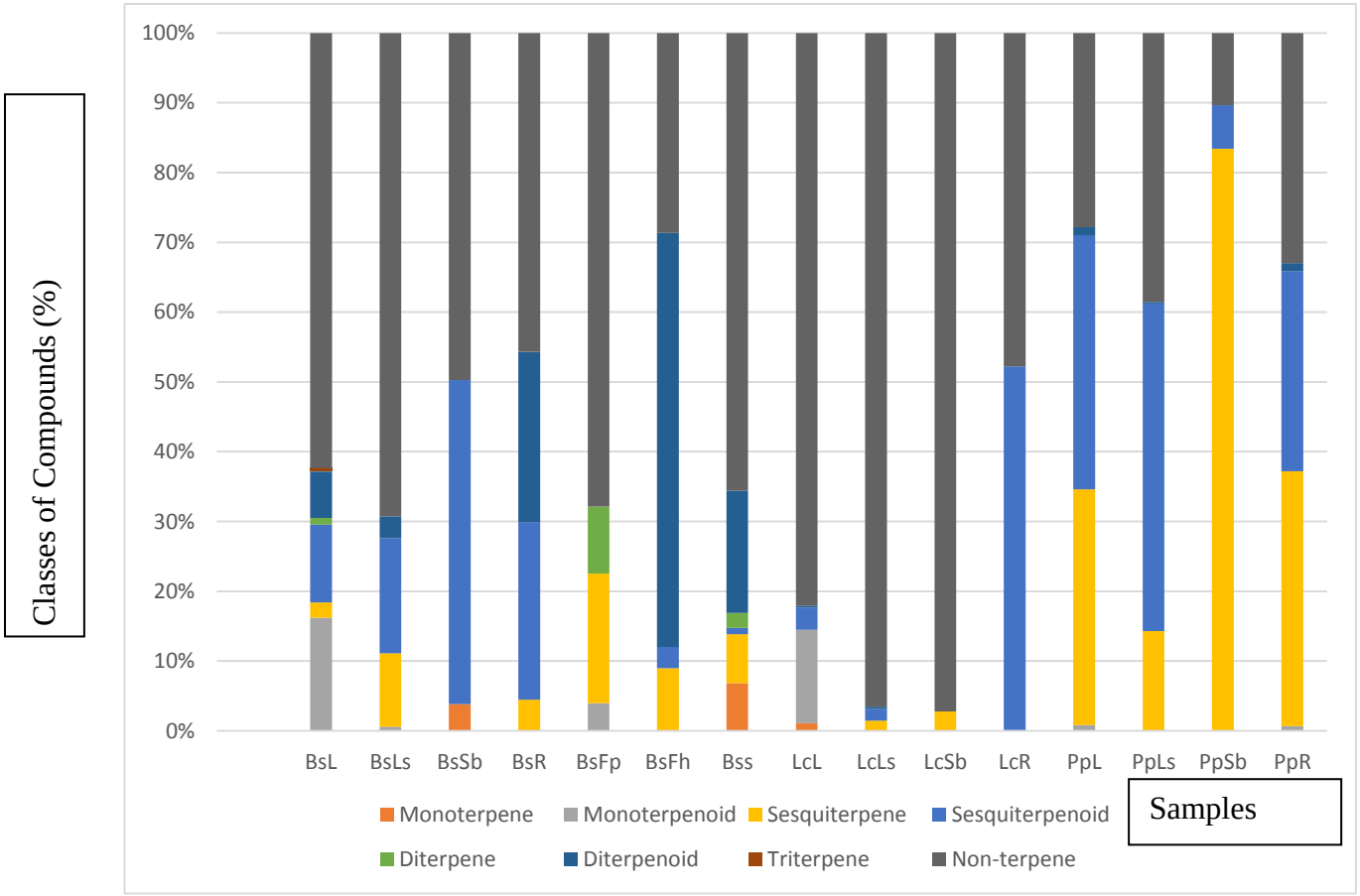


Figure 4.1: Classes of identified compounds of essential oils of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata*

Blighia sapida leaf (BsL), *Blighia sapida* leaf-stalk (BsLs), *Blighia sapida* stem-bark (BsSb), *Blighia sapida* root (BsR), *Blighia sapida* fruit-pulp (BsFp),*Blighia sapida* fruit-husk (BsFh), *Blighia sapida* seed (BsS), *Lecaniodiscus cupanioides* leaf (LcL), *Lecaniodiscus cupanioides* leaf-stalk (LcLs), *Lecaniodiscus cupanioides* stem-bark (LcSb), *Lecaniodiscus cupanioides* root (LcR), *Paullinia pinnata* leaf (PpL), *Paullinia pinnata* leaf-stalk (PpLs), *Paullinia pinnata* stem-bark (PpSb) and *Paullinia pinnata* root (PpR).

diterpenes while diterpenes were absent in all *Lecaniodiscus cupanioides* and *Paullinia pinnata* essential oils. *Blighia sapida* fruit husk was the highest in diterpenoids, triterpene was only present in *Blighia sapida* leaf while *Lecaniodiscus cupanioides* leaf stalk and stem bark had the highest content of non-terpenes.

Monoterpenes are common costituents of essential oils, they contain only carbon and hydrogen, they have two isoprene units with molecular formular of $C_{10}H_{16;}$ monoterpenes are acyclic (linear) and monocyclic or bicyclic (rings) while monoterpenoids are modified monoterpenes with functionality of oxygen like alcohols, phenols and carboxylic acids. Monoterpenes and monoterpenoids often have pharmacological functions such as antipruritic, antibacterial, antiinflammatory and hypotensive properties (Kabir *et al*., 2020).

Sesquiterpenes are also commonly found in essential oils. They have three isoprene units with molecular formular of $C_{15}H_{24}$. They are useful as antimalarial drugs, they also have antiplasmodial, anticancer, anti-inflammatory and anti-ulcer properties (Chadwick *et al*., 2013). Diterpenes rarely found in essential oils, they have four isoprene units with molecular formular $C_{20}H_{32}$. They have anti-inflammatory and antitumor properties (Vasas and Hohmann, 2014). Triterpenes have six isoprene units with molecular formular $C_{30}H_{48}$. They have antioxidant, anticancer and antiviral properties (Nazaruk and Borzym-Kluczyk, 2015).

4.3 Chemical constituents of essential oils of Sapindaceae

Many chemical compounds were identified in these three sapindaceae species as we have one hundred and ninety-seven (197) from seven (7) parts of *Blighia sapida*, one hundred and fifty-three (153) from four (4) parts of *Lecaniodiscus cupanioides* and one hundred and forty-two (142) from four (4) parts of *Paullinia pinnata* giving total of four hundred and ninety-two (492) compounds from *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata*. Some of the identified compounds as shown in Table 4.19 were repeatedly detected in two or three of these three sapindaceae species resulting to total of three hundred and fifty-eight (358) chemical compounds of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata* if similar compounds were not counted.

Table 4.19: Commonly present chemical constituents of Sapindaceae

S/N	Compounds	BsL	BsLs	BsSb	BsR	BsFp	BsFh	BsS	LcL	LcLs	LcSb	LcR	PpL	PpLs	PpSb	PpR
1	2 pentyl furan		0.48						0.34	0.27						0.87
2	Nonanal		0.57						0.41				0.50			
3	Methyl salicylate	0.66							0.79				1.42			
4	α- ionone	5.17							1.16				0.42			
5	α-caryophyllene					8.05		1.64		0.41			6.21	2.58	12.70	5.98
6	Hexahydrofarnesyl acetone	2.79	3.82	3.61			1.51	0.51	0.94	0.40			0.45	0.41		1.04
7	Phytol	4.03	1.74						0.18	0.29				0.51		

BsL - *Blighia sapida* Leaf, BbLs- *Blighia sapida* Leaf stalk, BsSb- *Blighia sapida* Stem bark, BsR- *Blighia sapida* Root, BsFp- *Blighia sapida* Fruit pulp, BsFh- *Blighia sapida* Fruit husk, BsS- *Blighia sapida* Seed, LcL- *Lecaniodiscus cupanioides* Leaf, LcLs- *Lecaniodiscus cupanioides* Leaf stalk, LcSb- *Lecaniodiscus cupanioides* Stem bark, LcR- *Lecaniodiscus cupanioides* Root, PpL- *Paullinia pinnata* Leaf, PpLs- *Paullinia pinnata* Leaf stalk, PpSb- *Paullinia pinnata* Stem bark, PpR- *Paulinia pinnata* Root.

Table 4.19: Commonly present chemical constituents of Sapindaceae Cont'd

S/N	Compounds	BsL	BsLs	BsSb	BsR	BsFp	BsFh	BsS	LcL	LcLs	LcSb	LcR	PpL	PpLs	PpSb	PpR
8	(E)-2-hexenal								32.68				0.66			
9	Eucalyptol								0.42	0.12						0.66
10	Linalool								6.41				0.76			
11	Caryophyllene								0.83	2.79			12.92	0.44	39.68	12.65
12	γ-muurolene										0.39		0.72		1.17	3.29
13	Cadina-1(10), 4-diene										0.40		1.40	1.14	3.44	1.36

BsL - *Blighia sapida* Leaf, BbLs- *Blighia sapida* Leaf stalk, BsSb- *Blighia sapida* Stem bark, BsR- *Blighia sapida* Root, BsFp- *Blighia sapida* Fruit pulp, BsFh- *Blighia sapida* Fruit husk, BsS- *Blighia sapida* Seed, LcL- *Lecaniodiscus cupanioides* Leaf, LcLs- *Lecaniodiscus cupanioides* Leaf stalk, LcSb- *Lecaniodiscus cupanioides* Stem bark, LcR- *Lecaniodiscus cupanioides* Root, PpL- *Paullinia pinnata* Leaf, PpLs- *Paullinia pinnata* Leaf stalk, PpSb- *Paullinia pinnata* Stem bark, PpR- *Paullinia pinnata* Root.

Table 4.19: Commonly present chemical constituents of Sapindaceae Cont'd

S/N	Compounds	BsL	BsLs	BsSb	BsR	BsFp	BsFh	BsS	LcL	LcLs	LcSb	LcR	PpL	PpLs	PpSb	PpR
14	Caryophyllene oxide								0.20				28.62	37.68	1.68	23.79
15	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene								0.07				12.18	13.08	0.47	7.64
16	Bis(2-ethylhexyl)phthalate								0.08	0.96			0.34			

BsL - *Blighia sapida* Leaf, BbLs- *Blighia sapida* Leaf stalk, BsSb- *Blighia sapida* Stem bark, BsR- *Blighia sapida* Root, BsFp- *Blighia sapida* Fruit pulp, BsFh- *Blighia sapida* Fruit husk, BsS- *Blighia sapida* Seed, LcL- *Lecaniodiscus cupanioides* Leaf, LcLs- *Lecaniodiscus cupanioides* Leaf stalk, LcSb- *Lecaniodiscus cupanioides* Stem bark, LcR- *Lecaniodiscus cupanioides* Root, PpL- *Paullinia pinnata* Leaf, PpLs- *Paullinia pinnata* Leaf stalk, PpSb- *Paullinia pinnata* Stem bark, PpR- *Paullinia pinnata* Root.

Hexahydrofarnesyl acetone which is an antimicrobial agent was most detected, it was found in ten (10) of fifteen (15) oils of these three (3) Sapindaceae species (*Blighia sapida* leaf, leaf-stalk, stem-bark, fruit-husk, seed; *Lecaniodiscus cupanioides* leaf, leaf-stalk and *Paulinia pinnata* leaf, leaf-stalk, root) as shown in Table 4.19 and its fragmentation pattern is shown in Fig. 4.2. McLafferty re-arrangement (Mass spectral cleavage) occurred in its fragmentation because there is a presence of hydrogen at γ-position to the carbonyl group (hydrogen on a carbon 3 atoms away from the carbonyl group) leading to the fragment at 58. Its base peak which is the most intensive peak was also 58 and its molecular ion which is the heaviest ion was the peak at 268, therefore, the molecular mass at m/z 268 [M^+] is diagnostic peak of hexahydrofarnesyl acetone to molecular formula $C_{18}H_{36}O$. Hexahydrofarnesyl acetone was calculated as 71.4%, 50.0% and 75.0% of the essential oils obtained from different plants' parts of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paulinia pinnata*, respectively.

α-caryophyllene which is a food additive and flavour was detected in seven (7) parts (*Blighia sapida* fruit-pulp, seed; *Lecaniodiscus cupanioides* leaf-stalk and *Paullinia pinnata* leaf, leaf-stalk, stem-bark and root). Caryophyllene which is an important agent in the treatment of seizures, relief of pain and anxiety, prevention of osteoporosis and reduction of cholesterol was detected in six (6) parts (*Lecaniodiscus cupanioides* leaf, leaf stalk and *Paullinia pinnata* leaf, leaf stalk, stem bark and root). Caryophyllene oxide, an anti-inflammatory, insect repellent, food additive and local anesthetic was discovered in five (5) parts (*Lecaniodiscus cupanioides* leaf and *Paullinia pinnata* leaf, leaf stalk, stem bark and root) likewise, phytol which is an antioxidant, antinociceptive and anti-inflammatory agent was also detected in five (5) parts (*Blighia sapida* leaf, leaf stalk; *Lecaniodiscus cupanioides* leaf, leaf stalk and *Paullinia pinnata* leaf stalk). Eucalyptol that was detected in *Lecaniodiscus cupanioides* leaf, leaf stalk and *Paullinia pinnata* root is known for its applications in flavouring, cosmetics, fragrance, insecticidal, cough suppressant, mouth wash and anti-inflammatory.

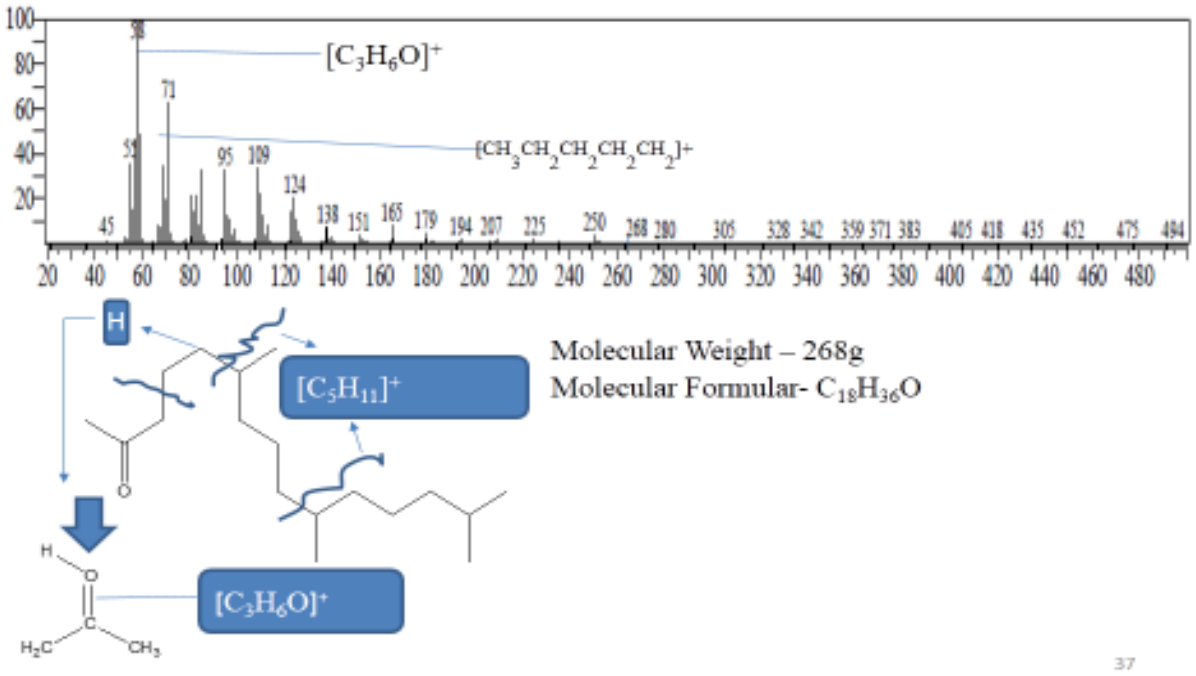


Figure 4.2: Fragmentation pattern of Hexahydrofarnesyl acetone

4.4 Applications of chemical constituents of essential oils

Essential oils are used as perfume, flavour and medicine as there were some of the chemical constituents that have been reported as perfumery, flavouring or medicinal agent (Alfrebro, 2018; Banu and Tomris, 2009; Bedoukan, 2017; Bedoukian, 2018; Janes et al., 2008; Jensen, 2010; Jirovetz, 2002; Lim, 2013; Lu et al., 2003; Nicoline et al., 2007; Siddiquee et al., 2012) detected in different plants' parts of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paulinia pinnata*. Some of the chemical constituents found in different plants' parts of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paulinia pinnata* essential oils have been established in literature to possess industrial and medical applications including applications in creams, perfumes and food flavouring. Some of their ethnomedicinal values cut across all the three plants such as in the treatment of skin infections, curing different types of fever, cough and dysentery (Alfa, 2018; Altman and Tyrer, 1980; Bloch, 1983; Bonikowski et al.,, 2015; Eric and Adamson, 2012; Fujita et al., 2007; Hills, 2004; Himejima and Kubo, 1993; James and Price, 2004; Justia, 2018; Kromidas *et al*.,2006; Lim, 2013; Listerine, 2015; Lock *et al*., 2004; Michael *et al*., 1987; Pappa, 2009; Schiestl and Roubik, 2004; Vic *et al*., 2007; Volker *et al*., 1984; Zeytinoglu *et al*., 2003; Zih-Rou, 2009) as shown in Tables 4.5, 4.11 and 4.17.

