HPLC, GC-MS PROFILING AND BACTERIAL INHIBITORY PROPERTY OF *ANTIARIS TOXICARIA VAR. AFRICANA* STEM BARK EXTRACT

BY

RITA OBIANUJU AGUBUEZE

PSC2105241

DEPARTMENT OF CHEMISTRY

FACULTY OF PHYSICAL SCIENCES

UNIVERSITY OF BENIN

OCTOBER, 2025

HPLC, GC-MS PROFILING AND BACTERIAL INHIBITORY PROPERTY OF *ANTIARIS TOXICARIA VAR. AFRICANA* STEM BARK EXTRACT

BY

RITA OBIANUJU AGUBUEZE

PSC2105241

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.Sc.) IN INDUSTRIAL CHEMISTRY

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project work HPLC, GC-MS profiling and bacterial inhibitory property of *Antiaris toxicaria var. africana* stem bark extract carried out by Rita Obianuju Agubueze with matriculation number PSC2105241 in the Department of Chemistry, University of Benin, Benin City.

_____ _____

Rita Obianuju Agubueze **Date**

(Student)

_____ _____

Dr. Anthony E. Aiwonegbe **Date**

(Project Supervisor)

_____ _____

Prof. E E.I. Irabor **Date**

(Head Of Department)

DEDICATION

I dedicate this research work to my beloved family; my parents, Mr. and Mrs. Agubueze, and my siblings, Chiamaka, Amarachi, and Onyebuchi Agubueze for their unwavering love, encouragement, and support throughout this journey.

ACKNOWLEDGEMENT

I am deeply grateful to the Almighty God for His grace, strength, and wisdom that guided me throughout this academic journey and made the successful completion of this research possible

My heartfelt appreciation goes to my supervisor, Dr. Anthony E. Aiwonegbe, for his guidance, unwavering support, and insightful feedback throughout this research work. His patience and dedication greatly contributed to the success of this project.

I would also like to extend my sincere gratitude to the Head of the Department of Chemistry, Prof. E. E. I. Irabor, and all the lecturers in the Department for their encouragement, guidance, and invaluable support, which played a vital role in the completion of this work.

My special thanks go to Dr. E. E. Odion and all the laboratory staff of the Department of Chemistry for their technical assistance and cooperation during the course of my experimental work.

I am profoundly grateful to my parents, Mr. and Mrs. Agubueze, for their love, prayers, and continuous encouragement, which have been a constant source of strength and motivation.

Finally, I wish to appreciate my project mates Omogbai Theophilus, Nicholas Lemuel, and Gwam Precious, for their friendship, teamwork, and shared efforts that made this research process both productive and enjoyable.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The search for new therapeutic agents derived from natural sources has intensified due to the global rise in antimicrobial resistance (AMR). Medicinal plants remain a significant source of bioactive compounds with diverse pharmacological potentials. This study examined the phytochemical components and bacterial inhibitory property of the dichloromethane (DCM) fraction of the stem bark of *Antiaris toxicaria var. africana*, a plant widely used in African ethnomedicine for treating various infections. The stem bark was extracted using 70% ethanol and subsequently fractionated with DCM using column chromatography. High-Performance Liquid Chromatography (HPLC) and Gas Chromatography–Mass Spectrometry (GC–MS) were employed for chemical characterization. GC–MS analysis revealed the presence of bioactive compounds with known antibacterial and antioxidant properties, including 2,4-di-tert-butylphenol (18.86%), hexadecanoic acid (14.35%), benzoic acid (7.39%), and methyl stearate (6.66%). HPLC profiling confirmed the presence of important phytochemicals such as flavonoids (11.36 µg/mL), proanthocyanidins (13.99 µg/mL), flavanones (9.93 µg/mL), tannins (3.70 µg/mL), cardiac glycosides (5.53 µg/mL), sapogenins (7.96 µg/mL), and steroids (4.70 µg/mL), indicating a rich diversity of secondary metabolites. Antimicrobial testing showed that the extract exhibited inhibitory activity against *Pseudomonas aeruginosa* (19 mm), *Bacillus subtilis* (18 mm), *Staphylococcus aureus* (19 mm), *Escherichia coli* (20 mm), and *Enterobacter cloacae* (17 mm), but not against *Bacillus cereus*. Although the extract demonstrated lower activity than the positive control, ciprofloxacin, it displayed broad-spectrum antibacterial potential. Overall, the findings provide scientific validation for the traditional use of *A. Toxicaria var. africana* in treating bacterial infections.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

1.1 BACKGROUND OF STUDY

Antimicrobial resistance (AMR) has emerged as one of the greatest threats to global health, as microorganisms increasingly develop resistance to existing drugs. The World Health Organization (2023) cautions that the continued spread of resistant pathogens could reverse decades of medical advancement, making previously curable infections harder to treat. It is estimated that millions of deaths each year are linked to AMR, and projections suggest this figure may rise to 10 million annually by 2050 if new antimicrobial solutions are not discovered (O'Neill, 2016). This growing concern underscores the need for innovative strategies and natural alternatives in the search for new therapeutic agents.

For centuries, medicinal plants have played a vital role in healthcare systems worldwide. They not only provide primary remedies for various ailments but also serve as the foundation for modern drug discovery. These plants produce a wide range of secondary metabolites—such as alkaloids, flavonoids, tannins, terpenoids, saponins, and phenolics—which serve as natural defense molecules against pathogens and environmental stress, while also exhibiting significant pharmacological benefits for humans (Yeshi *et al.,* 2022). The proven success of drugs like artemisinin and quinine demonstrates the invaluable contribution of plant-derived compounds in modern medicine.

The WHO Global Report on Traditional and Complementary Medicine (2019) highlights that many countries still rely on herbal medicine due to its affordability, accessibility, and cultural acceptance.