4.4.1 Applications of constituents of *Blighia sapida* essential oils

Some of the compounds that are medically and industrially useful from the leaf of *Blighia sapida* are methyl salicylate which can be applied as antispectic agent and for the treatment of pain (analgestic); β-cyclocitral as stabilizers, emulsifiers and surfactants; 1,2-dimethyl azetidine in drug design; β-ionone in vitamin A; (Z)-1,8(2H,5H)-hexahydro-8a-methyl-naphthalenedione in treatment of inflammation and microbial infection; myristaldehyde as emulsifier, surfactant, nutrient, membrane stabilizer and energy source; 1-dodecanol as antifungal agent, adhesive, plasticizer, lubricant and paint additives; pyrethrin as insecticidal agent; aspirin for treatment of pain, fever and inflammation; geranylgeraniol as antimicrobial agent; p-terphenyl in plastic production; farnesyl acetate as antimicrobial agent; squalene as precursor of steroids and skin lubrication. From leaf and leaf stalk essential oils are phytol, isophytol and phytanol as precursor of vitamin E and vitamin K1; 2-ethoxy-ethanol as multipurpose cleaner. 2-acetyl-5-methylfuran from leaf, leaf stalk and root as organoleptic agent. Hexadecanoic acid from leaf and root as thickener, stabilizer,

emulsifier and gelling agent. 1,3-di-n-propyladamantane from leaf stalk in drug design and as lubricant. Pentadecanal from leaf stalk, stem bark, fruit husk and seed as antimicrobial agent. Cadalene from leaf stalk and root to reduce adenomas. Farnesol from stem bark essential oil as pesticide, chemopreventative, anti-tumor and antibacterial agent. α-cadinol from root as antimicrobial agent. From seed essential oil, germacrene that was detected was reported as antimicrobial and insecticidal agents, (Z) –vaccenic acid as anticarcinogenic agents.

4.4.2 Applications of constituents of *Lecaniodiscus cupanioides* essential oils

Butyl oxirane, (E)-2-hexenal, heptanal, 6-methyl-5-heptene-2-one, n-hexyl acetate, (2E)-2-decenal, undecanal, (E)-β-ionone, caryophyllene oxide, isophytol and ethyl palmitate from leaf as disinfectant, antibacterial agent, lubricant, alarm pheromone, solvent, nematicide, antimycobacterial drug, antioxidant, antifungal agent, precursor of vitamin E and K, hair and skin conditioning agent respectively. D-limonene from leaf as clinical solvent, relief of heartburn and gastroesophageal reflux. Phenalacetaldehyde from leaf as insect and pest attractant, polymers for packaging and insulation. Linalool from leaf as antibacterial and antifungal agent, insecticidal agent, cleaning agent, anti-inflammatory, anti-epileptic and pain relief. α-terpineol from leaf as antihypertensive, anticonvulsant, antioxidant, antiulcer, antinociceptive and anticancer agent. Methyl salicylate from leaf as analgesic and antiseptic. Methyl isobutyl ketone, (8)-annulene, 1-octen-3-ol, (+/-)-(E)-nerolidol and palmitic acid from leaf stalk as solvent, rubber production, insecticide, antimicrobial agent and cosmetics to hide blemishes respectively. Nonanal from leaf stalk as cleaning agent, disinfectant, pesticide, polishes, waxes. Terpinen-4-ol from leaf stalk as anti-inflammatory, antioxidant and anticanceragent. n-butyl stearate from leaf stalk as solvent and plasticizer. 2-pentylfuran and hexahydrofarnesyl acetone from leaf and leaf stalk as insect repellent and antimicrobial agent respectively. Eucalyptol from leaf and leaf stalk as insecticidal, cough suppressant, mouth wash, traditional medicine. Phytol from leaf and leaf stalk as antioxidant, antinociceptive, antiallergic, diluent and anti-inflammatory agent. Caryophyllene from leaf stalk and stem bark as antibacterial, antifungal, antiseptic, anti-inflammatory, antioxidant, analgestic, cholesterol, seizure and osteoporosis treatment. Nonacosane from stem bark as insecticidal agent.

4.4.3 Applications of constituents of *Paullinia pinnata* essential oils

(E)-2-hexenal and methyl salicylate from leaf as antibacterial and analgesic agent respectively. Linalool from leaf as insecticidal, antimicrobial and cleaning agent. Phytol from leaf as diluent, antioxidant, antinociceptive, anti-inflammatory and antiallergic agent. Hexanal from leaf and root as antimicrobial agent. (-)-spathulenol from leaf and root as insect repellent, vasodilator and anaesthetic agent. Ambrosin from leaf stalk as mucolytic and mucokinetic agent. 1,6-dideoxy-l-mannitol from leaf stalk as antineoplastic drug and it also extends shelf life of fruits. (E)- geranyl geraniol and batilol from leaf stalk for synthesis of vitamins and prevention of radiation sicknesss respectively. Dotriacontane from leaf stalk for polystyrene synthesis, organogels formation and as solvent. Cedrene from stem bark as solvent and food additive. Germacrene B from stem bark for insecticidal and antimicrobial activities. 2-pentylfuran from root as insect repellent. Eucalyptol from root as cough suppressant, anti-inflammatory agent and mouth wash ingredient. Isopropyl palmitate from root as emollient and moisture. Germacrene D from leaf, stem bark and root as insecticidal and antimicrobial agent. Caryophyllene from leaf, leaf stalk, stem bark and root for the treatment of seizures, prevention of osteoporosis, reduction of cholesterol, relief of pain and anxiety. Caryophyllene oxide from leaf, leaf stalk, stem bark and root as anti-inflammatory, insect repellent, food additive and local anesthetic agent.

4.5 Phytochemical screening of *Blighia sapida* crude extracts

4.5.1 Qualitative phytochemical screening of *Blighia sapida* crude extracts

Preliminary qualitative phytochemical screening of hexane, ethylacetate and methanol extracts of leaf (BsLH, BsLEa, BsLM), stem-bark (BsSbH, BsSbEa, BsSbM), root (BsRH, BsREa, BsRM) from cold extraction and root extracts of soxhlet extraction (BRHH, BREH, BRMH) showed different phytochemical groups as nineteen (19) phytochemicals were investigated on each sample (Table 4:20) while 5 in quantitative analysis (Table 4.21). Leaf hexane gave positive results in the test for reducing sugars, alkaloids, saponins, steroids, resins and cardiac glycosides. Reducing sugars, alkaloids, saponins, steroids, resins, cardiac glycosides, anthocyanins and terpenoids were present in leaf ethyl acetate. Alkaloids, tannins, saponins, steroids, flavonoids, resins, cardiac glycosides, phenols, diterpenes and coumarin were positive in leaf methanol.

Table 4.20: Qualitative phytochemical screening of *Blighia sapida*

S/N	Test	BsLH	BsLEa	BsLM	BsSbH	BsSbEa	BsSbM	BsRH	BRHH	BsREa	BREH	BsRM	BRMH
1	Reducing sugars	+	+	-	+	+	+	+	+	+	+	-	-
2	Alkaloids (Hager's Test)	+	+	+	+	+	+	+	-	+	+	+	+
3	Quinones	-	-	-	+	+	+	+	+	+	+	+	+
4	Tannins (Braymer's Test)	-	-	+	-	-	+	-	-	-	-	+	+
5	Phlobatannin (Precipitate test)	-	-	-	-	-	-	-	-	-	-	-	-
6	Glycosides	-	-	-	+	+	+	+	+	+	+	+	+
7	Saponins (Froth's Test)	+	+	+	+	+	+	+	+	+	+	+	+
8	Steroids (Salkowaski's Test)	+	+	+	+	+	+	+	+	+	+	+	+
9	Flavonoids (Lead acetate Test)	-	-	+	+	+	-	+	+	-	-	-	-
10	Resins	+	+	+	-	-	-	-	-	+	+	+	+
11	Cardiac Glycosides	+	+	+	+	+	+	+	+	+	+	+	+
12	Phenols (Ferric Chloride's Test)	-	-	+	-	-	+	-	-	-	-	+	+

+ - Presence of the metabolite, - - Absence of the metabolite

Table 4.20: Qualitative phytochemical screening of *Blighia sapida* Cont'd

S/N	Test	BsLH	BsLEa	BsLM	BsSbH	BsSbEa	BsSbM	BsRH	BRHH	BsREa	BREH	BsRM	BRMH
13	Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
14	Anthocyanins (Reaction with Acid and Ammonia)	-	+	-	-	+	-	-	-	+	+	-	-
15	Diterpenes (Copper Acetate Test).	-	-	+	-	-	+	+	+	+	+	+	+
16	Terpenoid (Salkowaski's test)	-	+	-	-	+	-	+	+	+	+	+	+
17	Coumarin (Reaction with 10 % NaOH)	-	-	+	+	+	-	+	+	+	+	+	-
18	Charcones (Ammonium hydroxide's Test)	-	-	-	-	-	-	-	-	-	-	-	-
19	Protein	-	-	-	+	+	+	+	+	+	+	+	+

+ - Presence of the metabolite, - - Absence of the metabolite

Table 4.21: Quantitative phytochemical screening of *Blighia sapida*

S/N	Test	BsLH	BsLEa	BsLM	BsSbH	BsSbEa	BsSbM	BRH	BRHH	BsREa	BsREaH	BsRM	BSRMH
1	Alkaloids (mg of AE/g)	6.22 + 0.02	11.31 + 0.02	3.22 + 0.01	Nil	4.18 + 0.00	1.34 + 0.01	2.48 + 0.03	Nil	5.89 + 0.00	5.77 + 0.00	3.27 + 0.07	1.42 + 0.02
2	Tannins (mg of GAE/g)	Nil	Nil	0.415 + 0.000	Nil	Nil	0.756 + 0.000	Nil	Nil	Nil	Nil	6.097 + 0.153	0.643 + 0.000
3	% Saponins	2.08 + 0.00	4.18 + 0.01	7.18 + 0.02	2.13 + 0.04	2.55 + 0.00	2.18 + 0.01	3.25 + 0.07	3.80 + 0.00	3.22 + 0.00	0.91 + 0.01	3.86 + 0.12	4.08 + 0.00
4	Flavonoids (mg of QE/g)	Nil	Nil	3.16 + 0.000	2.220 + 0.002	6.111 + 0.002	Nil	7.037 + 0.423	1.667 + 0.007	Nil	Nil	45.555 + 0.481	Nil
5	Total Phenolics (mg of GAE/g)	Nil	Nil	2.845 + 0.039	Nil	Nil	5.354 + 0.103	Nil	Nil	Nil	Nil	10.809 + 0.114	1.299 + 0.039

Saponins and tannins were also reported positive in *Blighia sapida* leaf aqueous extract by Olayinka and Ozolua, (2021). Results on stem bark hexane showed the presence of reducing sugars, alkaloids, quinones, glycosides, saponins, steroids, flavonoids, cardiac glycosides, coumarin and protein. Reducing sugars, alkaloids, quinones, glycosides, saponins, steroids, flavonoids, cardiac glycosides, anthocyanins, terpenoid, coumarin and protein gave positive results in stem bark ethyl acetate. Reducing sugars, alkaloids, quinones, tannins, glycosides, saponins, steroids, cardiac glycosides, phenols, diterpenes and protein were present in stem bark methanol as Amira and Oloyede, (2017) also reported the presence of tannin, saponin, flavonoid, steroid, terpenoid, alkaloid and phenol in aqueous extract of *Blighia sapida* stem bark and Amira *et al*., (2019) reported saponins, flavonoids, steroids, terpenoids, alkaloids and phenols in hydro-ethanolic extract of *Blighia sapida* of stem bark.

Crude extracts were obtained from both successive cold extraction and successive soxhlet (hot) extraction of *Blighia sapida* root using hexane, ethylacetate and methanol. Reducing sugars, alkaloids, quinones, glycosides, saponins, steroids, flavonoids, cardiac glycosides, diterpenes, terpenoid, coumarin and protein were positive in root hexane from cold extraction while reducing sugars, quinones, glycosides, saponins, steroids, flavonoids, cardiac glycosides, diterpenes, terpenoid, coumarin and protein were present in extract of root hexane from soxhlet extraction. Reducing sugars, alkaloids, quinones, glycosides, saponins, steroids, resins, cardiac glycosides, anthocyanins, diterpenes, terpenoid, coumarin and protein were present in root ethyl acetate from cold extraction while reducing sugars, alkaloids, quinones, glycosides, saponins, steroids, resins, cardiac glycosides, anthocyanins, diterpenes, terpenoid, coumarin and protein were positive in extract of root ethyl acetate from soxhlet extraction. Root methanol from cold extraction gave positive results in the test for alkaloids, quinones, tannins, glycosides, saponins, steroids, resins, cardiac glycosides, phenols, diterpenes, terpenoid, coumarin and protein while extract from soxhlet extraction of root methanol also showed the presence of alkaloids, quinones, tannins, glycosides, saponins, steroids, resins, cardiac glycosides, phenols, diterpenes, terpenoid and protein.

Saponins, steroids and cardiac glycosides were present in all the extracts while phlobatannin, anthraquinones and charcones were not detected in all. Extracts from cold and soxhlet extractions of root ethyl acetate and root methanol had the highest number of secondary metabolites which was thirteen out of nineteen in each sample followed by cold extract of root hexane, soxhlet extract of root methanol and stem bark ethyl acetate with twelve while the least among them all was *Blighia sapida* leaf hexane with six secondary metabolites. Comparing the extracts from cold and soxhlet extraction of root, the results showed that hexane extract of root from cold extraction had one more metabolite which was alkaloid than extract from soxhlet extraction, likewise, methanol extract from cold extraction had one more metabolite which was coumarin than extract from soxhlet extraction but extracts from cold and soxhlet extractions of root ethyl acetate had the same secondary metabolites.

4.5.2 Quantitative phytochemical screening of *Blighia sapida* crude extracts

Quantitative phytochemical screening of alkaloids, tannins, saponins, flavonoids and total phenolics were investigated on these extracts to quantify these bioactive compounds. Results showed that these phytochemicals were in various amounts as reported in Table 4.21. Alkaloids which promote diuresis, cure malignant diseases, malaria and infections (Encyclopedia Britannica, 2017) had the highest content in *Blighia sapida* leaf ethyl acetate as 11.31 (mg of AE/g). % saponins that have been reported of anti-diabetic, hypocholesteremic activities and treatment of inflammation (Desai *et al*., 2009) had its highest content in *Blighia sapida* leaf methanol as 7.18. Tannin which is used in the prevention and treatment of ulcerated tissues, inflammation and cancer (Aiyegora and Okoh, 2010) was discovered as 6.097 (mg of AE/g) in *Blighia sapida* root methanol. Flavonoids have been reported to treat neurodegenerative diseases and prevention of deteriation in cognitive performance (Calabrece *et al*., 2003) and obtained as 45.555 (mg of QE/g) in *Blighia sapida* root methanol and total phenolics, antioxidant agents as 10.809 (mg of AE/g) in *Blighia sapida* root methanol.

Other phytochemical groups detected from the extracts in qualitative screening including quinones, glycosides, steroids, resins, cardiac glycosides, phenols, diterpenes, terpenoid, coumarin and protein have reported medicinal values.

4.6 Phytochemical analysis of ethylacetate fraction (BS6) from *Blighia sapida* root

4.6.1 Physical properties of BS6

The physical properties of isolated compound coded BS6 are stated on Table 4.22.

4.6.2 Characteristion of BS6

Compound BS6 was characterised using IR and NMR (1D and 2D) spectroscopic methods. Compound BS6 was identified as 7-hydroxy-6-methoxy-chromen-2-one (Scopoletin). As shown in Fig. 4.3 and Table 4.23, absorption bands of IR spectrum showed IR frequency of various functional groups present in BS6.

The ^{1}H NMR spectrum (400 MHz, DMSO) showed distinctive resonance of two one proton doublets at 7.91 (J = 9.47 Hz) and 6.23 (J = 9. 41Hz), these were assigned to protons on C4 and C3 of pyrone ring of the compound, the value of coupling constant indicated that the protons on the ring were at ortho position; two one proton singlets at 7.21 and 6.78 which are aromatic protons were assigned as the singlet proton on C5 and C8; also a three proton singlet due to methoxy protons at 3.82 ppm. This is shown in Fig. 4.4 and Table 4.24

The ^{13}C NMR (101 MHz, DMSO) with different sub–spectra showed number of carbons and number of attached protons which were indicated by different phases of the signals produced. Attached Proton Test Experiment (APT) spectrum indicated that 10 carbons were present in the compound out of which 5 were positive peaks, these 5 peaks were quartenary carbons (C) while methylene (CH_2) suppose positive peak too was absent based on Distortionless Enhancement by Polarization Transfer (DEPT), also 5 negative peaks, that is, methine (CH) and methyl (CH_3) were present. DEPT 135 spectra showed all protonated carbons present but inverted from APT, methine and methyl (CH and CH_3) are positive peaks and from this compound, 5 positive peaks were present, 4 out of them were attached to single proton, 1 was methyl group while methylene (CH2) that suppose to be negative peaks on DEPT was absent which indicated that the remaining 5 carbons on APT spectrum were quartenary carbons which include one O-methyl group and one phenolic hydroxyl group as shown in Fig. 4.5-4.7 and Table 4.25.

Table 4.22: Physical properties of BS6

Physical Properties	Characteristics
Colour	Biege
State	Solid/Crystalline powder
UV Visual	245nm
Weight	71.5 mg
Percentage Yield	0.06%
Melting point	202-202.5^{o}C

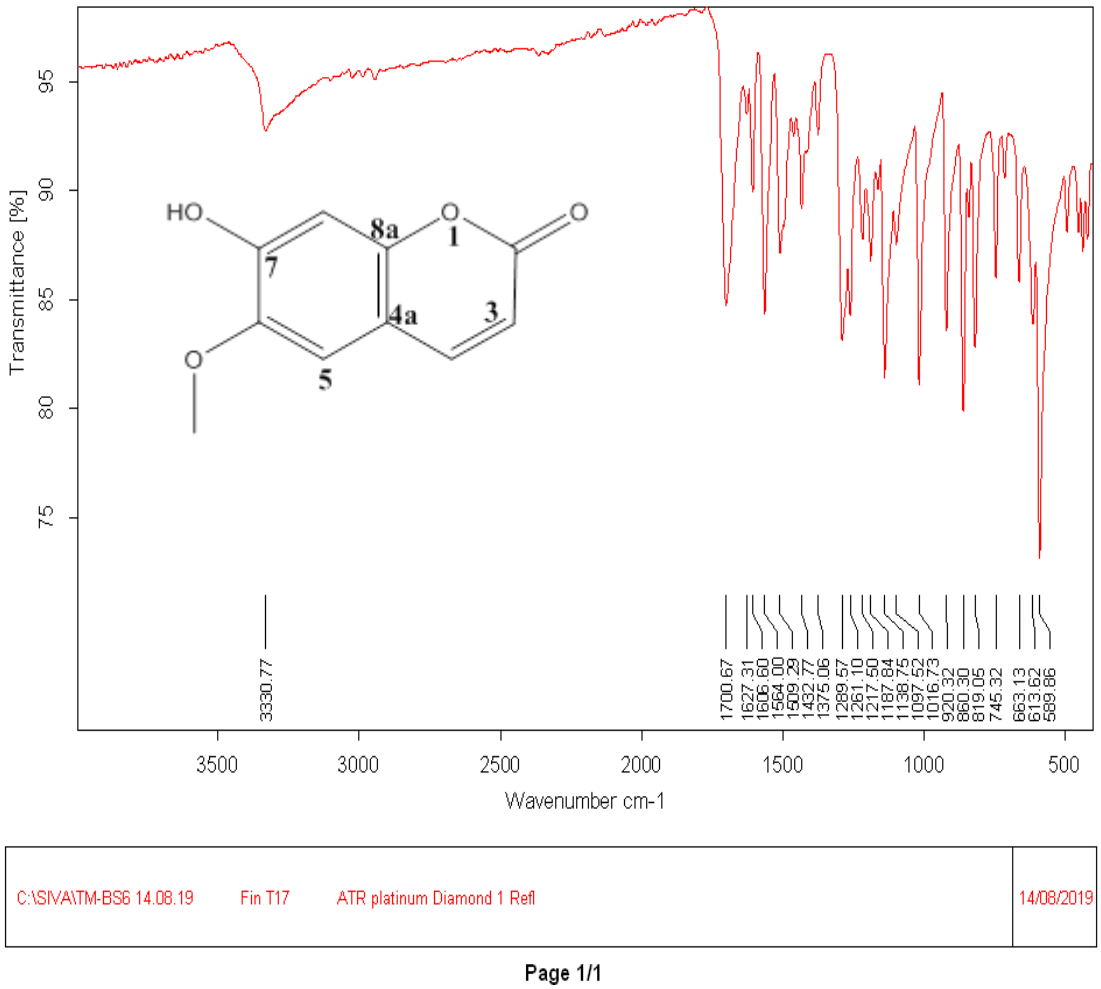


Figure 4.3: Infrared spectrum (IR) of BS6

Table 4.23: IR frequency of BS6

S/N	IR Frequency (cm^{-1})	Functional group
1	3330. 77	OH – stretching vibration of phenol
2	1700. 67	Carbonyl group
3	1627. 31, 1606.60	Aromatics stretching (C=C)
4	1564, 1509.29, 1432. 77	Benzene ring
5	1375.06	C-H methyl group bending
6	1187. 84	C-O group
7	745. 32	Ortho – disubstituted benzene ring

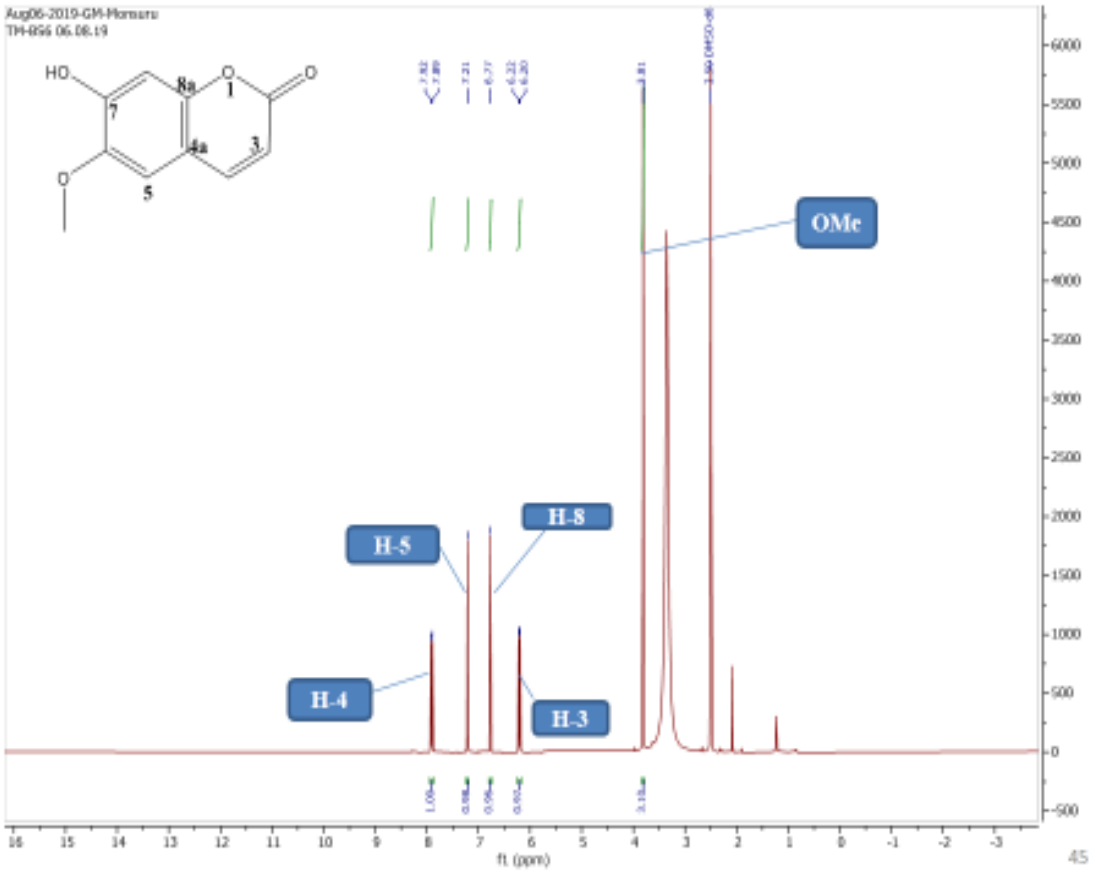


Fig. 4.4: Proton NMR (^{1}H-NMR) of BS6

Table 4.24: ^{1}H - NMR of BS6

Proton Number	Chemical shift of H-NMR (δ)	Coupling constant (J_{HZ})
H-3	6.23	9.41
H-4	7.91	9.47
H-5	7.21	
H-8	6.78	
H- OMe	3.82	

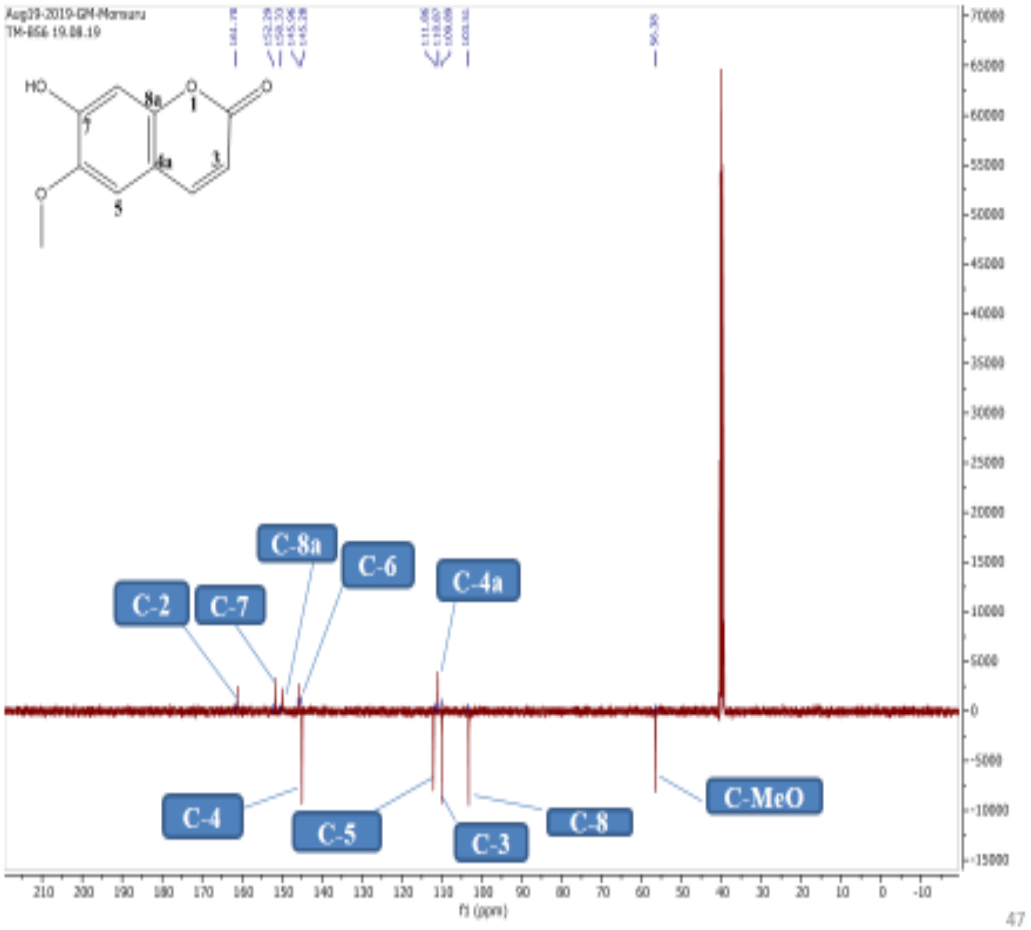


Figure 4.5: ^{13}C-NMR (Attached Proton Test (APT)) spectrum for BS6

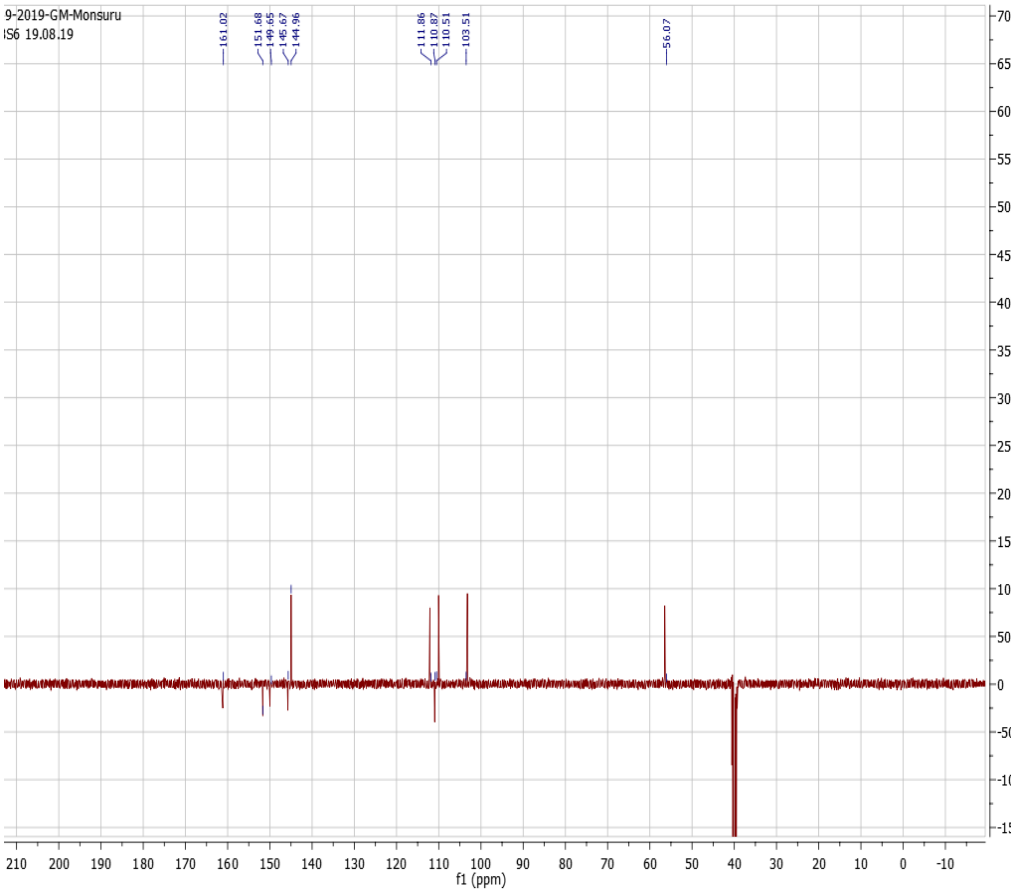


Figure 4.6: Inverted ^{13}C-NMR (APT) spectrum for BS6

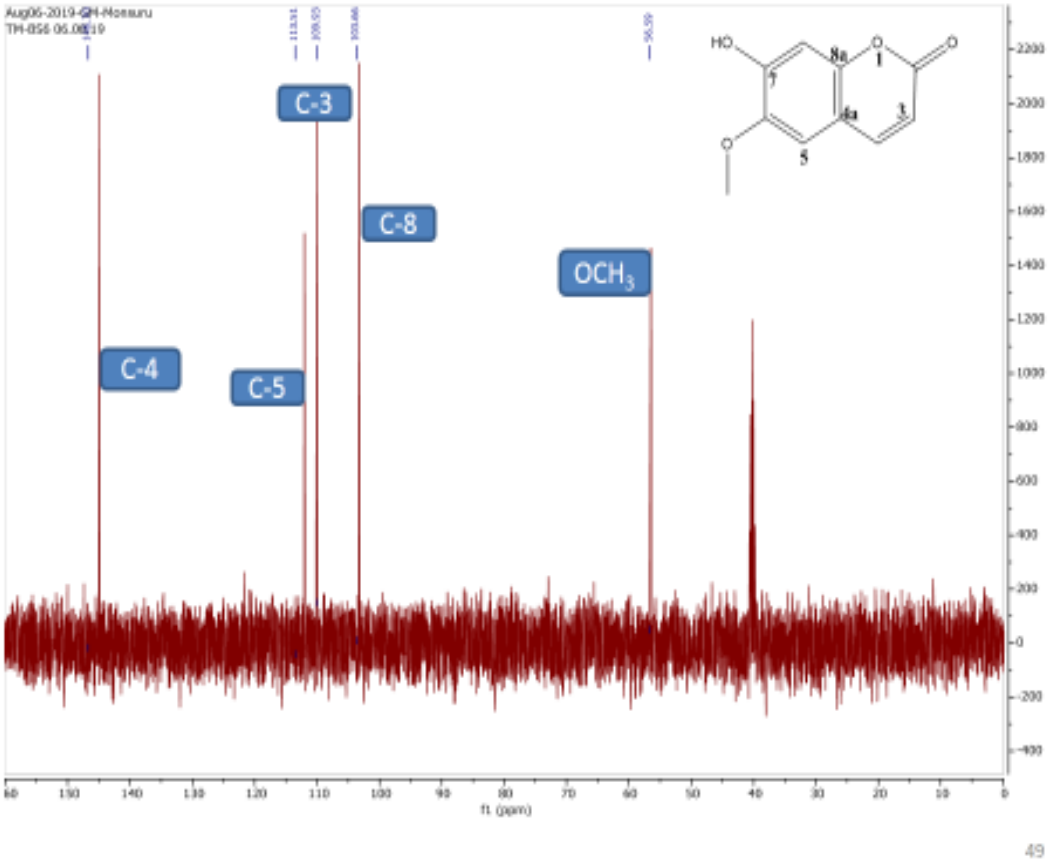


Figure 4.7: Distortionless Enhancement of Polarization Transfer (DEPT) 135 of BS6

Table 4.25: ^{13}C - NMR of BS6

Carbon Number	Multiplicity (DEPT)	Chemical shift of 13C-NMR (δ)
C-2	C	161.82
C-3	CH	111.16
C-4	CH	144.96
C-5	CH	112.10
C-6	C	145.20
C-7	C	149.60
C-8	CH	103.22
C-8a	C	150.20
C-4a	C	111.40
C-OMe	CH_3	56.47

$^{1}H - {}^{1}H$ COSY shows correlation between protons 3 and 4 in the ^{1}H NMR spectrum where protons 3 and 4 are coupled to each other because they form a box pattern symmetric to their diagonal (Fig. 4.8). $^{1}H - {}^{13}C$ HSQC that showed which carbons are directly attached to which protons had it that carbon of methoxy at 56. 43 is directly attached to three protons at 3.82, C3 at 111.16 to proton at 6.21, C8 at 103.22 to proton at 6.78, C5 at 112.10 to proton at 7.22 and C4 at 144.96 is directly attached to proton at 7.91 (Fig. 4.9). $^{1}H - {}^{13}C$ HMBC shows correlations between protons and carbons that are separated by multiple bonds (Fig. 4.10). C2 at 161.13 correlating to H-4 at 7.91, C4 at 143.06 correlating to H-5 at 7.21, C6 at 145.20 correlating to H-Methoxy (H-OMe) at 3.82, C6 also correlating to H-8 at 6.78. NOESY spectrum showed resonance that were spacially close, it is similar to COSY experiment (Fig. 4.11). The High Resolution Time of Flight Mass Spectrum of BS6 (Fig. 4.12) showed the molecular mass at m/z 192 which was 215 [M + Na] which is diagnostic peak of scopoletin to molecular formula $C_{10}H_8O_4$.

4.6.3 Confirmation of BS6 from reported data

Structure of BS6 (Fig. 4.13) was also confirmed by comparing IR, ^{1}H NMR and ^{13}C NMR data to the reported data of scopoletin isolated from *Cardiospermum corindum L*. from Sapindaceae family (Silva *et al*., 2014), *Ipomoea digitata* from Convolvulaceae family (Khan and Hossain, 2015) and *Macaranga gigantifolia* (Euphorbiaceae) (Darmawan, *et al*. 2012) as shown in Table 4.28.