One such plant of ethnomedicinal value is *Antiaris toxicaria var. africana*, a member of the *Moraceae* family. It is a large deciduous tree found throughout tropical Africa and parts of Asia, and its various parts—particularly the stem bark, leaves, and latex—are used traditionally for the treatment of ailments such as fevers, skin infections, microbial diseases, and inflammatory conditions (Djerdjouri *et al.,* 2024). Among these, the stem bark has received notable attention for its potential antimicrobial and anti-inflammatory properties.

Previous investigations on species within the *Antiaris* genus have revealed the presence of several phytochemicals, including cardenolides, triterpenes, flavonoids, alkaloids, and phenolic compounds, many of which are associated with antimicrobial, antioxidant, and cytotoxic activities (Huang *et al.,* 2022). Nonetheless, there is still a lack of detailed chemical and antimicrobial characterization of the stem bark of *A. toxicaria var. africana*, which limits its potential application in pharmaceutical and therapeutic development.

This research therefore focuses on the stem bark of *A. toxicaria var. africana*, subjecting it to extraction, fractionation, and phytochemical analyses using Gas Chromatography–Mass Spectrometry (GC–MS) and High-Performance Liquid Chromatography (HPLC). Ethanol was used as the solvent because it efficiently extracts a broad range of bioactive molecules.

Through this study, scientific evidence supporting the antimicrobial potential of *A. toxicaria var. africana* stem bark will be provided, offering a basis for its traditional medicinal use and contributing to ongoing efforts to identify novel natural compounds capable of addressing antimicrobial resistance.

1.2 STATEMENT OF PROBLEM

The growing challenge of antimicrobial resistance (AMR) has created an urgent need for new and effective antimicrobial agents. Although antibiotics have long been a cornerstone of modern medicine, their frequent misuse and overuse have accelerated the development of resistant strains of microorganisms, making many common infections increasingly difficult to treat. As a result, morbidity and mortality rates linked to drug-resistant infections continue to rise globally.

Medicinal plants, rich in structurally diverse secondary metabolites, represent a valuable source of potential antimicrobial compounds. For generations, these plants have been central to traditional medicine for managing various diseases, and scientific research continues to explore their bioactive constituents for drug discovery (Dias *et al.,* 2012).

In Nigeria*, Antiaris toxicaria var. africana* is widely recognized in traditional medicine for treating fever, wounds, and skin infections. However, while previous studies have explored certain parts of the plant—such as its bark and latex—there remains a scarcity of detailed investigations focusing specifically on the stem bark and its antimicrobial potential (Singh *et al.,* 2010). This lack of

comprehensive data limits our understanding of the plant's pharmacological properties and its possible contribution to overcoming antimicrobial resistance.

Without thorough scientific evaluation, the therapeutic claims associated with *Antiaris toxicaria var. africana* remain largely anecdotal. Thus, this study seeks to bridge that gap by assessing the phytochemical composition and antimicrobial activity of the plant's stem bark, thereby providing scientific evidence to support or refute its traditional use in infection management.

1.3 JUSTIFICATION OF STUDY

The increasing prevalence of antimicrobial resistance (AMR) has become a major public health issue worldwide, making the treatment of infections that were once easily curable more difficult and costly. Although synthetic antibiotics have saved countless lives, their widespread and often inappropriate use has hastened the development of resistant pathogens. This growing resistance has resulted in longer hospital stays, higher medical costs, and a rise in infection-related deaths.

Medicinal plants provide a valuable and natural alternative for combating microbial resistance. Their diverse chemical structures make them an important source of bioactive compounds with potential therapeutic applications. Over the years, many communities have relied on these plants for disease management due to their accessibility, cultural relevance, and relatively low cost.

In Nigeria*, Antiaris toxicaria var. africana* holds a long-standing reputation in traditional medicine for treating ailments such as fever, wounds, and skin infections. Despite this traditional knowledge, there is still limited scientific data to validate these claims, particularly regarding the plant's antimicrobial and phytochemical properties. Scientific verification is therefore essential to determine whether the plant's traditional uses are supported by measurable biological activity.

Furthermore, most existing studies on *A. toxicaria var. africana* have focused on its bark or latex, while few have examined its stem bark in detail. This lack of comprehensive data restricts full understanding of the plant's pharmacological potential and its role in addressing antimicrobial resistance. Conducting this research will not only provide scientific evidence to support its ethnomedicinal use but also contribute to the search for new natural compounds that may be developed into effective antimicrobial agents.

1.4 SCOPE OF WORK

This research is specifically focused on the phytochemical characterization and bacterial inhibitory property of the dichloromethane (DCM) fraction obtained from the stem bark *of Antiaris toxicaria var. africana*. The study begins with the proper collection and identification of the plant material to ensure scientific accuracy and reproducibility of results.

The stem bark was air-dried, pulverized, and extracted using 70% ethanol. The crude extract obtained was fractionated using dichloromethane (DCM) and subsequently analysed using Gas Chromatography–Mass Spectrometry (GC–MS) and High-Performance Liquid Chromatography (HPLC) to identify and quantify its phytochemical constituents.

In addition, the bacterial inhibitory property of the DCM fraction was assessed against selected bacterial and fungal species through standard laboratory assays. These tests aimed to determine the extent of bacterial inhibition and provide insights into the possible relationship between the extract's chemical composition and its biological activity.

By concentrating on the DCM fraction of the stem bark, this study narrows its investigation to a specific segment of the plant's medicinal profile. The outcome is expected to provide scientific validation for its traditional applications, highlight its potential as a natural antimicrobial source, and form a foundation for future studies aimed at isolating and developing novel therapeutic compounds.

1.5 LIMITATIONS OF STUDY

Although this study provides insightful information on the antibacterial properties, of the (DCM) fraction from the stem bark of *Antiaris toxicaria var. africana*, there are some limitations that should be acknowledged.

Firstly, the study focused solely on the stem bark of *Antiaris toxicaria var africana*, even though other parts of the plant, such as the leaves, roots, and latex, might also contain bioactive compounds with medicinal benefits. Limiting the investigation to just one part of the plant may therefore restrict a fuller understanding of its overall therapeutic properties.

Secondly, the antimicrobial tests were carried out against a relatively small selection of bacterial and fungal strains available at the time. This means the results might not fully represent how effective the extract could be against a broader range of harmful microorganisms.

Thirdly, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests, which are important for determining the exact potency of antimicrobial agents, were not conducted. Without these measurements, the study can only confirm the presence of antimicrobial activity, but cannot pinpoint the precise concentrations needed for effective treatment.

In addition, environmental factors such as soil conditions, climate, and the timing of sample collection could have influenced the chemical composition of the stem bark. Such natural variations might lead to differences in the results if the plant material is sourced from other locations or collected at different times.

Finally, this study was limited to in vitro (laboratory) experiments. While these are useful for initial screening, the results cannot be directly applied to living organisms without further pharmacological and toxicological studies.

Despite these limitations, this research provides a strong basis for future work and emphasises the need for broader and more detailed studies to fully explore the pharmacological and therapeutic promise of *Antiaris toxicaria var. africana*.

1.6.1 AIM

This study aims to investigate the phytochemical composition and bacterial inhibitory property of the dichloromethane (DCM) fraction of the ethanol extract of *Antiaris toxicaria var. africana* stem bark using advanced analytical methods such as Gas Chromatography–Mass Spectrometry (GC–MS) and High-Performance Liquid Chromatography (HPLC).

1.6.2 OBJECTIVES

The specific objectives of this study are to:

1. collect, identify, and authenticate the stem bark of *Antiaris toxicaria var. africana*.

2. extract the powdered stem bark using ethanol and fractionate the extract using dichloromethane (DCM).
3. characterize and identify the phytochemical constituents of the DCM fraction using Gas Chromatography–Mass Spectrometry (GC–MS) and High-Performance Liquid Chromatography (HPLC).
4. evaluate the bacterial inhibitory property of the DCM fraction against selected pathogenic microorganisms.

1.7 LITERATURE REVIEW

1.7.1 DESCRIPTION OF PLANT

Fig1.1: Antiaris toxicaria var. africana

The genus *Antiaris* belongs to the *Moraceae* family, which includes several economically and medicinally significant plants such as *Ficus* and *Morus*. Among them, *Antiaris toxicaria var. africana*—commonly known as the African poison tree—is particularly well known for its toxic latex and historical importance in traditional medicine (Huang *et al.,* 2025).

Antiaris toxicaria var. africana is a large deciduous tree that typically grows between 25 and 40 m in height, with a straight cylindrical trunk up to 20 m before branching. The bark is greyish-brown,

rough, and deeply fissured, exuding a milky latex when cut (World Agroforestry Centre, 2025). The tree's crown is broad and spreading, forming a dense canopy that provides significant shade in forest environments. Its leaves are simple, alternate, and broadly ovate with serrated margins. The upper leaf surface is rough in texture, while the underside is covered with soft hairs—features that help reduce water loss and enhance photosynthesis.

Antiaris toxicaria var. africana is commonly found across tropical regions of Africa and Southeast Asia, where it thrives particularly in moist lowland forests and along riverbanks. The species grows best in fertile, well-drained soils and plays an important ecological role in its environment. Its wide crown provides shade, helps regulate local microclimates, and supports biodiversity by serving as a habitat for smaller plants and epiphytes. Additionally, its root system contributes to soil stabilization and forest regeneration. (World Agroforestry, 2025)

Culturally, *A. Toxicaria var. africana* holds great significance in many African communities. The milky latex was historically used as a potent arrow poison during hunting and warfare, earning it the name "Poison Tree." Despite its toxicity, local healers have long utilized various parts of the plant, particularly the bark, for medicinal purposes. Decoctions and powdered bark preparations are traditionally used to treat fevers, wounds, skin infections, ulcers, rheumatism, and inflammatory conditions.

1.7.2TAXONOMICAL CLASSIFICATION OF ANTARIS TOXICARIA

The taxonomic position of *Antiaris toxicaria var. africana* (African poison tree) is as follows;

Kingdom: *Plantae*

Division (Phylum): *Magnoliophyta* (Angiosperms)

Class: *Magnoliopsida* (Dicotyledons)

Subclass: *Dilleniidae*

Order: *Rosales*

Family*: Moraceae*

Genus: *Antiaris*

Species: *Antiaris toxicaria*

Variety: *Antiaris toxicaria var. Africana* (Engl.) C.C. Berg

(Royal Botanic Gardens, Kew, 2024)

Plant name (Scientific name): *Antiaris toxicaria var. africana*

Common names;

English: African poison tree, Bark cloth tree

Yoruba (Nigeria): *Ori*

Igbo (Nigeria): *Odo*

Hausa (Nigeria): *Farin katu*

Other regions: Upas tree

This classification places *Antiaris toxicaria var. africana* among dicotyledonous flowering plants, characterized by broad leaves and net-like venation. Members of the *Moraceae* family are recognized for their latex-producing tissues, which often contain bioactive compounds such as triterpenes, flavonoids, and alkaloids that contribute to their pharmacological properties (Huang *et al.,* 2022). The genus *Antiaris* shares close phylogenetic relationships with *Ficus and Artocarpus*, genera that also possess medicinally active species. These similarities suggest that *A. toxicaria var. africana* may possess comparable bioactive compounds, warranting further exploration into its phytochemical and antimicrobial properties.