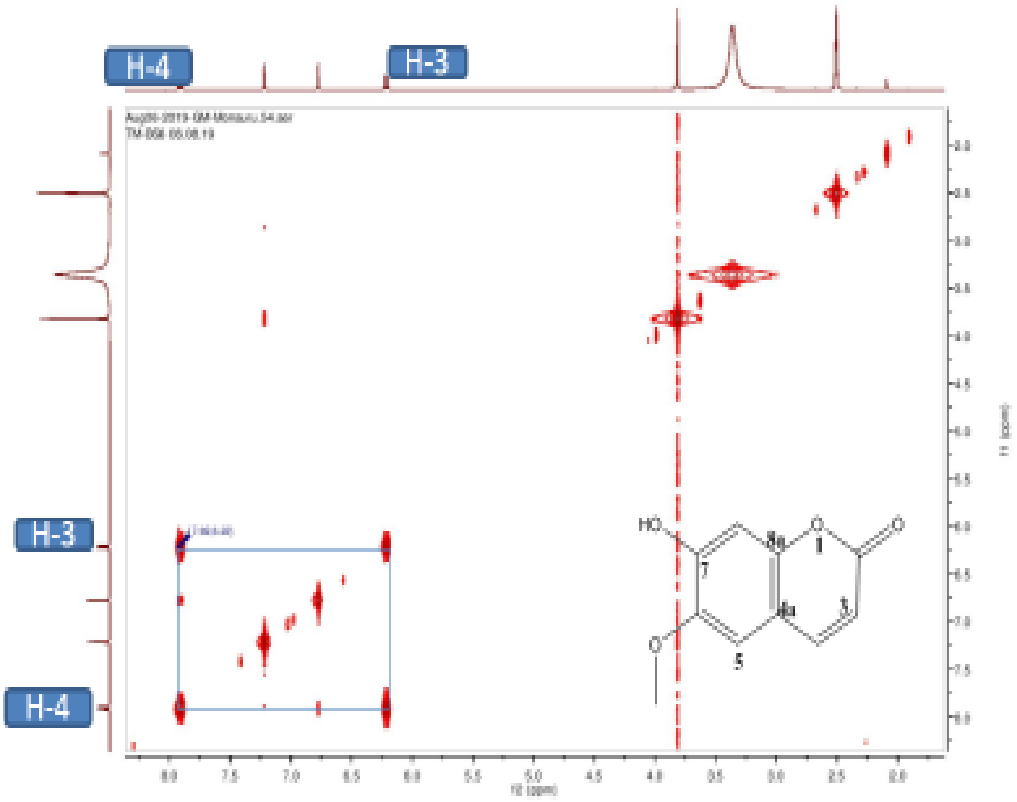


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Figure 4.8: ^{1}H – ^{1}H Correlation Spectroscopy (^{1}H – ^{1}H COSY) of BS6

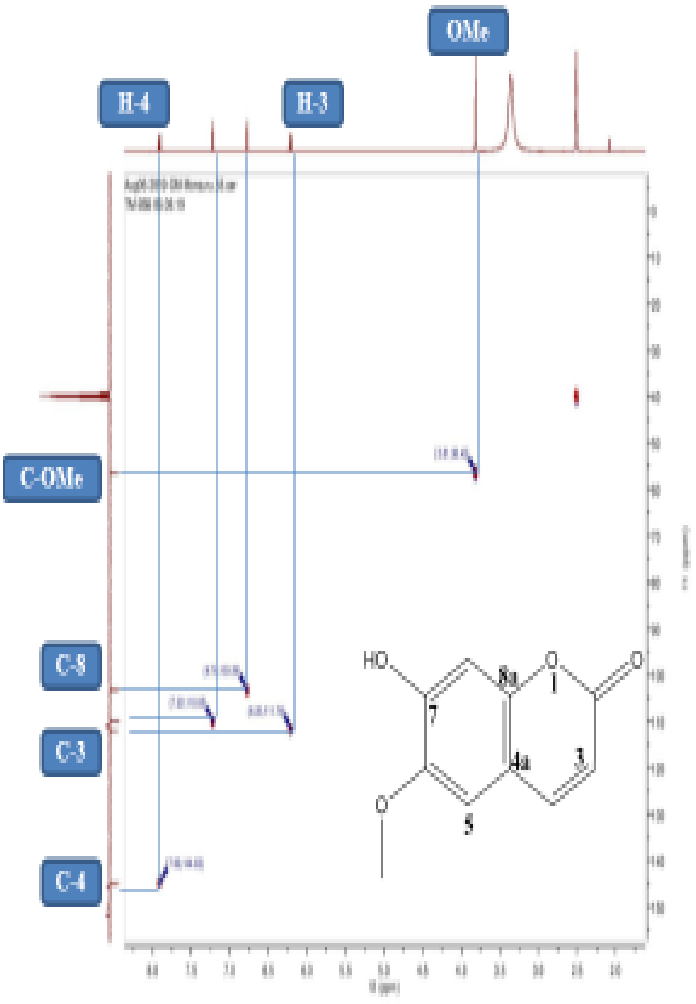

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Figure 4.9: ^{1}H - ^{13}C Heteronuclear Single Quantum Coherence Spectroscopy (HSQC) of BS6

Table 4.26: NMR Spectra of BS6 (HSQC)

Carbon Number	Multiplicity (DEPT)	Chemical shift of 13C-NMR (δ)	Chemical shift of H-NMR (δ)	Coupling constant (J_{HZ})
C-2	C	161.82	-	
C-3	CH	111.16	6.21	9.41
C-4	CH	144.96	7.91	9.47
C-5	CH	112.10	7.22	
C-6	C	145.20	-	
C-7	C	149.60	-	
C-8	CH	103.22	6.78	
C-8a	C	150.20	-	
C-4a	C	111.40	-	
C-OMe	CH_3	56.47	3.82	

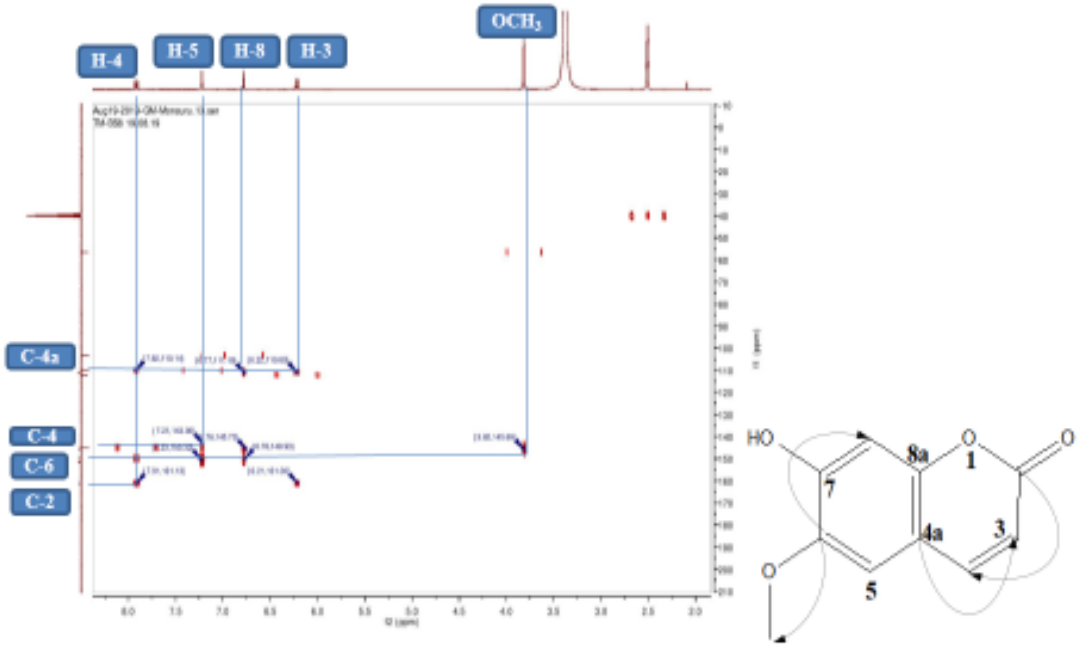


Figure 4.10: ^{1}H - ^{13}C Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC) of BS6

Table 4.27: NMR Spectra of BS6 (HMBC)

Carbon Number	Chemical shift of 13C-NMR (δ)	Proton Number	Chemical shift of H-NMR (δ)
C-2	161.13	H-4	7.91
C-4	143.06	H-5	7.21
C-4a	110.60	H-3	6.23
C-6	145.20	H- OMe	3.82
C-6	145.20	H-8	6.78

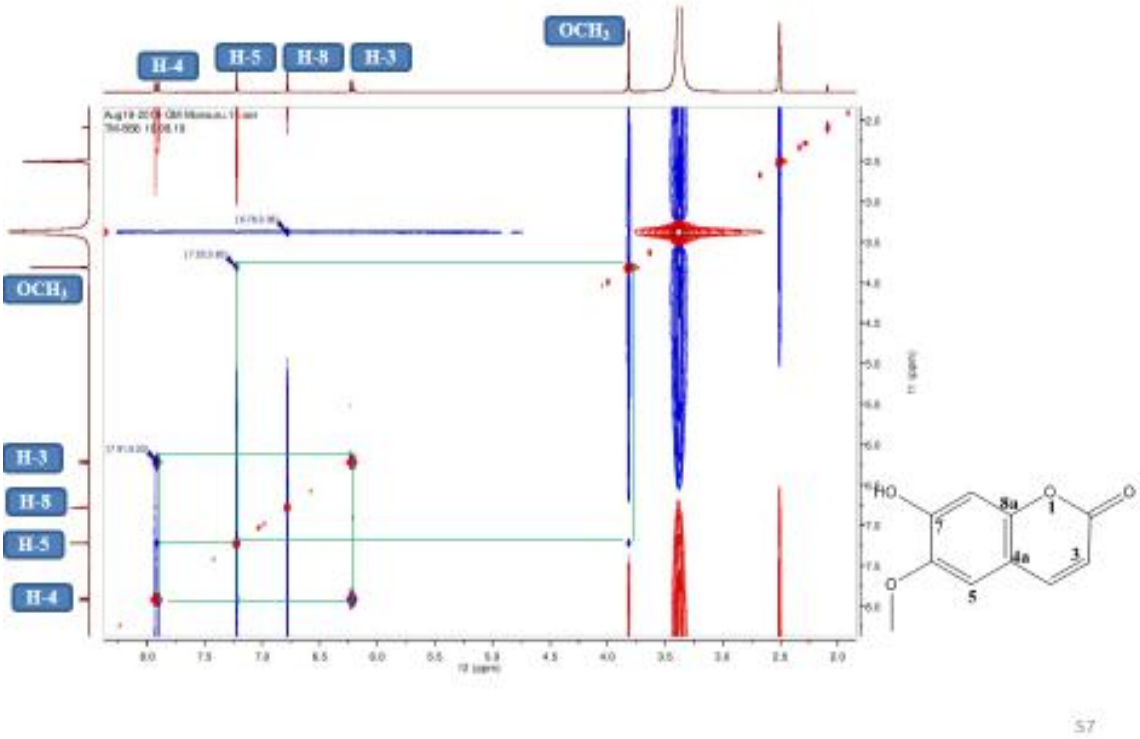


Figure 4.11: $^{1}H - ^{1}H$ Nuclear Overhauser Effect Spectroscopy (NOESY) spectrum of BS6

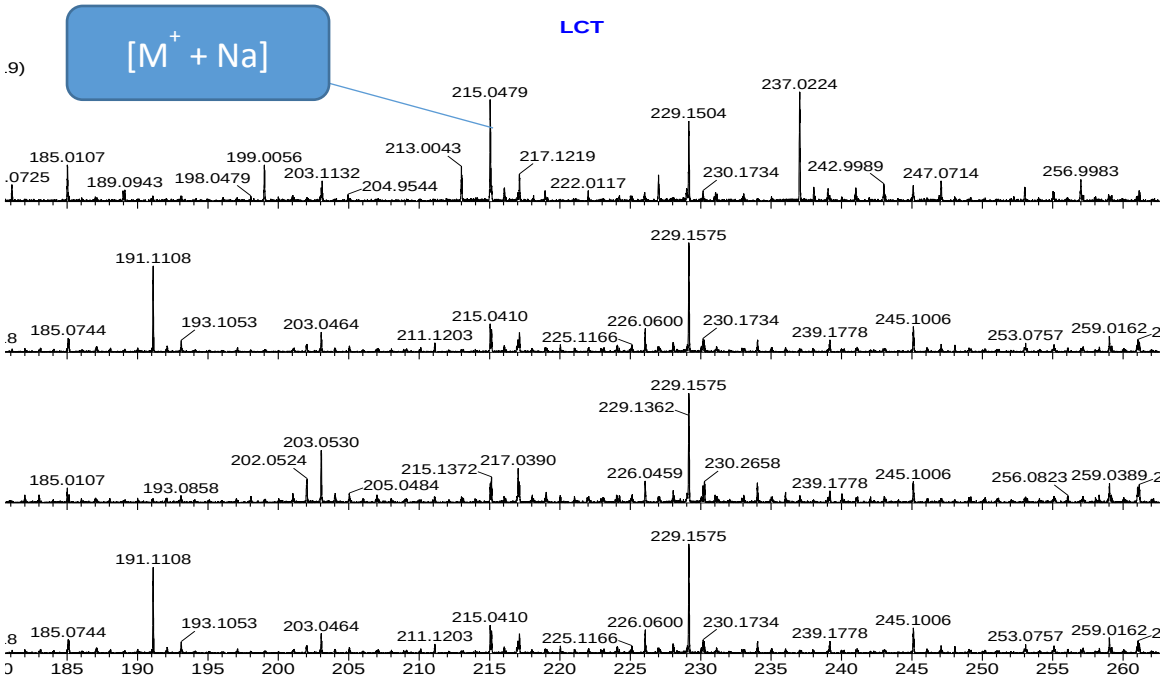


Figure 4.12: High Resolution Time of Flight Mass Spectrum (TOF-ESI-MS) of BS6

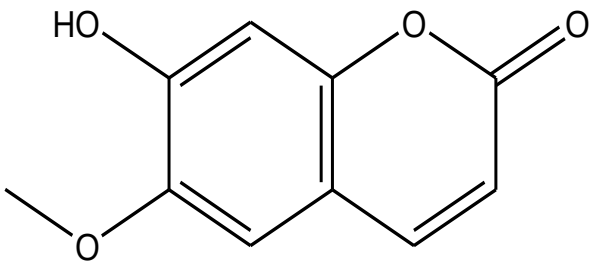


Figure 4.13: Chemical structure of Scopoletin

Table 4.28: Comparison of BS6 data with literature

Carbon Number	Multiplicity (DEPT)	Chemical shift of 13C-NMR (δ)			Chemical shift of H-NMR (δ)			Coupling constant (J_{HZ})		
		Bs	Id	Mg	Bs	Id	Mg	Bs	Id	Mg
C-2	C	161.82	162.60	160.8	-					
C-3	CH	111.16	108.10	113.3	6.21(d,^{1}H)	6.20(d,^{1}H)	6.25(d,^{1}H)	9.41	9.20	9.75
C-4	CH	144.96	144.20	144.7	7.91(d,^{1}H)	7.70(d,^{1}H)	7.84(d,^{1}H)	9.47	9.20	9.75
C-5	CH	112.10	111.60	109.9	7.22(s,^{1}H)	6.90(s,^{1}H)	7.19(s,^{1}H)			
C-6	C	145.20	145.30	146.0	-					
C-7	C	149.60	151.00	151.9	-					
C-8	CH	103.22	102.90	103.7	6.78(s,^{1}H)	6.80(s,^{1}H)	6.79(s,^{1}H)			
C-8a	C	150.20	149.80	151.2	-					
C-4a	C	111.40	110.90	112.1	-					
C-OMe	CH_3	56.47	55.80	56.7	3.82(s,^{3}H)	3.88(s,^{3}H)	3.90(s,^{3}H)			

Bs – *Blighia sapida*. Id - *Ipomoea digitata* (Khan and Hossain, 2015), Mg - *Macaranga gigantifolia* (Darmawan, *et al*. 2012)

4.7 Biological assessments of essential oils

4.7.1 Antimicrobial assessments of essential oils

4.7.1.1 Antimicrobial activities of *Blighia sapida* essential oils

Essential oils from leaf, leaf-stalk, stem-bark, root, fruit-pulp, fruit-husk and seed of *Blighia sapida* showed antibacterial and antifungal activities (Table 4.29) although, lower than Gentamicin and Tioconazole which are standard drugs. Among these essential oils, *Blighia sapida* root essential oil is the most potent against all the ten test micro organisms, followed by stem-bark, leaf-stalk, seed, fruit-pulp, leaf and then fruit-husk. Leaf and leaf-stalk oils are also of high potency against *S. aureus*.

Leaves essential oil displayed antimicrobial activities against all the test microorganisms except *Salmonellae typhi* and *Klebisiella pneumoniae*; this essential oil exhibited antimicrobial activities against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 6.25-100 mg/mL by 10-24 mm, 12.5-100 mg/mL by 10-16 mm, 25-100 mg/mL by 10-16 mm, 25-100 mg/mL by 12-18 mm, 25-100 mg/mL by 10-16 mm, 50-100 mg/mL by 10-12 mm, 50-100 mg/mL by 10-12 mm and 50-100 mg/mL by 10-12 mm Diameters of Inhibition Zone (DIZ) respectively.

Leaf-stalk, stem-bark, root, fruit-pulp and seed essential oils showed growth inhibitory effects on all the ten test organisms. Leaf-stalk exhibited inhibition against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 6.25-100 mg/mL by 10-24 mm, 6.25-100 mg/mL by 10-20 mm, 12.5-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-16 mm, 50-100 mg/mL by 10-12 mm, 50-100 mg/mL by 10-12 mm, 12.5-100 mg/mL by 10-18 mm, 25-100 mg/mL by 10-14 mm, 50-100 mg/mL by 10-12 mm and 50-100 mg/mL by 10-12 mm DIZ respectively.