1.7.3 MEDICINAL USE OF ANTARIS TOXICARIA VAR AFRICANA

In Nigeria, *Antiaris toxicaria var. africana* continues to hold an important place in traditional medicine despite its highly toxic latex. Ethnopharmacological surveys across various regions reveal its diverse uses among different ethnic groups. Among the Yoruba, decoctions made from the bark are commonly used to treat fever, convulsions, and inflammatory conditions, while poultices from the leaves are applied externally to ease swellings and rheumatism. In Igbo communities, extracts from the bark and roots are traditionally used to manage coughs, asthma, and other respiratory ailments, and they are sometimes incorporated into spiritual practices for

protection. Meanwhile, the Hausa people rely on bark preparations to relieve body pains, malaria symptoms, and gastrointestinal problems (Evbuomwan *et al.,* 2023).

Recent pharmacological research strongly supports these traditional applications. Bioactive compounds such as flavonoids, alkaloids, tannins, and terpenoids found in the bark and leaves exhibit a wide range of biological activities. Extracts of *Antiaristoxicaria* have demonstrated antimicrobial, anti-inflammatory, antioxidant, analgesic, and anti-plasmodial effects, which validate their use in treating infections, pain, and malaria (Evbuomwan *et al.,* 2023). For example, (Sathishkumar *et al.*, 2009) showed that bark extracts acted as powerful reducing agents in the green synthesis of silver and zinc nanoparticles with significant antibacterial properties, opening up new possibilities for therapeutic applications.

However, alongside these medicinal benefits, the plant also presents notable toxicological risks. Its milky latex contains cardiac glycosides such as antiarin, compounds known to disrupt heart function, potentially causing arrhythmias or even cardiac arrest if ingested in high amounts. This dual nature—as both a healing agent and a poison—highlights the importance of careful, controlled use and further pharmacological studies to isolate safe and effective therapeutic compounds while minimizing toxic effects.

Therefore, in Nigerian traditional medicine, *Antiaris toxicaria var. africana* holds a unique and complex role. It is both respected as a versatile natural remedy and regarded with caution as a dangerous toxic plant, reflecting the delicate balance between its therapeutic potential and inherent risks in ethnomedicinal practice.

1.7.4 CHEMICAL COMPOSITION/ PHYTOCHEMICALS PRESENT IN ANTARIS TOXICARIA VAR AFRICANA

The phytochemical composition of *Antiaris toxicaria var. africana* is notably rich and varied, which explains its wide range of biological and pharmacological activities. Phytochemicals are naturally occurring secondary metabolites that plants produce, not for their immediate growth or reproduction, but primarily as protective agents against environmental stress, pathogens, and herbivores (Divekar *et al., 2022)*. In recent years, these compounds have received considerable

attention due to their therapeutic significance, particularly their antimicrobial, antioxidant, anti-inflammatory, and anticancer potentials.

Screening of *Antiaris toxicaria var. africana* has revealed the presence of several important groups of phytochemicals, including alkaloids, flavonoids, tannins, saponins, cardiac glycosides, terpenoids, and phenolic compounds (Subiono *et al.*, 2023). Each class of compound contributes specific biological effects — for instance, flavonoids and phenolics are associated with antioxidant and anti-inflammatory properties, while alkaloids and terpenoids often exhibit antimicrobial and analgesic effects.

The diversity of these bioactive molecules provides scientific support for the plant's long-standing ethnomedicinal use in Nigeria and other parts of Africa. Their combined actions are believed to enhance the plant's therapeutic efficacy, justifying its continued use in traditional medicine for treating various infections and inflammatory diseases.

ALKALOIDS

Alkaloids are nitrogen-containing compounds known for their broad pharmacological activities, including analgesic, antimalarial, and antimicrobial properties (Grijalva *et al*., 2020). In *Antiaris toxicaria*, the alkaloid fraction has been associated with antimicrobial activity against both Gram-positive and Gram-negative organisms, supporting its traditional use in the treatment of infectious diseases.

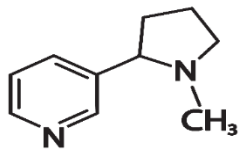


Fig 1.2: Nicotine

FLAVONOIDS

Flavonoids, another major class of phytochemicals, are polyphenolic compounds with strong antioxidant, anti-inflammatory, and antimicrobial effects (Ullah *et al*., 2020). These compounds play a critical role in neutralizing free radicals and reducing oxidative stress, which is a key factor in chronic diseases. Studies have shown that flavonoid-rich extracts from *Antiaris* species exhibit significant antibacterial and antifungal activity (Dubale *et al*., 2023).

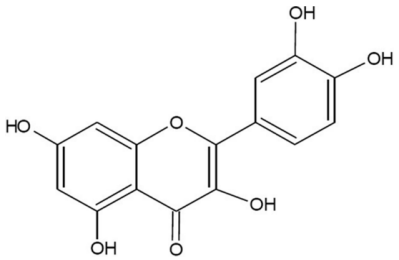


Fig 1.3: Quercetin

TANNINS

Tannins are polyphenolic compounds with astringent properties. They exert antimicrobial action by binding to bacterial proteins and disrupting cell membranes (Huang *et al*., 2018). In *Antiaris toxicaria var. africana*, tannins have been implicated in wound healing and the treatment of gastrointestinal infections, which aligns with its ethnomedicinal applications in Nigeria.

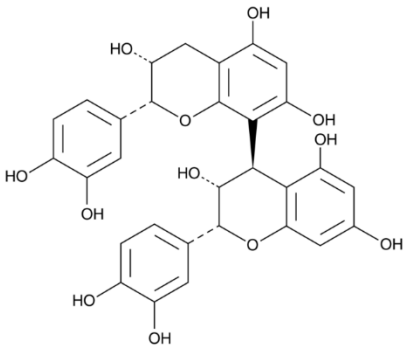


Fig 1.4: Procyanidin

SAPONINS

Saponins are glycosides characterized by their ability to form foams in aqueous solution. Saponins exhibit antimicrobial effects against a wide range of pathogens, including bacteria, viruses, fungi, and protozoa. (Timilsena *et al*., 2023). Additionally, saponins have been linked to immunomodulatory, anti-inflammatory, and cholesterol-lowering properties.

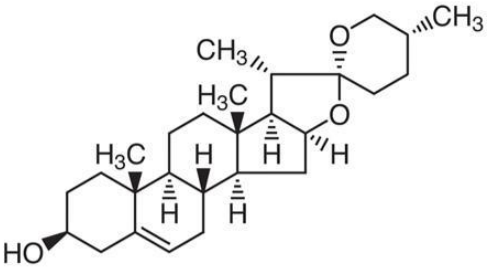


Fig 1.5: Diosgenin

CARDIAC GLYCOSIDES

Cardiac glycosides, particularly cardenolides, are also present in *Antiaris toxicaria*. These compounds act on the Na^+/K^+-ATPase pump in cardiac tissues and are known for their cardiotonic activity (Nath *et al*., 2020). However, their presence also accounts for the plant's toxicity, as excessive intake can lead to cardiac arrest. This dual nature—therapeutic at low doses and toxic at high doses—underscores the need for careful dosage in medicinal use.

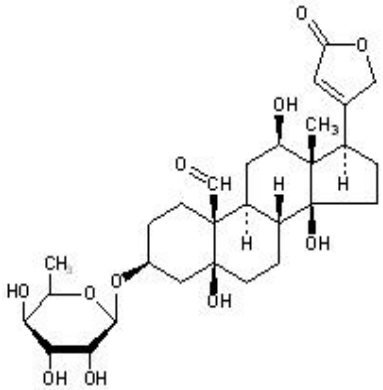


Fig 1.6: Antiarin

TERPENOIDS AND STEROIDS

Terpenoids and steroids have been reported as additional constituents of *Antiaris toxicaria*. These compounds exhibit diverse biological activities, including antimicrobial, anticancer, and anti-inflammatory effects (Roy *et al.,* 2022).

PHENOLIC COMPOUNDS

Phenolic compounds, which are abundant in the plant, contribute strongly to its antioxidant activity. Phenolics are well-documented for their role in disease prevention, particularly in combating oxidative stress and inflammation (Rudrapal *et al*., 2022).

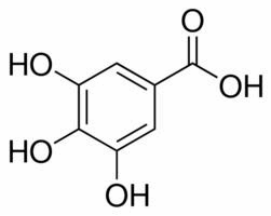


Fig 1.7: Gallic Acid

1.7.5 TOXICITY STUDY

The traditional medicinal plant *Antiaris toxicaria var. Africana* contains strong bioactive substances that have the ability to alter cellular activity. The plant has both medicinal value and inherent toxicity when not properly controlled, as evidenced by studies that show strong cytotoxic and pro-apoptotic effects in extracts from the plant (Thiam *et al.*, 2022).

There have also been cases of accidental poisoning in people, especially when the latex or bark was used without proper preparation. In some severe instances, this has led to heart failure and even death, which explains why the plant is sometimes called the “Poison Tree”. Researchers have found that compounds like antiarin disrupt the sodium-potassium pump in cells, which messes up the heart’s rhythm and can be very dangerous (Nath *et al*., 2020).

Despite these risks, traditional healers have long known how to use the plant safely. They prepare the extracts carefully, often diluting them and controlling the amount given. This knowledge,

passed down over generations, has allowed communities to benefit from the plant's healing powers while avoiding the worst toxic effects.

This fine line between healing and harm makes *Antiaris toxicaria var. africana* a fascinating plant. It demands respect because it can be both a powerful medicine and a deadly poison, depending on how it's used. That's why ongoing research is so important—to better understand its safety, set safe dosages, and find ways to use its medicinal benefits without putting anyone at unnecessary risk.

1.7.6 COLUMN CHROMATOGRAPHY

Column chromatography is a purification technique used to separate different compounds in a plant extract based on their polarity. It works by passing the extract through a column packed with a stationary phase, usually silica gel, while a solvent or mixture of solvents (the mobile phase) flows through it. Compounds that are less polar move faster through the column, while more polar ones move slower, leading to their separation into different fractions.

In phytochemical studies, such as with *Antiaris toxicaria var. africana,* column chromatography helps isolate the active components of the crude extract for further analysis using techniques like Gas Chromatography–Mass Spectrometry (GC–MS). This process makes it easier to identify and study the specific compounds responsible for the plant's biological activities.

1.7.7 ANTIMICROBIAL SUSCEPTIBILITY TESTING

An essential stage in assessing the biological activity of plant extracts and isolated phytochemicals is antimicrobial susceptibility testing. It validates the potential therapeutic uses of these extracts by providing information on their capacity to inhibit or eradicate harmful bacteria. The two most popular approaches are the broth dilution procedures and the agar diffusion techniques (such disc diffusion and well diffusion).

Plant extracts are added to wells or impregnated discs that are set on agar plates that have been infected with test microorganisms in agar diffusion techniques. To determine the antibacterial potency, the zone of inhibition—the clear area surrounding the extract—is assessed after

incubation. This approach is straightforward and economical, but it is mostly qualitative and cannot precisely determine the concentrations needed to stop microbial growth.