Table 4.29: Antimicrobial activities of *Blighia sapida* essential oils

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
1	Leaf	100	24	16	16	18	n.a.	n.a.	14	12	12	12
		50	20	14	12	14	n.a.	n.a.	12	10	10	10
		25	18	12	10	12	n.a.	n.a.	10	n.a.	n.a.	n.a.
		12.5	14	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	Leaf-stalk	100	24	20	18	16	12	12	18	14	12	12
		50	20	18	14	14	10	10	14	12	10	10
		25	18	14	12	12	n.a.	n.a.	12	10	n.a.	n.a.
		12.5	14	12	10	10	n.a.	n.a.	10	n.a.	n.a.	n.a.
		6.25	10	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. - No Activity, - -Not Applicable

Table 4.29: Antimicrobial activities of *Blighia sapida* essential oils Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
3	Stem-bark	100	20	18	18	20	22	18	16	16	16	14
		50	18	16	16	18	18	16	14	14	14	12
		25	14	14	14	14	14	12	12	12	12	10
		12.5	12	12	12	12	12	10	10	10	10	n.a.
		6.25	10	10	10	10	10	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	Root	100	24	24	22	24	26	24	18	18	16	16
		50	20	20	18	20	22	20	16	16	14	14
		25	18	16	16	18	18	18	14	14	12	12
		12.5	14	14	14	14	14	14	12	12	10	10
		6.25	12	10	10	12	10	12	10	10	n.a.	n.a.
		3.125	10	n.a.	n.a.	10	n.a.	10	n.a.	n.a.	n.a.	n.a.

n.a. - No Activity, - -Not Applicable

Table 4.29: Antimicrobial activities of *Blighia sapida* essential oils Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
5	Fruit-pulp	100	20	18	18	14	16	12	14	12	12	12
		50	18	16	16	12	12	10	12	10	10	10
		25	14	12	14	10	10	n.a.	10	n.a.	n.a.	n.a.
		12.5	12	10	12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	10	n.a.	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Fruit-husk	100	16	16	14	12	12	12	14	14	n.a.	n.a.
		50	14	14	12	10	10	10	12	12	n.a.	n.a.
		25	12	12	10	n.a.	n.a.	n.a.	10	10	n.a.	n.a.
		12.5	10	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. - No Activity, - -Not Applicable

Table 4.29: Antimicrobial activities of *Blighia sapida* essential oils Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
7	Seed	100	20	18	16	18	18	18	16	14	14	14
		50	18	14	14	14	14	16	14	12	12	12
		25	14	12	12	12	12	14	12	10	10	10
		12.5	12	10	10	10	10	10	10	n.a.	n.a.	n.a.
		6.25	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Solvent	Hexane		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	DMSO		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Standard	Gentamicin	10 µg/mL	40	38	38	40	38	40	-	-	-	-
	Tioconazole	10 µg/mL	-	-	-	-	-	-	28	28	26	28

n.a. - No Activity, - -Not Applicable

Stem-bark essential oil displayed antimicrobial activities at 6.25-100 mg/mL by 10-20 mm, 6.25-100 mg/mL by 10-18 mm, 6.25-100 mg/mL by 10-18 mm, 6.25-100 mg/mL by 10-20 mm, 6.25-100 mg/mL by 10-22 mm, 12.5-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-16 mm, 12.5-100 mg/mL by 10-16 mm, 12.5-100 mg/mL by 10-16 mm and 25-100 mg/mL by 10-14 mm DIZ respectively. Essential oil from root displayed antimicrobial activities at 3.125-100 mg/mL by 10-24 mm, 6.25-100 mg/mL by 10-24 mm, 6.25-100 mg/mL by 10-22 mm, 3.125-100 mg/mL by 10-24 mm, 3.125-100 mg/mL by 10-26 mm, 6.25-100 mg/mL by 12-26 mm, 6.25-100 mg/mL by 10-18 mm, 6.25-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-16 mm and 12.5-100 mg/mL by 10-16 mm DIZ respectively.

Fruit-pulp essential oil showed antimicrobial activities at 6.25-100 mg/mL by 10-20 mm, 12.5-100 mg/mL by 10-18 mm, 6.25-100 mg/mL by 10-18 mm, 25-100 mg/mL by 10-14 mm, 25-100 mg/mL by 10-16 mm, 50-100 mg/mL by 10-12 mm, 25-100 mg/mL by 10-14 mm, 50-100 mg/mL by 10-12 mm, 50-100 mg/mL by 10-12 mm and 50-100 mg/mL by 10-12 mm DIZ respectively. Fruit-husk essential oil displayed growth inhibitory effects on all the organisms except *Rhizhopus stolonifer* and *Penicillium notatum*; this essential oil exhibited antimicrobial activities against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonellae typhi, Candida albicans* and *Aspergillus niger* at 12.5-100 mg/mL by 10-16 mm, 12.5-100 mg/mL by 10-16 mm, 25-100 mg/mL by 10-14 mm, 50-100 mg/mL by 10-12 mm, 50-100 mg/mL by 10-12 mm, 50-100 mg/mL by 10-12 mm, 25-100 mg/mL by 10-14 mm and 25-100 mg/mL by 10-14 mm DIZ respectively. Essential oil from seed displayed antimicrobial activities at 6.25-100 mg/mL by 10-20 mm, 12.5-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-16 mm, 12.5-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-16 mm, 25-100 mg/mL by 10-14 mm, 25-100 mg/mL by 10-14 mm and 25-100 mg/mL by 10-14 mm DIZ respectively.

4.7.1.2 Antimicrobial activities of *Lecaniodiscus cupanioides* essential oils

Essential oils from leaf, leaf-stalk, stem-bark and root of *Lecaniodiscus cupanioides* showed antibacterial and antifungal activities although, lower than Gentamicin and Tioconazole. Among these essential oils, *Lecaniodiscus cupanioides* leaves stalk essential oil is the most potent against all test micro organisms, followed by stem-bark

which is the next in antibacterial activities except on *Klebisiella pneumoniae* followed by leaf while leaf is the next after leaf-stalk in antibacterial activity on *Klebisiellae pneumonae* and antifungal activities followed by stem-bark as shown in Table 4.30. Root essential oil is the least potent against all the ten test micro organisms and there is no activity at all on *Klebisiella pneumoniae*.

Leaves essential oil showed antimicrobial activities against all ten test organisms: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 3.125-100 mg/mL by 10-20 mm, 6.25-100 mg/mL by 10-17 mm, 6.25-100 mg/mL by 10-19 mm, 6.25-100 mg/mL by 10-19 mm, 6.25-100 mg/mL by 10-17 mm, 6.25-100 mg/mL by 10-17 mm, 6.25-100 mg/mL by 10-17 mm, 12.5 – 100 mg/mL by 10-18 mm, 12.5 – 100 mg/mL by 10 - 17 and 12.5-100 mg/mL by 10-18 mm Diameters of Inhibition Zone (DIZ) respectively.

Leaf-stalk essential oil exhibited antimicrobial activities against all ten test organisms which were *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 3.125-100 mg/mL by 12-28 mm, 3.125-100 mg/mL by 10-26 mm, 3.125-100 mg/mL by 10-24 mm, 3.125-100 mg/mL by 10-25 mm, 3.125-100 mg/mL by 10-24 mm, 3.125-100 mg/mL by 10-25 mm, 3.125-100 mg/mL by 10-20 mm, 3.125-100 mg/mL by 10-19 mm, 3.125-100 mg/mL by 10 - 18 and 3.125-100 mg/mL by 10-19 mm Diameters of Inhibition Zone (DIZ) respectively.

Table 4.30: Antimicrobial activities of *Lecaniodiscus cupanioides* essential oils

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
1	Leaf	100	20	17	19	19	17	17	17	18	17	17
		50	18	16	16	17	15	14	16	15	14	14
		25	15	13	14	15	13	13	14	13	12	12
		12.5	14	11	12	12	11	12	12	10	10	10
		6.25	12	10	10	10	10	10	10	n.a.	n.a.	n.a.
		3.125	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	Leaf-stalk	100	28	26	24	25	24	25	20	19	18	19
		50	24	23	21	22	21	22	18	18	17	17
		25	21	20	18	18	18	19	16	16	15	15
		12.5	18	17	16	15	15	17	14	14	13	13
		6.25	14	13	14	13	12	13	12	12	12	11
		3.125	12	10	10	10	10	11	10	10	10	10

n.a. - No Activity, - -Not Applicable

Table 4.30: Antimicrobial activities of *Lecaniodiscus cupanioides* essential oils Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
3	Stem-bark	100	23	20	19	18	17	19	18	16	15	15
		50	20	18	18	16	14	17	14	14	13	13
		25	17	16	16	14	12	14	12	12	10	10
		12.5	15	14	14	12	10	12	10	10	n.a.	n.a.
		6.25	13	12	12	10	n.a.	10	n.a.	n.a.	n.a.	n.a.
		3.125	10	10	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
4	Root	100	18	17	15	15	n.a.	16	15	13	13	15
		50	16	14	13	13	n.a.	13	13	10	10	13
		25	13	12	10	10	n.a.	10	10	n.a.	n.a.	10
		12.5	10	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Solvent	Hexane		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	DMSO		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Gentamicin	10 µg/mL	38	39	38	38	40	38	-	-	-	-
	Tioconazole	10 µg/mL	-	-	-	-	-	-	27	27	28	28

n.a. - No Activity, - -Not Applicable

Essential oil from stem-bark displayed antimicrobial activities against all the ten organisms at 3.125-100 mg/mL by 10-23 mm, 3.125-100 mg/mL by 10-20 mm, 3.125-100 mg/mL by 10-19 mm, 6.25-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-17 mm, 6.25-100 mg/mL by 10-19 mm, 12.5-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-16 mm, 25-100 mg/mL by 10 - 15 and 25-100 mg/mL by 10-15 mm Diameters of Inhibition Zone (DIZ) respectively. Root essential oil exhibited antimicrobial activities against all ten test organisms except *Klebisiella pneumoniae*. It displayed activities against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 12.5-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-17 mm, 25-100 mg/mL by 10-15 mm, 25-100 mg/mL by 10-15 mm, 25-100 mg/mL by 10-16 mm, 25-100 mg/mL by 10-15 mm, 50-100 mg/mL by 10–13, 50-100 mg/mL by 10-13 and 25-100 mg/mL by 10-15 mm Diameters of Inhibition Zone (DIZ) respectively.

4.7.1.3 Antimicrobial activities of *Paullinia pinnata* essential oils

Essential oils from leaf, leaf-stalk, stem-bark and root of *Paullinia pinnata* displayed antibacterial and antifungal activities (Table 4.43) with their activities lower than Gentamicin and Tioconazole. *Paullinia pinnata* stem-bark essential oil is the most active among all, followed by root followed by leaf-stalk and leaf is not far from the activities of leaf-stalk, although, leaf is not active at all against *Rhizhopus stolonifer*. Essential oil from leaves showed antimicrobial activities against all ten test organisms except *Rhizhopus stolonifer,* the activities were against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger* and *Penicillium notatum* at 6.25-100 mg/mL by 10-19 mm, 25-100 mg/mL by 10-17 mm, 12.5-100 mg/mL by 10-17 mm, 25-100 mg/mL by 10-15 mm, 25-100 mg/mL by 10-17 mm, 25-100 mg/mL by 10-14 mm, 50-100 mg/mL by 10-13 mm, 50 – 100 mg/mL by 10-13 mm and 50-100 mg/mL by 10-13 mm Diameters of Inhibition Zone (DIZ) respectively.

Table 4.31: Antimicrobial activities of *Paullinia pinnata* essential oils

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
1	Leaf	100	19	17	17	15	17	14	13	13	n.a.	13
		50	17	15	15	13	15	12.	10	10	n.a.	10
		25	15	12	13	10	13	10	n.a.	n.a.	n.a.	n.a.
		12.5	13	n.a.	10	n.a.	11	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	10	n.a.	n.a.	n.a.	10	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	Leaf - stalk	100	17	18	19	15	17	17	14	15	15	14
		50	15	15	16	13	14	14	12	13	13	12
		25	13	13	14	10	12	12	10	10	10	10
		12.5	11	12	12	n.a.	10	10	n.a.	n.a.	n.a.	n.a.
		6.25	10	10	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. - No Activity, - -Not Applicable

Table 4.31: Antimicrobial activities of *Paullinia pinnata* essential oils Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
3	Stem-bark	100	26	25	28	24	24	26	20	19	18	18
		50	23	21	23	21	21	24	18	18	16	17
		25	19	18	19	18	18	20	16	16	14	15
		12.5	14	16	17	15	15	17	14	14	12	13
		6.25	12	14	14	13	13	14	12	12	11	12
		3.125	10	12	10	10	10	11	10	10	10	10
4	Root	100	19	17	19	18	16	19	17	18	17	17
		50	17	15	18	16	14	17	14	14	14	15
		25	14	12	16	14	12	15	12	12	12	12
		12.5	12	10	14	12	10	12	10	10	10	10
		6.25	10	n.a.	12	10	n.a.	10	n.a.	n.a.	n.a.	n.a.
		3.125	n.a.	n.a.	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Solvent	Hexane		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	DMSO		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	Gentamicin	10 µg/mL	38	39	39	40	40	39	-	-	-	-
	Tioconazole	10 µg/mL	-	-	-	-	-	-	28	27	27	26

n.a. - No Activity, - -Not Applicable

Leaf-stalk essential oil showed antimicrobial activities against all ten test organisms at 6.25-100 mg/mL by 10-17 mm, 6.25-100 mg/mL by 10-18 mm, 6.25-100 mg/mL by 10-19 mm, 25-100 mg/mL by 10-15 mm, 12.5-100 mg/mL by 10-17 mm, 12.5-100 mg/mL by 10-17 mm, 25-100 mg/mL by 10-14 mm, 25-100 mg/mL by 10-15 mm, 25-100 mg/mL by 10 - 15 and 25-100 mg/mL by 10-14 mm Diameters of Inhibition Zone (DIZ) respectively. Stem-bark essential oil exhibited antimicrobial activities against all the ten organisms at 3.125-100 mg/mL by 10-26 mm, 3.125-100 mg/mL by 10-25 mm, 3.125-100 mg/mL by 10-28 mm, 3.125-100 mg/mL by 10-24 mm, 3.125-100 mg/mL by 10-24 mm, 3.125-100 mg/mL by 10-26 mm, 3.125-100 mg/mL by 10-20 mm, 3.125-100 mg/mL by 10-19 mm, 3.1255-100 mg/mL by 10 - 18 and 3.125-100 mg/mL by 10-18 mm Diameters of Inhibition Zone (DIZ) respectively. Essential oil of root exhibited antimicrobial activities against all ten test organisms at 6.25-100 mg/mL by 10-19 mm, 12.5-100 mg/mL by 10-17 mm, 3.125-100 mg/mL by 10-19 mm, 6.25-100 mg/mL by 10-18 mm, 12.5-100 mg/mL by 10-16 mm, 6.25-100 mg/mL by 10-19 mm, 12.5-100 mg/mL by 10 – 17, 12.5-100 mg/mL by 10 – 18, 12.5-100 mg/mL by 10 - 17 and 12.5-100 mg/mL by 10-17 mm Diameters of Inhibition Zone (DIZ) respectively.

Antibacterial and antifungal activities of essential oils of three (3) studied Sapindaceae plants were established which support ethno-medicinal uses of these Sapindaceae plants.

4.7.2 Antioxidant assessments of essential oils

4.7.2.1 Antioxidant activity of *Blighia sapida* essential oils

Antioxidant activities of essential oils of *Blighia sapida* leaf (BsL), leaf stalk (BsLs), stem bark (BsSb), root (BsR) and fruit husk (BsFh) were compared with known antioxidants: α-tocopherol (TP), butylated hydroxyanisole (BHA) and ascorbic acid (ASA) as shown in Fig. 4.14. The decrease in absorption at 517 nm shows that the samples can scavenge free radical with hydrogen donating ability. Using % inhibition for the concentrations between 62.5 – 1000 µg/mL, the scavenging effect of the *Blighia sapida* essential oils and standards on the DPPH radical decreased in the order of BHA > ASA > BsSb> BsL > BsLs > BsFh > TP > BsR and were 95% - 97%, 81% - 96%, 23% - 71%, 21% - 67%, 22% - 60%, 20% - 44%, 28% - 38% and 19% - 30% respectively, *Blighia sapida* stem bark had highest % inhibition as reported by Oloyede *et al*., (2022) on their study on leaves, stem bark and root of *Blighia sapida*.

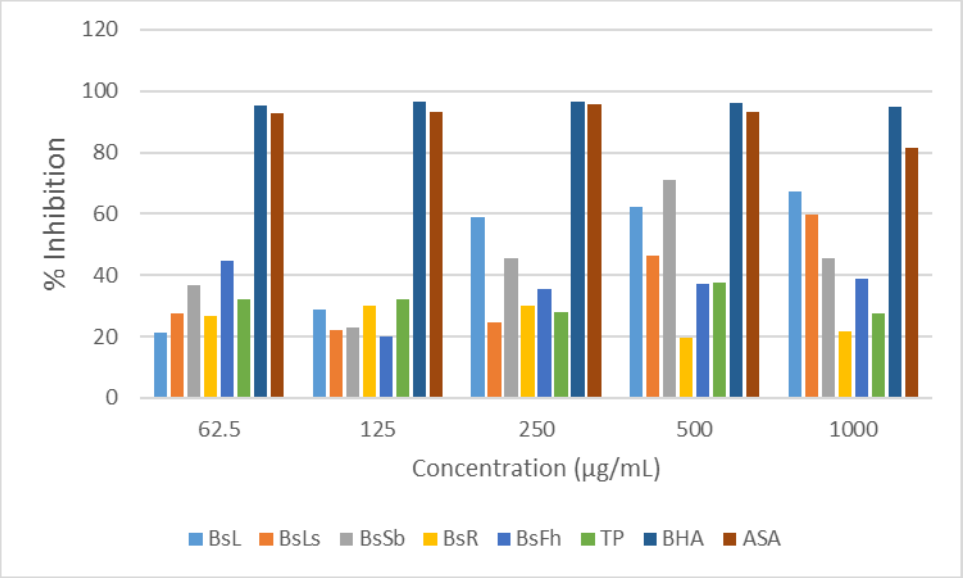


Figure 4.14: DPPH radical scavenging activity of the essential oils of *Blighia sapida*.