When comparing the relative potencies of plant-derived antimicrobials and conventional antibiotics, these values are crucial. Furthermore, in order to guarantee a broad-spectrum assessment, the choice of test organisms is essential and frequently includes both Gram-positive and Gram-negative bacteria in addition to fungus. Significantly antibacterial plant extracts could be used as building blocks for new medications, particularly in light of the rise in multidrug resistance. The ethnomedicinal value of plants is thus validated by antimicrobial susceptibility testing, which also helps to close the gap between traditional

1.7.8 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

One of the most effective analytical methods for identifying and measuring volatile and semi-volatile substances in plant extracts is gas chromatography-mass spectrometry (GC-MS). The technique combines mass spectrometry's capacity for structural elucidation with the high separation efficiency of gas chromatography. In GC, an inert gas (like helium) vaporizes the sample and transports it into a capillary column, where its components are separated according to their volatility and interactions with the stationary phase of the column. After being separated, each compound is sent into the mass spectrometer, where it undergoes ionization, fragmentation, and detection to create a distinct mass spectrum. To identify compounds, this spectrum is compared to standard libraries (such as those maintained by the National Institute of Standards and Technology, or NIST).

GC-MS has been extensively applied in phytochemical studies to detect bioactive compounds such as alkaloids, flavonoids, terpenoids, phenolics, and fatty acids. Its high sensitivity, selectivity, and reproducibility make it invaluable for characterizing plant metabolites, especially in antimicrobial and pharmacological research. However, since it requires volatility and thermal stability, non-volatile compounds may need derivatization before analysis.

1.7.9 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High Performance Liquid Chromatography (HPLC) is a widely used technique for the separation, quantification, and purification of non-volatile and thermolabile compounds in plant extracts. Unlike GC-MS, HPLC operates in the liquid phase, where samples are dissolved in a suitable solvent and pumped under high pressure through a column packed with stationary phase particles. The interaction between analytes, mobile phase, and stationary phase governs separation.

Detection in HPLC is often achieved using UV-VIS detectors, fluorescence detectors, or mass spectrometry (LC-MS) for higher sensitivity and accuracy. This allows precise quantification of compounds such as alkaloids, tannins, flavonoids, phenolic acids, and glycosides.

HPLC is particularly valuable in standardization studies, enabling researchers to determine the concentration of active phytochemicals and assess extract purity. It is also essential for quality control in herbal formulations, ensuring reproducibility and consistency of bioactive constituents.

CHAPTER TWO

MATERIALS AND METHOD

2.1 MATEEIALS

Measuring cylinder

Beakers

Glass jar

Spatula

Test tubes

Funnel

Analytical balance

Wash bottle

Filter paper

Heating mantle

Foil paper

Pipette

Sample bottles

Chromatographic column

Industrial blender

Water bath

Thermometer

Masking tape

Hand gloves

Face mask

Cotton wool

Tissue paper

Mortar and pestle

Retort stand

Burette clamp (Boss clamp)

2.2 REAGENTS

Distilled water

70% Ethanol (BDH, England)

Dichloromethane (DCM) (Sigma-Aldrich, Germany)

Silica gel 60-120 mesh (Merck, Germany)

2.3 METHOD

2.3.1 SAMPLE COLLECTION AND AUTHENTICATION

Antiaris toxicaria var. Africana (commonly *Antiaris toxicaria*) used in this study was collected on March 21, 2025, from a mature tree in front of the Faculty of Pharmacy (New Building), University of Benin, Ugbowo Campus, Benin City, Edo State, Nigeria (6°23'36"N 5°37'17"E) . The site, located within the tropical rainforest zone is noted for its high humidity, rainfall, and rich biodiversity—factors supporting medicinal plant growth and phytochemical variation.

Stem bark was chosen as the study material due to its ethnomedicinal and pharmacological significance. Immediately after harvesting, fresh bark samples were placed in sterile polythene bags, labelled with the date and location to prevent contamination.

The plant sample was authenticated and identified by Prof. E. I. Aigbokhan at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. Following taxonomic evaluation, the plant was confirmed as *Antiaris toxicaria var. africana* (Engl.) C.C.

Berg,, and a voucher specimen (EIA02063) was prepared and deposited in the departmental herbarium, ensuring traceability and scientific credibility for this research.

2.3.2 EXTRACTION OF ANTARIS TOXICARIA STEM BARK

After being carefully cleaned with tap water to get rid of any sand or debris, the harvested stem bark of *Antiaris toxicaria var. africana* was allowed to air dry for two weeks in the shade. A clean electric grinder was used to grind the dried bark into a fine powder, which was then moved to a sterile, airtight container and kept at ambient temperatures until it was extracted. A 400g sample of the finely ground bark was macerated for 120 hours at ambient temperature in 2 × 2.5 L of 70% ethanol. To enhance solvent penetration, the resulting mixture was shaken every 30 minutes for the first two hours. After that, it was allowed to stand with sporadic hand shaking to guarantee complete extraction. The resulting mixture was separated by decanting and filtered through the Whatman No. 1 filter paper in order to clarify the extract.

A hot water bath set at 60°C was used to concentrate the mixed filtrates until they were completely dry and the solvent had mostly evaporated, leaving behind a crude extract. After being moved to labelled sample vials, this extract was kept at 4°C for subsequent analysis.

2.4 FRACTIONATION OF CRUDE EXTRACT USUNG COLUMN CHROMATOGRAPHY

The fractionation of the crude extract was carried out using column chromatography. A clean glass chromatography column was mounted vertically on a retort stand and held securely with clamps. A small plug of cotton wool was first placed at the bottom of the column to prevent the loss of stationary phase material.

The column was then packed with dry silica gel (60–120 mesh) serving as the stationary phase. Approximately 50–100 g of silica gel was added carefully into the column in portions while gently tapping the sides to allow proper settling and uniform packing. The dried crude extract was thoroughly mixed with a small amount of silica gel to form a fine, free-flowing mixture and was gently placed on top of the packed column.

Elution was performed using dichloromethane (DCM) as the mobile phase. The solvent was passed gradually through the column, allowing the components of the extract to separate based on their polarity and affinity for the stationary phase.

Eluates were collected in beakers as distinct fractions based on observable color changes. The collected fractions were concentrated by allowing the solvent to evaporate, yielding semi-solid residues. These DCM fractions were stored in labeled sample vials at 4°C for subsequent analysis, Gas Chromatography–Mass Spectrometry (GC–MS), High-Performance Liquid Chromatography (HPLC), and antimicrobial activity testing.

2.5 GC-MS SPECTROMETRY ANALYSIS

2.5.1 EXTRACTION OF THE PHYTOCHEMICAL FOR GC-MS ANALYSIS.

A test tube was filled with 1g of the dichloromethane (DCM) fraction and 25mL of ethanol. The mixture was cooked for 90 minutes at 60 °C on a hotplate. After cooling, the material was moved into a funnel for separation. The test tube was filled sequentially with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water, and 3mL of hexane, the rinses were mixed with the extract in the funnel. The organic layer was then dried over anhydrous sodium sulfate, and the solvent was evaporated to obtain a residue. The residue was reconstituted in 1 mL of pyridine, and 200 µL of this solution was transferred into a GC vial for analysis.

2.5.2 GC–MS ANALYSIS CONDITION.

A BUCK M910 (Agilent 6890 gas chromatography equipped with mass spectroscopy) gas chromatograph, coupled with a mass spectrometer and an HP-5MS capillary column (30 m 0.25 mm 0.25 m film thickness), was used to conduct the phytochemical analysis. At 70 eV, the electron ionization (EI) mode was used. As the carrier gas, high-purity helium (99.995%) was used at a steady flow rate of 1.0 mL/min. The initial temperature of the oven was set at 50 °C, ramped up to 150 °C (10 min hold) at 3 °C/min, and then increased to 300 °C at 10 °C/min. The produced extract (1% solution in acetonitrile) was delivered in split-less mode in a microlitre (1 µL).

Relative abundances of the phytochemicals were calculated as peak area percentages from the total ion chromatogram.

2.5.3 IDENTIFICATION OF CONSTITUENTS.

The bioactive compounds were identified by comparing their retention times with those of reference standards and by matching their mass spectra with data available in the GC–MS system libraries (Replib and Mainlab data of GC–MS systems).

2.6 HPLC ANALYSIS

2.6.1 EXTRACTION OF PHYTOCHEMICALS FOR HPLC ANALYSIS.

10 mL of a 50% m/v potassium hydroxide solution and 15 mL of ethanol were added to a test tube containing 0.2 g of the extract. The mixture spent three hours at 60 °C in a water bath. After that, the reaction mixture was moved to a separatory funnel, and the test tube was rinsed with 3 mL of hexane, 10 mL of cold water, 10 mL of hot water, and 20mL of ethanol. 10 mL of a 10% v/v ethanol aqueous solution was used to wash the mixture three times after these washings were mixed in the separatory funnel. Following the removal of the ethanol solvent, the residue was dissolved in 1000 µL of pyridine, 200 µL of which was then put into a vial for HPLC analysis.

2.6.2 HIGH-PERFOMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS CONDITION.

A Shimadzu LC-10AD dual binary pump system, a Shimadzu CTO-10AS column oven, and a Shimadzu Prominence SPD-20A UV/VIS detector was used for the HPLC study. Using a C-12 normal phase column (Phenomenex, Gemini 5 µm, 200 mm × 4.8 mm i.d.), separation was accomplished. At a flow rate of 0.8 mL/min, the mobile phase was composed of acetonitrile as solvent B and acetic acid-acidified deionized water (pH 2.8) as solvent A. A 20 µL injection volume and a 38 °C column temperature was maintained. At 280 nm, phenolic chemicals were detected, identified, and measured by contrasting peak areas and retention durations with real standards. The external standard method was utilized in the construction of calibration curves.

The gradient elution program was as follows:

0–5 min: 5–9% solvent B

5–15 min: 9% solvent B

15–22 min: 9–11% solvent B

22–38 min: 11–18% solvent B

38–43 min: 18–23% solvent B

43–44 min: 23–90% solvent B

44–45 min: 90–80% solvent B

45–55 min: equilibration phase

2.7 ANTIBACTERIAL ACTIVITY OF ANTARIS TOXICARIA VAR AFRICANA

2.7.1 SPECIMEN COLLECTION

The bacterial isolates used in this study were obtained from the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. The test organisms included: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Enterobacter cloacae, and Bacillus cereus*.

2.7.2 PREPARATION OF TEST ORGANISMS

The isolates were kept at -20°C in a broth containing 20% glycerol until they were needed. Before the experiment began, each isolate was sub cultured onto sterile nutrient agar plates, and the colonies were obtained by incubating them overnight at 37 °C. After that, colonies were suspended in nutritional broth that had been sterilized and cultured for 12 hours. Adjusting the suspension's turbidity to 0.5 McFarland standard meant that the inoculum size was roughly 1.0×10^7 CFU/mL.

2.7.3 ANTIMICROBIAL SUSCEPTIBILITY TEST AND DETERMINATION OF INHIBITION ZONE DIAMETER (IZD)

Using the agar well diffusion method described by CLSI (2016), the antibacterial activity of the DCM fraction of *Antiaris toxicaria var. africana* was assessed. To solidify, 30 millilitres of Mueller-Hinton agar were put into sterilised Petri dishes. Then, to get rid of any residual surface moisture, the plates were dried for five minutes at 40 °C in a hot-air oven. In order to prevent

cross-contamination, a sterile inoculating loop that was flamed in between uses was used to streak the agar surface of each plate with 0.5 McFarland standardized bacterial suspension.