Blighia sapida leaf (BsL), *Blighia sapida* leaf-stalk (BsLs), *Blighia sapida* stem-bark (BsSb), *Blighia sapida* root (BsR), *Blighia sapida* fruit-husk (BsFh), α-tocopherol (TP), Butylated hydroxyanisole (BHA) and Ascorbic acid (AsA)

The inhibitory concentration (IC_{50}) was in the order of BHA > ASA> BsL > BsLs > BsSb >BsFh > TP > BsR with IC_{50} values of 0.95 µg/ mL, 0.97 µg/ mL, 0.98 µg/ mL, 0.99 µg/ mL, 1.00 µg/ mL, 1.01 µg/ mL, 1.02 µg/ mL and 1.03 µg/ mL respectively. *Blighia sapida* leaf had IC_{50} value of 0.98 µg/ mL, so it showed the highest activity among *Blighia sapida* oils though lower than butylated hydroxyanisole (0.95 µg/ mL) and ascorbic acid (0.97 µg/ mL) which made it most potent as antioxidant; followed by *Blighia sapida* leaf-stalk with IC_{50} of 0.99 µg/ mL; *Blighia sapida* stem-bark (1.00 µg/ mL) and fruit-husk (1.01 µg/ mL) which were more potent than α-tocopherol (1.02 µg/ mL) and *Blighia sapida* root (1.03 µg/ mL) which was the least potent among all. This showed antioxidant activities of *Blighia sapida* oils at IC_{50} of 0.98 - 1.03 µg/ mL compared with control at IC_{50} of 0.95 - 1.02 µg/ mL.

4.7.2.2 Antioxidant activity of *Lecaniodiscus cupanioides* essential oils

Antioxidant activities of essential oils of *Lecaniodiscus cupanioides* leaf (LcL), leaf stalk (LcLs), stem bark (LcSb) and root (LcR) were also compared with the known antioxidants as shown in Fig. 4.15. Decrease in absorption at 517 nm shows that the samples except the root can scavenge free radical with hydrogen donating ability. Scavenging effect of *Lecaniodiscus cupanioides* essential oils and standards on DPPH radical decreased in the order of BHA > ASA > LcL > TP > LcSb >LcLs and were 95% - 97%, 81% - 96%, 24% - 53%, 28% - 38%, 7% - 19% and 3% - 8% respectively while IC_{50} was in the order of BHA > ASA > TP >LcL > LcSb >LcLs with IC_{50} values of 0.95 µg/ mL, 0.97 µg/ mL, 1.02 µg/ mL, 1.03 µg/ mL, 1.04 µg/ mL and 1.05 µg/ mL respectively. *Lecaniodiscus cupanioides* leaf had IC_{50} value of 1.03 µg/ mL, so it showed the highest activity among *Lecaniodiscus cupanioides* oils though lower than butylated hydroxyanisole (0.95 µg/ mL) and ascorbic acid (0.97 µg/ mL) but close to α-tocopherol (1.02 µg/ mL) which made it most potent; followed by *Lecaniodiscus cupanioides* stem-bark (1.04 µg/ mL), then leaf-stalk (1.05 µg/ mL) and *Lecaniodiscus cupanioides* root had no activity among all. This results showed antioxidant activities of *Lecaniodiscus cupanioides* oils at IC_{50} of 1.03 – 1.05 µg/ mL compared with control at IC_{50} of 0.95 - 1.02 µg/ mL. .

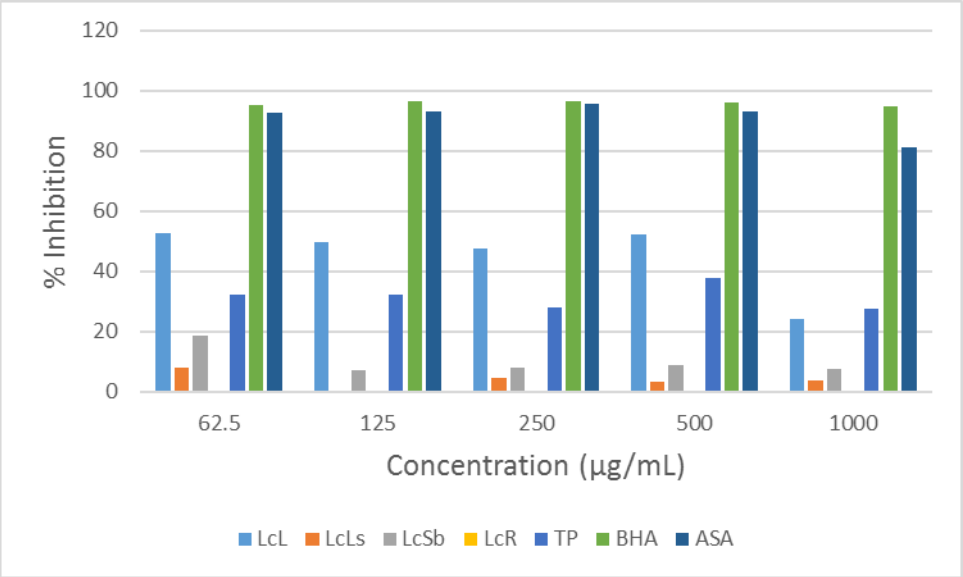


Figure 4.15: DPPH radical scavenging activity of the essential oils of *Lecaniodiscus cupanioides*

Lecaniodiscus cupanioides leaf (LcL), *Lecaniodiscus cupanioides* leaf-stalk (LcLs), *Lecaniodiscus cupanioides* stem-bark (LcSb), *Lecaniodiscus cupanioides* root (LcR), α-tocopherol (TP), Butylated hydroxyanisole (BHA) and Ascorbic acid (AsA).

4.7.2.3 Antioxidant activity of *Paullinia pinnata* essential oils

Comparison of antioxidant activities of essential oils of *Paullinia pinnata* leaf (PpL), leaf stalk (PpLs), stem bark (PpSb) and root (PpR) with known antioxidants was done as shown in Fig. 4.16. The decrease in absorption at 517 nm based on our findings shows that only *Paullinia pinnata* essential oils of leaf and root can scavenge free radical with hydrogen donating ability. Scavenging effect of *Paullinia pinnata* essential oils and standards on DPPH radical decreased in the order of BHA > ASA > PpR > TP > PpL and were 95% - 97%, 81% - 96%, 27% - 43%, 28% - 38% and 19% - 29% respectively while IC_{50} was in the order of BHA > ASA > PpR > TP = PpL with IC_{50} values of 0.95 µg/ mL, 0.97 µg/ mL, 1.01 µg/ mL, 1.02 µg/ mL, 1.02 µg/ mL respectively. *Paullinia pinnata* root had IC_{50} value of 1.01 µg/ mL and it was most potent, it showed the highest activity among *Paullinia pinnata* oils though lower than butylated hydroxyanisole (0.95 µg/ mL) and ascorbic acid (0.97 µg/ mL) but close to α-tocopherol and *Paullinia pinnata* leaf which had the same IC_{50} value (1.02 µg/ mL) while *Paullinia pinnata* leaf-stalk and stem-bark had no activity among all. *Paullinia pinnata* oils showed antioxidant activities at IC_{50} of 1.01 – 1.02 µg/ mL compared with control at IC_{50} of 0.95 - 1.02 µg/ mL.

4.7.2.4 Antioxidant activities of *Blighia sapida, Lecaniodiscus cupanioides* and *Paullinia pinnata* essential oils

Comparing the antioxidant activities of essential oils of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata* with standards using IC_{50} as shown in Fig. 4.17, results showed that two (2) out of the three (3) standards, that is, butylated hydroxyanisole and ascorbic acid used had least IC_{50} values 0.95 and 0.97µg/mL respectively, indicating them as highest in activities because the lower the IC_{50} value, the better the activity while the third standard, α-tocopherol (1.02µg/mL) was competing with the samples of which some the samples had better activity than the α-tocopherol. Among the samples, *Blighia sapida* leaf was most active with IC_{50} 0.98 µg/mL while the least active was *Lecaniodiscus cupanioides* leaf stalk with IC_{50} 1.05 µg/ mL. Antioxidant activities of these three (3) sapindaceae plants were established which could be linked with antioxidant agents detected from their GC-MS results. Therefore, these results support the ethno-medicinal and industrial applications of the three plants.

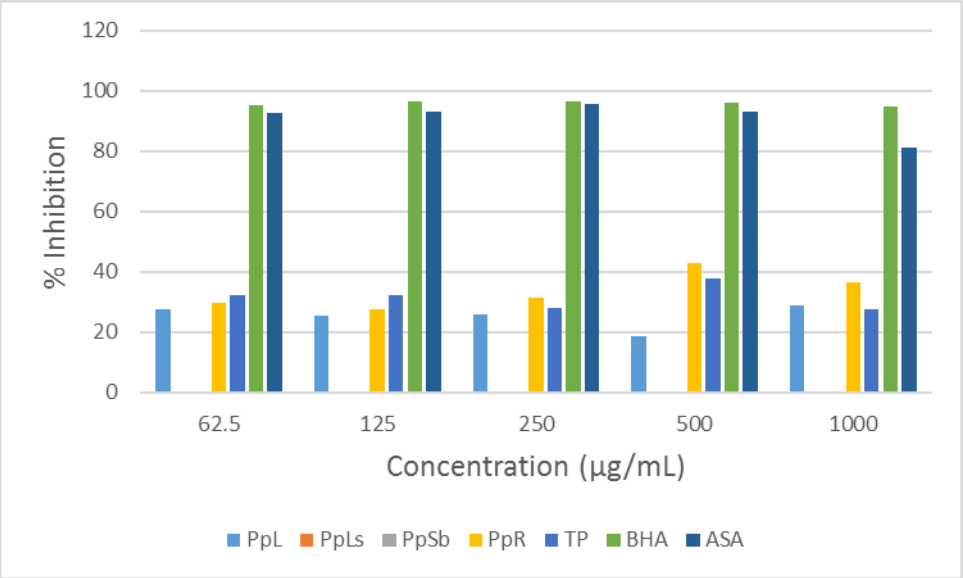


Figure 4.16: DPPH radical scavenging activity of the essential oils of *Paullinia pinnata*

Paullinia pinnata leaf (PpL), *Paullinia pinnata* leaf-stalk (PpLs), *Paullinia pinnata* stem-bark (PpSb), *Paullinia pinnata* root (PpR), α-tocopherol (TP), Butylated hydroxyanisole (BHA) and Ascorbic acid (AsA)

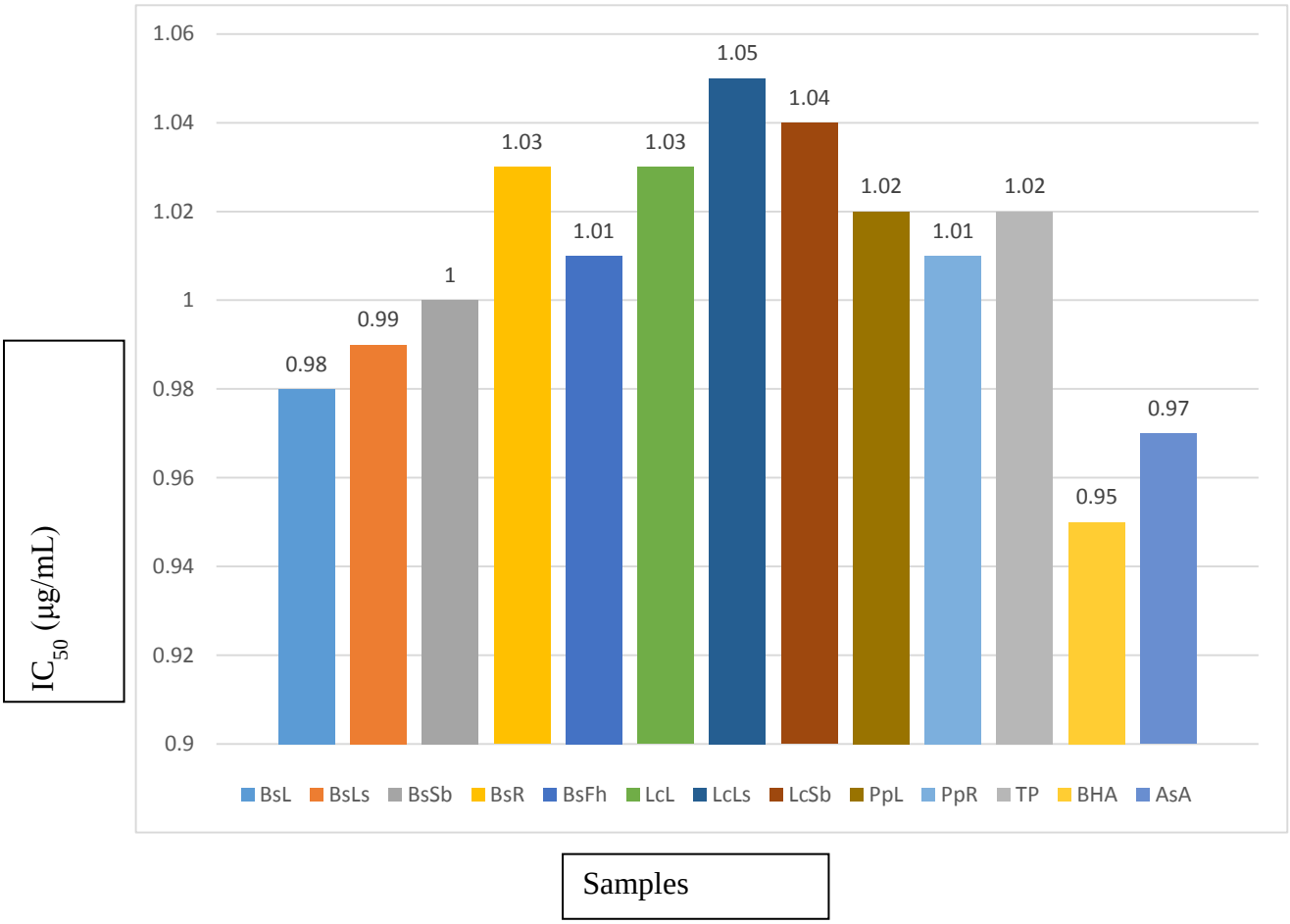


Figure 4.17: IC_{50} (µg/mL) of essential oils

Blighia sapida leaf (BsL), *Blighia sapida* leaf-stalk (BsLs), *Blighia sapida* stem-bark (BsSb), *Blighia sapida* root (BsR), *Blighia sapida* fruit-husk (BsFh), *Lecaniodiscus cupanioides* leaf (LcL), *Lecaniodiscus cupanioides* leaf-stalk (LcLs), *Lecaniodiscus cupanioides* stem-bark (LcSb), *Lecaniodiscus cupanioides* root (LcR), *Paullinia pinnata* leaf (PpL), *Paullinia pinnata* leaf-stalk (PpLs), *Paullinia pinnata* stem-bark (PpSb), *Paullinia pinnata* root (PpR), α-tocopherol (TP), Butylated hydroxyanisole (BHA) and Ascorbic acid (AsA)

4.8 Biological assessments of *Blighia sapida* crude extracts

4.8.1 Antimicrobial activities of *Blighia sapida* crude extracts

The activities of hexane, ethyl acetate and methanol extracts obtained from leaf, stem-bark and root of *Blighia sapida* were found to be lower than the activities of the standard drugs used as shown in Table 4.32. On the activities against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer and Penicillium notatum, Blighia sapida* root methanol extract was the most potent against all the ten test micro organisms with the zone of inhibition in the range of 11-27, 10-24, 10-26, 10-24, 10-23, 10-23, 10-23, 10-21, 10-18 and 10-19 mm, respectively, at concentration 6.25-200 mg/mL, followed by leaf methanol extract, then stem-bark methanol extract which is not far from activity of stem-bark hexane extract, followed by leaf ethyl acetate extract, then root hexane extract (no activity against *Rhizhopus stolonifer*) and root ethyl acetate extract (no activity against *Salmonellae typhi* and *Klebisiella pneumoniae*), followed by stem-bark ethyl acetate extract (no activity against *Klebisiella pneumoniae* and *Penicillium notatum*) which its activity is not far from leaf hexane extract (no activity against *Klebisiella pneumoniae*).

Leaf hexane extract exhibited antimicrobial activities against all the ten test microorganisms except *Klebisiella pneumoniae, that is, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer and Penicillium notatum* at 25-200 mg/mL by 10-18 mm, 12.5-200 mg/mL by 10-19 mm, 25-200 mg/mL by 10-17 mm, 50-200 mg/mL by 10-15 mm, 50-200 mg/mL by 10-16 mm, 50-200 mg/mL by 10-17 mm, 50-200 mg/mL by 10-14 mm, 50-200 mg/mL by 10-14 mm and 50-200 mg/mL by 10-17 mm Diameters of Inhibition Zone (DIZ) respectively.

Table 4.32: Antimicrobial activities of *Blighia sapida* crude extracts

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
1	Leaf Hexane	200	18	19	17	15	n.a.	16	17	14	14	17
		100	15	17	15	13	n.a.	14.	13	13	13	13
		50	13	15	13	10	n.a.	10	10	10	10	10
		25	10	12	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		12.5	n.a.	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	Leaf Ethyl acetate	200	19	16	22	17	19	15	16	17	16	17
		100	17	15	18	14	17	13	15	15	14	14
		50	15	13	14	10	14	10	13	13	12	12
		25	12	10	12	n.a.	12	n.a.	10	10	10	10
		12.5	10	n.a.	10	n.a.	10	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. - No Activity, - -Not Applicable

Table 4.32: Antimicrobial activities of *Blighia sapida* crude extracts Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
3	Leaf Methanol	200	27	23	23	24	24	22	23	20	19	18
		100	25	21	21	17	17	18	20	18	17	16
		50	21	18	18	14	14	16	18	16	14	14
		25	18	16	16	12	12	13	16	14	12	12
		12.5	14	14	13	10	10	10	13	12	10	10
		6.25	12	10	10	n.a.	n.a.	n.a.	10	10	n.a.	n.a.
4	Stem-bark Hexane	200	26	22	26	25	21	19	21	20	21	18
		100	23	19	23	23	19	17	20	18	18	17
		50	19	16	19	19	17	14	18	16	16	14
		25	17	14	17	15	12	12	16	14	14	12
		12.5	14	12	13	13	13	10	13	12	12	10
		6.25	10	10	10	10	10	n.a.	10	10	10	n.a.

n.a. - No Activity, - -Not Applicable

Table 4.32: Antimicrobial activities of *Blighia sapida* crude extracts Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
5	Stem-bark Ethyl acetate	200	20	19	17	17	n.a.	17	20	19	17	n.a.
		100	15	17	15	15	n.a.	15	17	15	14	n.a.
		50	13	14	13	13	n.a.	13	14	13	12	n.a.
		25	10	11	10	10	n.a.	10	10	10	10	n.a.
		12.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6	Stem-bark Methanol	200	23	27	23	24	19	21	18	20	20	19
		100	21	24	20	22	17	17	17	18	19	17
		50	18	20	17	17	14	14	15	15	17	15
		25	16	18	15	14	12	12	12	12	15	12
		12.5	14	14	12	12	10	10	10	10	12	10
		6.25	11	11	10	10	n.a.	10	n.a.	n.a.	10	n.a.

n.a. - No Activity, - -Not Applicable

Table 4.32: Antimicrobial activities of *Blighia sapida* crude extracts Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
7	Root Hexane	200	18	17	18	17	15	17	17	16	n.a.	14
		100	17	15	17	16	13	15	14	14	n.a.	13
		50	14	13	14	13	10	13	12	12	n.a.	10
		25	10	10	12	11	n.a.	10	10	10	n.a.	n.a.
		12.5	n.a.	n.a.	10	10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		6.25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	Root Ethyl acetate	200	20	18	17	16	n.a.	n.a.	21	19	18	17
		100	18	17	15	14	n.a.	n.a.	18	16	14	14
		50	14	14	13	12	n.a.	n.a.	15	14	12	12
		25	12	12	10	10	n.a.	n.a.	13	12	10.	10
		12.5	10	10	n.a.	n.a.	n.a..	n.a.	10	10	n.a.	n.a.
		6.25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.a. - No Activity, - -Not Applicable

Table 4.32: Antimicrobial activities of *Blighia sapida* crude extracts Cont'd

S/N	Plant Parts	Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
9	Root Methanol	200	27	24	26	24	23	23	23	21	18	19
		100	25	23	24	23	20	19	20	18	17	16
		50	21	18	20	19	18	17	18	14	14	14
		25	18	16	17	17	16	15	15	10	12	12
		12.5	15	13	13	13	13	13	12	10	10	10
		6.25	11	10	10	10	10	10	10	10	n.a.	n.a.
Solvent	Hexane		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	DMSO		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Standard	Gentamicin	10 µg/mL	38	38	38	38	38	38	-	-	-	-
	Tioconazole	10 µg/mL	-	-	-	-	-	-	27	26	28	28

n.a. - No Activity, - -Not Applicable

Minimum Inhibotory Concentration (MIC) also varied as seen in Table 4.33; MIC assessments also determine the efficiency of therapeutic strategy against infections (Kowalska-Krochmal and Dudek-Wicher, 2021). The Minimum Inhibotory Concentration (MIC) of leaf hexane extract at the range of 1.25 mg/mL to 10 mg/mL on the ten organisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer and Penicillium notatum* showed 10 mg/mL, 5 mg/mL, 10 mg/mL, none, none, none, 10mg/mL, none, none and 10 mg/mL respectively.

Leaf ethyl acetate extract exhibited antimicrobial activities against all the test microorganisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 12.5-200 mg/mL by 10-19 mm, 25-200 mg/mL by 10-16 mm, 12.5-200 mg/mL by 10-22 mm, 50-200 mg/mL by 10-17 mm, 12.5-200 mg/mL by 10-19 mm, 50-200 mg/mL by 10-15 mm, 25-200 mg/mL by 10-16 mm, 25-200 mg/mL by 10-17 mm, 25-200 mg/mL by 10-16 mm and 25-200 mg/mL by 10-17 mm Diameters of Inhibition Zone (DIZ) respectively. The Minimum Inhibotory Concentration (MIC) of this sample at the range of 1.25 mg/mL to 10 mg/mL on the ten organisms used showed 5 mg/mL, 10 mg/mL, 5 mg/mL, 10 mg/mL, 5 mg/mL, none, 10 mg/mL, 10 mg/mL, 10 mg/mL and 10 mg/mL respectively.

Table 4.33: Minimum Inhibitory Concentration (MIC) on the antimicrobial activities of *Blighia sapida* crude extracts

S/N	Plant Parts	Concentration (mg/mL)	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
1	Leaf Hexane	10	-	-	-	+	+	+	-	+	+	-
		5	+	-	+	+	+	+	+	+	+	+
		2.5	+	+	+	+	+	+	+	+	+	+
		1.25	+	+	+	+	+	+	+	+	+	+
2	Leaf Ethyl acetate		-	-	-	-	-	+	-	-	-	-
		5	-	+	-	+	-	+	+	+	+	+
		2.5	+	+	+	+	+	+	+	+	+	+
		1.25	+	+	+	+	+	+	+	+	+	+
3	Leaf Methanol	10	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	+	+	+	-	-	+	+
		1.25	-	-	+	+	+	+	+	+	+	+
4	Stem-bark Hexane	10	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	+	-	-	-	+
		1.25	-	+	+	+	+	+	+	+	+	+

- Inhibition (No growth of micro organism), + No Inhibition (Growth of micro organism)

Table 4.33: Minimum Inhibitory Concentration on the antimicrobial activities of *Blighia sapida* crude extracts Cont'd

S/N	Plant Parts	Concentration mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
5	Stem-bark Ethyl acetate	10	-	-	-	-	+	-	-	-	-	+
		5	-	+	+	+	+	+	+	+	+	+
		2.5	+	+	+	+	+	+	+	+	+	+
		1.25	+	+	+	+	+	+	+	+	+	+
6	Stem-bark Methanol	10	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	+	+	+	+	-	+
		1.25	+	+	+	+	+	+	+	+	+	+
7	Root Hexane	10	-	-	-	-	+	-	-	+	+	+
		5	-	+	-	+	+	+	+	+	+	+
		2.5	+	+	+	+	+	+	+	+	+	+
		1.25	+	+	+	+	+	+	+	+	+	+

- Inhibition (No growth of micro organism), + No Inhibition (Growth of micro organism)

Table 4.33: MIC on the antimicrobial activities of *Blighia sapida* crude extracts Cont'd

S/N	Plant Parts	Concentration mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
8	Root Ethyl acetate	10	-	-	-	-	+	+	-	-	-	-
		5	-	-	+	+	+	+	-	-	+	+
		2.5	+	+	+	+	+	+	+	+	+	+
		1.25	+	+	+	+	+	+	+	+	+	+
9	Root Methanol	10	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	-
		2.5	-	-	-	-	-	-	-	+	+	+
		1.25	-	-	+	+	+	+	+	+	+	+

- Inhibition (No growth of micro organism), + No Inhibition (Growth of micro organism)

Leaf methanol extract exhibited antimicrobial activities against all the test microorganisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi*, *Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 6.25-200 mg/mL by 12-27 mm, 6.25-200 mg/mL by 10-23 mm, 6.25-200 mg/mL by 10-23 mm, 12.5-200 mg/mL by 10-24 mm, 12.5-200 mg/mL by 10-24 mm, 12.5-200 mg/mL by 10-22 mm, 6.25-200 mg/mL by 10-23 mm, 6.25-200 mg/mL by 10-20 mm, 12.5-200 mg/mL by 10-19 mm and 12.5-200 mg/mL by 10-18 mm Diameters of Inhibition Zone (DIZ) respectively. The Minimum Inhibotory Concentration (MIC) of this sample at the range of 1.25 mg/mL to 10 mg/mL on the ten organisms used shows 1.25 mg/mL, 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, 5 mg/mL, 5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 5 mg/mL and 5 mg/mL respectively. Ologundudu *et al.*, (2018) also reported antimicrobial activities of *Blighia sapida* leaf extracts against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumoniae*, although, no activity of *Blighia sapida* leaf hexane extract against *Klebsiella pneumoniae* in our study.

Stem-bark hexane extract exhibited antimicrobial activities against all the test microorganisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi*, *Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 6.25-200 mg/mL by 10-26 mm, 6.25-200 mg/mL by 10-22 mm, 6.25-200 mg/mL by 10-26 mm, 6.25-200 mg/mL by 10-25 mm, 6.25-200 mg/mL by 10-21 mm, 12.5-200 mg/mL by 10-19 mm, 6.25-200 mg/mL by 10-21 mm, 6.25-200 mg/mL by 10-20 mm, 6.25-200 mg/mL by 10-21 mm and 12.5-200 mg/mL by 10-18 mm Diameters of Inhibition Zone (DIZ) respectively. MIC of this sample at the range of 1.25 mg/mL to 10 mg/mL on the ten organisms used shows 1.25 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 2.5 mg/mL and 5 mg/mL respectively.

Stem-bark ethyl acetate extract exhibited antimicrobial activities against all the test microorganisms except *Klebisiella pneumoniae* and *Penicillium notatum*; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa,*

Salmonellae typhi, *Candida albicans, Aspergillus niger* and *Rhizhopus stolonifer* at 25-200 mg/mL by 10-20 mm, 25-200 mg/mL by 11-19 mm, 25-200 mg/mL by 10-17 mm, 25-200 mg/mL by 10-17 mm, 25-200 mg/mL by 10-17 mm, 25-200 mg/mL by 10-20 mm, 25-200 mg/mL by 10-19 mm and 25-200 mg/mL by 10-17 mm Diameters of Inhibition Zone (DIZ) respectively. MIC of this sample at the range of 1.25 mg/mL to 10 mg/mL on the ten organisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* shows 5 mg/mL, 10 mg/mL, 10 mg/mL, 10 mg/mL, none, 10 mg/mL, 10 mg/mL, 10 mg/mL, 10 mg/mL and none respectively.

Stem-bark methanol extract exhibited antimicrobial activities against all the test microorganisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi*, *Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 6.25-200 mg/mL by 11-23 mm, 6.25-200 mg/mL by 11-27 mm, 6.25-200 mg/mL by 10-23 mm, 6.25-200 mg/mL by 10-24 mm, 12.5-200 mg/mL by 10-19 mm, 6.25-200 mg/mL by 10-21 mm,12.5-200 mg/mL by 10-18 mm, 12.5-200 mg/mL by 10-20 mm, 6.25-200 mg/mL by 10-20 mm and 12.5-200 mg/mL by 10-19 mm Diameters of Inhibition Zone (DIZ) respectively. MIC of this sample at the range of 1.25 mg/mL to 10 mg/mL on the ten organisms used shows 2.5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 5 mg/mL, 5 mg/mL, 5 mg/mL, 5 mg/mL, 2.5 mg/mL and 5 mg/mL respectively. *Blighia sapida* stem bark extracts had antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumoniae* according to Ologundudu *et al.,* (2018).

Root hexane extract exhibited antimicrobial activities against all the test microorganisms except *Rhizhopus stolonifer*; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger* and *Penicillium notatum* at 25-200 mg/mL by 10-18 mm, 25-200 mg/mL by 10-17 mm, 12.5-200 mg/mL by 10-18 mm, 12.5-200 mg/mL by 10-17 mm, 50-200 mg/mL by 10-15 mm, 25-200 mg/mL by 10-17 mm, 25-200 mg/mL by 10-17 mm, 25-200 mg/mL by 10-16 mm and 50-200 mg/mL by 10-14 mm Diameters of Inhibition Zone (DIZ) respectively. MIC of this sample at the range

of 1.25 mg/mL to 10 mg/mL on the ten organisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* shows 5 mg/mL, 10 mg/mL, 5 mg/mL, 10 mg/mL, none, 10 mg/mL, 10 mg/mL, none, none and none respectively.

Root ethyl acetate extract exhibited antimicrobial activities against all the test microorganisms except *Salmonellae typhi* and *Klebisiella pneumoniae*; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 12.5-200 mg/mL by 10-20 mm, 12.5-200 mg/mL by 10-18 mm, 25-200 mg/mL by 10-17 mm, 25-200 mg/mL by 10-16 mm, 12.5-200 mg/mL by 10-21 mm, 12.5-200 mg/mL by 10-19 mm, 25-200 mg/mL by 10-18 mm and 25-200 mg/mL by 10-17 mm Diameters of Inhibition Zone (DIZ) respectively. MIC of this sample at the range of 1.25 mg/mL to 10 mg/mL on the ten organisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* shows 5 mg/mL, 5 mg/mL, 10 mg/mL, 10 mg/mL, none, none, 5 mg/mL, 5 mg/mL, 10 mg/mL and 10 mg/mL respectively.

Root methanol extract exhibited antimicrobial activities against all the test microorganisms; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebisiella pneumoniae, Salmonellae typhi, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* at 6.25-200 mg/mL by 11-27 mm, 6.25-200 mg/mL by 10-24 mm, 6.25-200 mg/mL by 10-26 mm, 6.25-200 mg/mL by 10-24 mm, 6.25-200 mg/mL by 10-23 mm, 6.25-200 mg/mL by 10-23 mm, 6.25-200 mg/mL by 10-23 mm, 6.25-200 mg/mL by 10-21 mm, 12.5-200 mg/mL by 10-18 mm and 12.5-200 mg/mL by 10-19 mm Diameters of Inhibition Zone (DIZ) respectively. MIC of this sample at the range of 1.25 mg/mL to 10 mg/mL on the ten organisms used shows 1.25 mg/mL, 1.25 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 2.5 mg/mL, 5 mg/mL, 5 mg/mL and 5 mg/mL respectively.

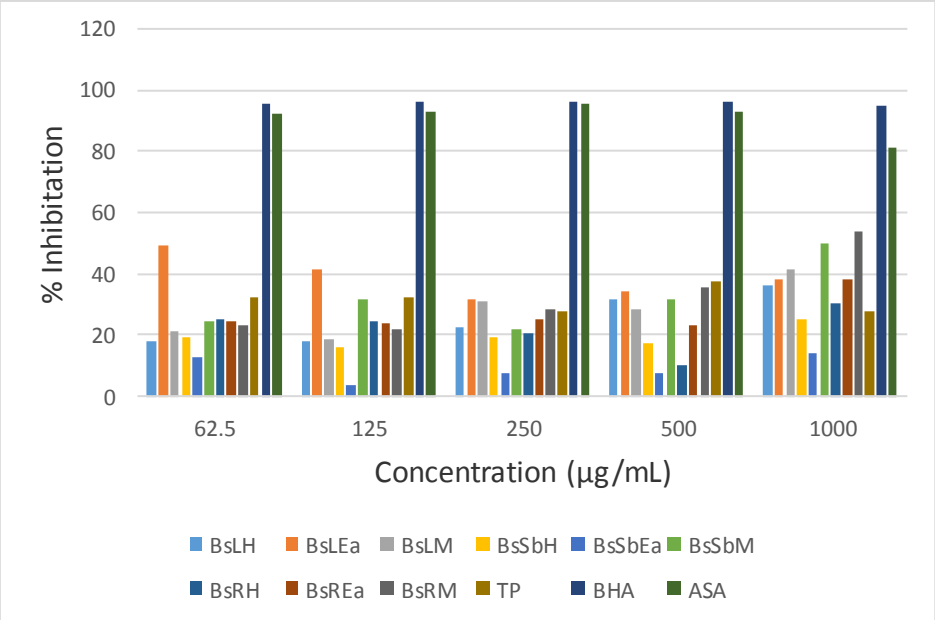
Antibacterial and antifungal activities of *Blighia sapida* extracts were established by using various established protocols which support ethno-medicinal uses of the plant.

4.8.2 Antioxidant activities of *Blighia sapida* crude extracts

Antioxidant activities of hexane, ethyl acetate and methanol extracts of *Blighia sapida* crude extracts of leaf (BsLH, BsLEa, BsLM), stem-bark (BsSbH, BsSbEa, BsSbM) and root (BsRH, BsREa, BsRM) were compared with known antioxidants: α-tocopherol (TP), butylated hydroxyanisole (BHA) and ascorbic acid (ASA) as shown in Fig. 4.18. The decrease in absorption at 517 nm shows that the samples can scavenge free radical with hydrogen donating ability as also reported by Amira *et al*., (2019) on *Blighia sapida* stem bark extract. Using % inhibition for the concentrations between 62.5 – 1000 µg/mL, the scavenging effect of the *Blighia sapida* crude extracts and standards on the DPPH radical decreased in the order of BHA > ASA > BsRM > BsLEa > BsSbM > BsLM > BsREa > TP > BsLH > BsRH > BsSbH > BsSbEa and were 95% - 97%, 81% - 96%, 22% - 54%, 31 – 50%, 22% - 50%, 18 – 41%, 23 – 39%, 28% - 38%, 18 – 36%, 10 – 30%, 16 – 25% and 4 – 14% respectively.

Considering only the crude extracts from above order to know the extracts that will be more promising, BsRM > BsLEa > BsSbM > BsLM > BsREa > BsLH > BsRH > BsSbH > BsSbEa and were 22% - 54%, 31 – 50%, 22% - 50%, 18 – 41%, 23 – 39%, 18 – 36%, 10 – 30%, 16 – 25% and 4 – 14% respectively after 10 minutes. These crude extracts were re-examined again after 30 minutes to compare the order and this showed the decreasing order of BsSbM >BsRM > BsLEa > BsLM > BsSbH >BsREa > BsRH > BsLH > BsSbEa and were 29% - 64%, 25% - 54%, 30 – 53%, 22 – 51%, 34 – 48%, 29 – 46%, 35 – 43%, 23 – 36% and 9 – 21% respectively which is almost similar to the order after 10 minutes. Among these crude extracts, *Blighia sapida* root methanol, *Blighia sapida* leaf ethyl acetate and *Blighia sapida* stem-bark methanol were the most potent antioxidants both at 10 and 30 minutes while *Blighia sapida* stem-bark ethyl acetate was the least potent antioxidant both at 10 and 30 minutes.

Inhibitory concentration (IC_{50}) of these extracts at 10 minutes was in the order of BHA > ASA > BsRM > BsSbM > BsLM > BsREa = BsLEa > BsLH > BsRH > TP > BsSbH > BsSbEa and were 0.950%, 0.966%, 0.996%, 1.000%, 1.009%, 1.012% = 1.012%, 1.014%, 1.020%, 1.023%, 1.026%, 1.036%. All extracts were active though lower than the standards butylated hydroxyanisole and ascorbic acid but they were all active than α-tocopherol except the stem-bark hexane and stem-bark ethyl acetate.



Blighia sapida leaf hexane extract (BsLH), *Blighia sapida* leaf ethyl acetate extract (BsLEa), *Blighia sapida* leaf methanol extract (BsLM), *Blighia sapida* stem-bark hexane extract (BsSbH), *Blighia sapida* stem-bark ethyl acetate extract (BsSbEa), *Blighia sapida* stem-bark methanol extract (BsSbM), *Blighia sapida* root hexane extract (BsRH), *Blighia sapida* root ethyl acetate extract (BsREa), *Blighia sapida* root methanol extract (BsRM), α-tocopherol (TP), Butylated hydroxyanisole (BHA) and Ascorbic acid (ASA).

Figure 4.18: DPPH radical scavenging activity of the crude extracts of *Blighia sapida*.

Among the extracts, methanol extracts (root, stem-bark and leaf) were most active, followed by ethyl acetate extracts (root and leaf) except stem-bark while the least active were the hexane extracts (leaf, root, stem bark) using IC_{50}. In all, root methanol extract was the most potent while the least was the stem-bark ethyl acetate.

Antioxidant activities of *Blighia sapida* extracts were established using % inhibition and inhibitory concentration (IC_{50}) to assess DPPH radical scavenging activity. Phenolic compounds such as flavonoids and nitrogen compounds such as alkaloids are antioxidants (Amira *et al*., 2019) and these were present from the results of phytochemical screening of *Blighia sapida* extracts. Therefore, the antioxidant results support ethno-medicinal uses of the plant.

4.9 Biological assessments of isolate (Scopoletin)

4.9.1 Antimicrobial activities of Scopoletin (BS6)

Scopoletin (BS6) showed antibacterial and antifungal activities against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonellae typhi, Klebisiella pneumoniae, Candida albicans, Aspergillus niger, Rhizhopus stolonifer* and *Penicillium notatum* (Table 4.34). It was potent at varied concentration between 3.125 to 100 mg/mL except at 3.125 mg/mL on *Salmonellae typhi, Klebisiella pneumoniae* and 12.5 mg/mL on *Rhizhopus stolonifer* and *Penicillium notatumi.* The Minimum Inhibitory Concentration (MIC) was also evaluated (Table 4.35) which showed the lowest concentration of the antimicrobial after overnight incubation that can inhibit the visible growth of microorganism. Scopoletin has many biological activities such as antifeedant (Barrero *et al*., 2013; Tripathi *et al*., 2011), antioxidant (Malik *et al*., 2011), antifungal (Carpinella *et al*., 2005; Lee *et al*., 2010; Valle, 1997), antitumoural (Arcos *et al*., 2006; Khuda-Bukhsh, 2010), anti-inflammatory (Mahattanadul, 2011; Shah, 2011), hepatoprotective (Kang *et al*., 1998; Kwon *et al*., 2011; Mohamed *et al*., 2005; Noh *et al*., 2011), neurological (Capra, 2010; Hornick *et al*., 2011; Mishra, 2010), insecticidal (Sharma *et al*., 2006) and radical scavenging (Thuong, 2010) activities.

Table 4.34: Antimicrobial activities of Scopoletin

Serial Dilution mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
100	24	21	26	20	18	18	20	20	14	14
50	21	16	24	18	16	16	18	18	12	12
25	18	14	21	16	14	14	16	16	10	10
12.5	16	12	17	14	12	12	14	14	n.a.	n.a.
6.25	14	10	14	12	10	10	12	12	n.a.	n.a.
3.125	10	10	10	10	n.a.	n.a.	10	10	n.a.	n.a.
DMSO	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Gentamicin (10 µg/mL)	38	36	36	38	38	36	-	-	-	-
Tioconazole (10 µg/mL)	-	-	-	-	-	-	28	28	26	28

n.a. - No Activity, - -Not Applicable

Table 4.35: Minimum Inhibitory Concentration on the antimicrobial activities of Scopoletin

Concentration mg/mL	*S. aureus*	*E. coli*	*B. subtilis*	*P. auruginosa*	*K. pneumoniae*	*S. typhi*	*C. albicans*	*A. niger*	*R. stolonifer*	*P. notatum*
50	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	+	+
12.5	-	-	-	-	-	+	-	-	+	+
6.25	-	-	-	+	+	+	+	+	+	+
3.125	+	+	+	+	+	+	+	+	+	+

- Inhibition (No growth of micro organism), + No Inhibition (Growth of micro organism)

Scopoletin, a fluorescent compound is a coumarin found in *Scopolia carniolica*, *Scopolia japonica*, chicory, *Artemisia scoparia*, *Urtica dioica*, passion flower, *Brunfelsia*, *Viburnum prunifolium*, *Solanum nigrum*, *Mallotus resinosus*, *Kleinhovia hospita*, fenugreek, vinegar, whiskies, dandelion coffee and cardiospermum corundum L. (Ma *et al*., 2004; Ouzir *et al*, 2016; Silva *et al*., 2014; Zhao *et al*., 2010).

Antibacterial and antifungal activities of isolated and characterised scopoletin (7-hydroxy-6-methoxy-chromen-2-one) were established which support ethno-medicinal uses of *Blighia sapida* plant from where it was isolated.

4.9.2 Antioxidant activity of Scopoletin (BS6)

Antioxidant activity of scopoletin was compared with known antioxidants: α-tocopherol (TP), butylated hydroxyanisole (BHA) and ascorbic acid (ASA) as shown in Fig. 4.19. The result showed that the isolate (scopoletin) can scavenge free radical with hydrogen donating ability. Using % inhibition for the concentrations between 62.5 – 1000 µg/mL, the scavenging effect of BS6 and standards on the DPPH radical decreased in the order of BHA > ASA > TP > BS6 and were 95% - 97%, 81% - 96%, 28% - 38%, 24 – 34% respectively while IC_{50} was also in the order of BHA > ASA > TP > BS6 and were 0.95 µg/mL, 0.97 µg/mL, 1.02 µg/mL, 1.03 µg/mL. Though, BHA (0.95 µg/mL) and ASA (0.97 µg/mL) standards were more active than isolated and characterised scopoletin (1.03 µg/mL) but the activity of this isolate was very close to the third standard, TP (1.02 µg/mL) used as shown in Fig. 4.20.

Antioxidant activity of scopoletin (7-hydroxy-6-methoxy-chromen-2-one) was established and this isolate is a flavonoid; flavonoids are known to be antioxidants (Amira *et al*., 2019). Therefore, the antioxidant results support ethno-medicinal uses of *Blighia sapida* from where the compound was isolated.

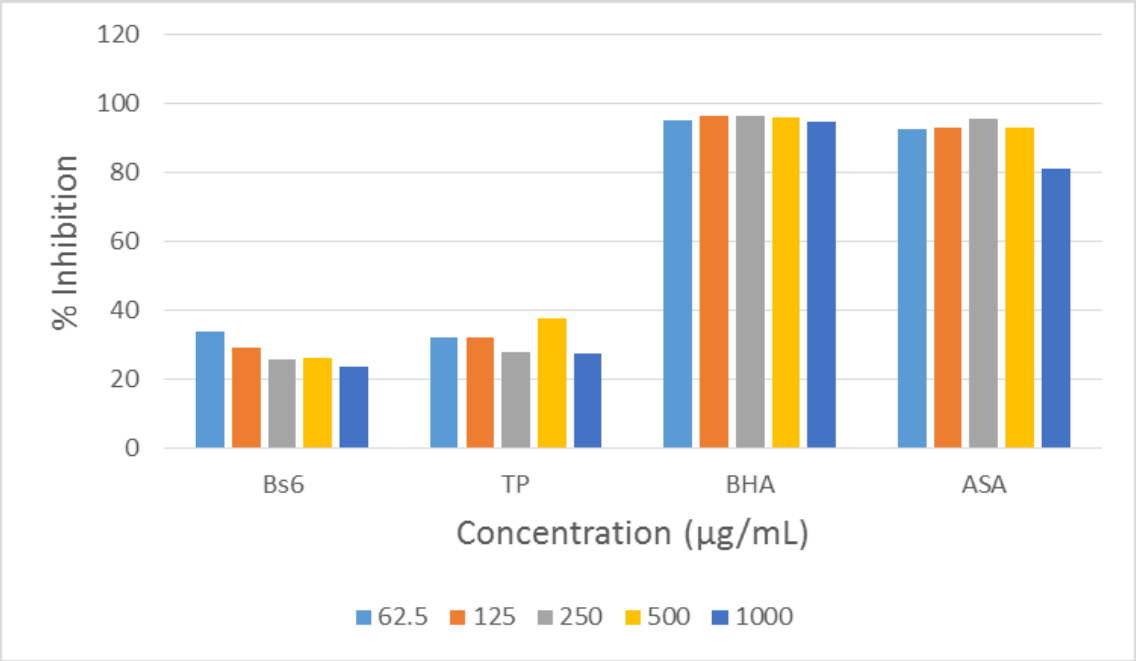


Figure 4.19: DPPH radical scavenging activity of BS6

Scopoletin (BS6), α-tocopherol (TP), Butylated hydroxyanisole (BHA) and Ascorbic acid (ASA).

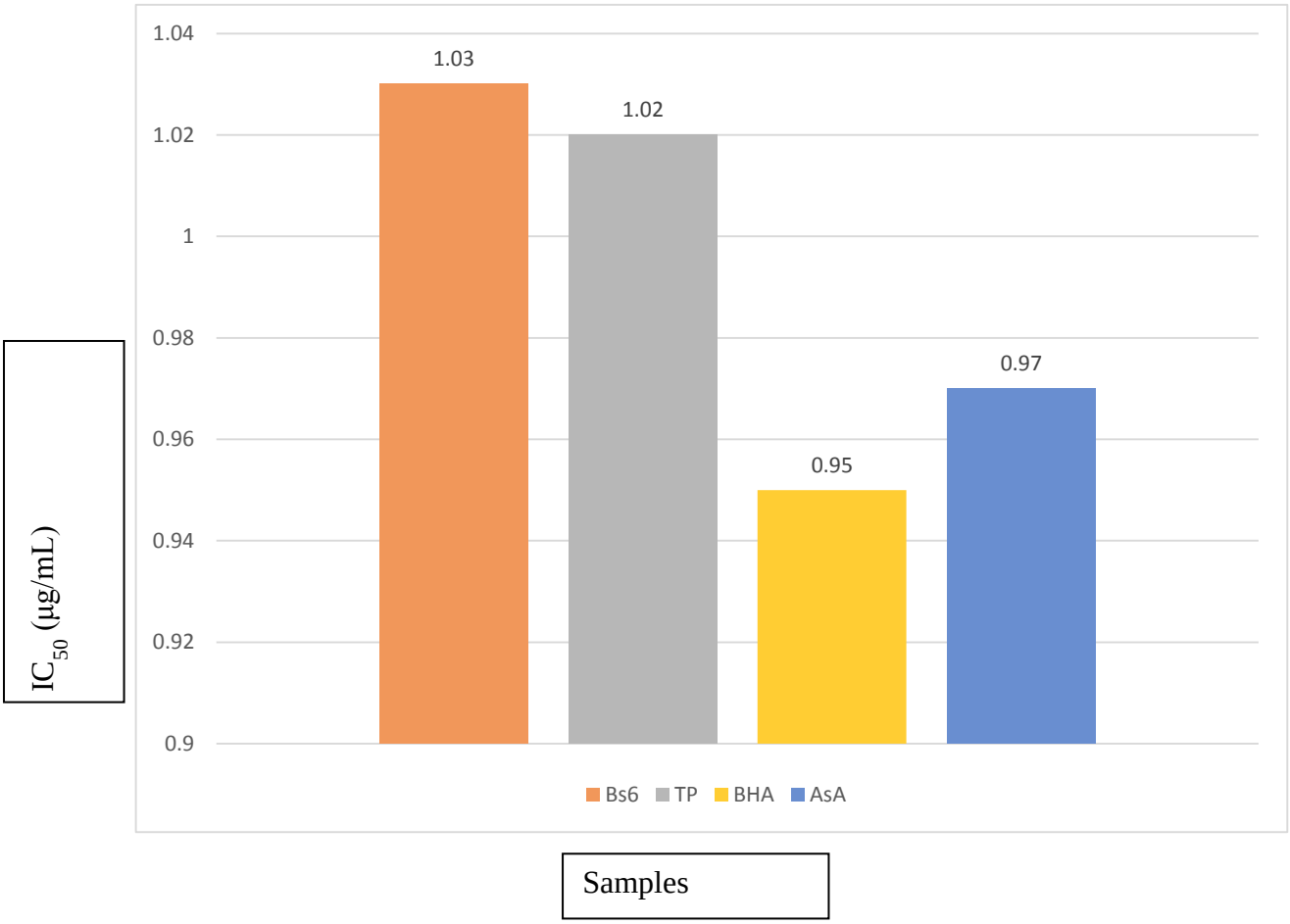


Figure 4.20: IC_{50} (µg/mL) of BS6

Scopoletin (BS6), α-tocopherol (TP), Butylated hydroxyanisole (BHA) and Ascorbic acid (AsA)

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

Blighia sapida, *Lecaniodiscus cupanioides* and *Paullinia pinnata* that are sapindaceae species are used in ethno-medicine to treat different types of ailment but with limited information on literature, these plants were analysed to establish their chemical compounds.

Essential oils were extracted and characterised from *Blighia sapida* leaf, leaf stalk, stem bark, root, fruit pulp, fruit husk, seed; *Lecaniodiscus cupanioides* leaf, leaf stalk, stem bark, root and *Paullinia pinnata* leaf, leaf stalk, stem bark and root using GC/GC-MS.

Blighia sapida root that had scarce information on isolation and characterisation was subjected to isolation using column chromatographic methods and characterisation of isolated compound using spectroscopic techniques of IR, NMR and MS.

Fifteen (15) essential oils extracted were subjected to antibacterial, antifungal and antioxidant activities. Extracts from successive extractions of *Blighia sapida* leaf, stem bark and root were also subjected to phytochemical screening, antibacterial and antioxidant activities, likewise, the isolated compound (scopoletin) from *Blighia sapida* root was also assessed on antibacterial, antifungal and antioxidant activities.

The results supported the use of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata* for treatment of microbial infections and oxidative stress.

5.2 Conclusion

Four hundred and ninety-two (492) compounds were established in *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata* essential oils and are newly reported in most of these plants' parts and were consistent with the plant's taxonomy. These chemical constituents are important agents which include:

Medical agents- such as hexahydrofarnesyl acetone (antimicrobial), methyl salicylate (antispectic, analgestic), eucalyptol (cough suppressant), phytol (treatment of inflammation), 1-dodecanol (antifungal), geranylgeraniol, pentadecanal, linalool, hexanal (antimicrobial), α-terpineol (antihypertensive, antioxidant), caryophyllene oxide (anti-inflammatory, local anaesthetic), caryophyllene (treatment of seizures, pain and anxiety).

Industrial agents- such as β-cyclocitral (emulsifiers), pyrethrin, 2-pentylfuran and hexahydrofarnesyl acetone (insecticidal), Hexadecanoic acid (emulsifier, gelling agent, thickner), farnesol (pesticide), caryophyllene oxide, phenalacetaldehyde (insect and pest attractant), palmitic acid (insecticide, cosmetics), ethyl palmitate (hair and skin conditioning agent).

Flavouring agents- such as 2, 4-Decadienal, 3-Hexanol, ethyl palmitate, decanal, farnesyl acetone, hexahydrofarnesyl acetone, α-ionone, nonanal, 2-Pentylfuran.

Compound isolated and characterised by various spectroscopic techniques from *Blighia sapida* root was identified as scopoletin (7-hydroxy-6-methoxy-chromen-2-one). 7-hydroxy-6-methoxy-chromen-2-one is not a new compound but first reported from *Blighia sapida*.

Blighia sapida, *Lecaniodiscus cupanioides* and *Paullinia pinnata* essential oils exhibited significant antibacterial, antifungal and antioxidant activities. The bioactivities of the oils support the use of the plants in ethnomedicine for the treatment of ailments associated with microbial infections and oxidative stress. 7-hydroxy-6-methoxy-chromen-2-one (scopoletin) also has bioactivities which support the ethno-medicinal uses of the plant.

5.3 Recommendations

1. Despite the findings above, more work needs to be done on isolation of bioactive compounds from *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata* because of their wide use in ethno-medicine.
2. Synthesis of important compounds that were established from these plants are necessary for improved activity and availability.
3. Improving essential oils yield through biotechnology research should be explored.
4. Other biological investigations of the plants are recommended to support other claims of ethno-medicinal uses.

5.4 Contributions to knowledge

1. This research established 492 chemical compounds from the fifteen (15) essential oils: 197 compounds from seven oils of *Blighia sapida* (leaf, leaf-stalk, stem-bark, root, fruit-pulp, fruit-husk and seed), 153 compounds from four oils of *Lecaniodiscus cupanioides* (leaf, leaf-stalk, stem-bark and root) and 142 compounds from four oils of *Paullinia pinnata* (leaf, leaf-stalk, stem-bark and root).
2. Scopoletin (7-hydroxy-6-methoxy-chromen-2-one) isolated was reported for the first time from *Blighia sapida*.
3. Results from chemical constituent's identification, antibacterial, antifungal and antioxidant assessments justify the local uses of *Blighia sapida*, *Lecaniodiscus cupanioides* and *Paullinia pinnata* in ethno-medicine, soap making and as insecticidal agents.

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