Using a sterile cork borer, wells (10 mm in diameter) were aseptically drilled into the agar. 0.02 mL of molten Mueller-Hinton agar was then added to the base of each well. 0.2 mL of a stock solution containing 15 mg/mL of the DCM fraction was added to one well of each plate. The second well of each plate was filled with 0.2 mL of ciprofloxacin (1.6 µg/mL) as a reference control.

18 to 24 hours were spent incubating the plates at 37 °C. By measuring the inhibition zone diameters (IZD) surrounding the wells in millimeters after incubation, the antibacterial activity was determined. The inhibition zones were then compared to those of the common antibiotic to ascertain each bacterial isolate's susceptibility or resistance to the ethanolic fraction of *A. toxicaria.*

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS RESULT

Table 1: Putative compounds identified by GC-MS in DCM fraction of *Antiaris toxicaria var. africana* stem bark

peak	RT (min)	% Area	Compound Name	Molecular Formular	Molecular weight
1	5.422	7.39%	Benzoic acid, methyl ester	$C_7H_6O_2$ / $C_8H_8O_2$	122.12 /136.15
2	10.543	18.86%	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32
3	11.298	3.15%	7-Hexadecene	$C_{16}H_{32}$	224.43
4	12.328	4.44%	Cyclododecane	$C_{12}H_{24}$	168.32
5	13.273	5.48%	9-Octadecene	$C_{18}H_{36}$	252.48
6	14.491	14.35%	Hexadecanoic acid, methyl ester	$C_{16}H_{36}O_2$	256.43
7	14.846	17.69%	n-Hexadecanoic acid	$C_{16}H_{36}O_2$	256.43
8	15.064	6.39%	1-Nonadecene	$C_{19}H_{38}$	266.51
9	16.048	5.02%	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.47
10	16.294	6.66%	Methyl stearate	$C_{19}H_{38}O_2$	298.51
11	16.471	6.64%	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.47
12	17.032	3.94%	1-Docosene	$C_{22}H_{44}$	308.58

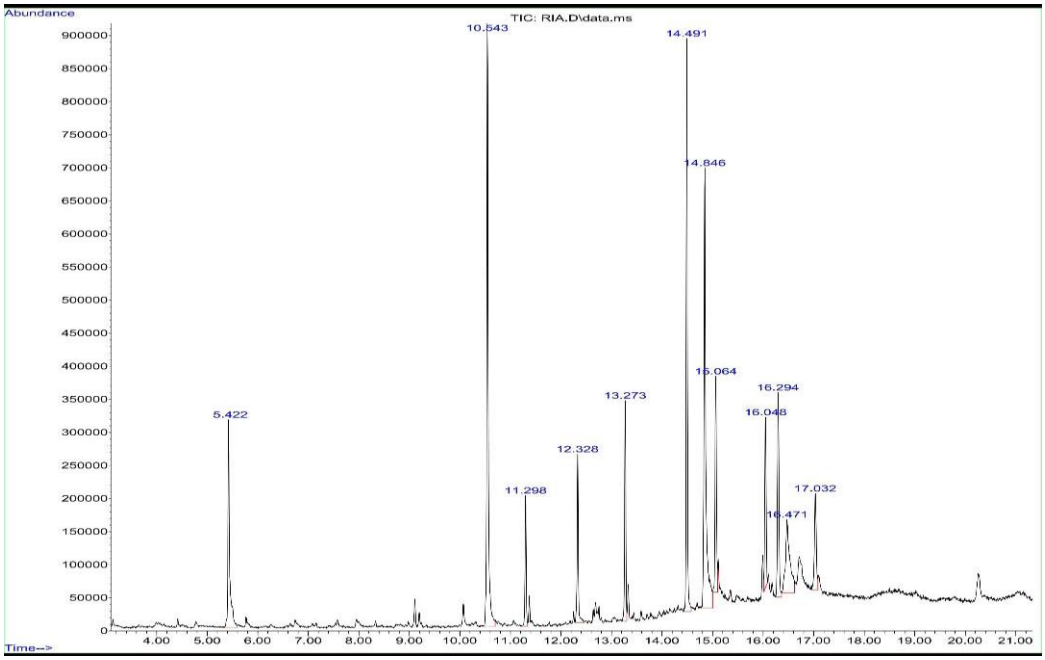


Fig 3.1: GC MS graph of the DCM fraction of Antiaris toxicaria var. Africana

The GC–MS analysis of the dichloromethane fraction of *Antiaris toxicaria var. Africana* revealed several important bioactive compounds. Among these were phenolics like benzoic acid derivatives and 2,4-di-tert-butylphenol, fatty acids and their esters such as hexadecanoic acid methyl ester and methyl stearate, as well as long-chain hydrocarbons including 7-hexadecene and 1-nonadecene. Notably, 2,4-di-tert-butylphenol made up the largest portion (18.86%) of the extract and is known for its strong antibacterial effects, especially against Gram-positive bacteria like *Cutibacterium acnes and Bacillus cereus.*

The considerable amounts of fatty acid derivatives, such as hexadecanoic acid methyl ester (14.35%) and n-hexadecanoic acid (17.69%), add to the extract's antimicrobial potential. These fatty acids are known to disrupt bacterial membranes, making it harder for bacteria—both Gram-positive and Gram-negative—to grow.

Finding these compounds gives us a clearer picture of why the extract showed antimicrobial activity in lab tests. While the phenolics like 2,4-di-tert-butylphenol likely play a leading role because of their direct ability to kill bacteria, the fatty acids and their esters help boost this effect by weakening bacterial membranes and allowing the compounds to penetrate better. Even the

hydrocarbons, which are less powerful on their own, might help by improving the extract's stability and solubility, supporting its overall effectiveness.

These results not only back up the traditional use of *Antiaris toxicaria var. africana* as a medicinal plant but also make it an interesting candidate for future research into new antimicrobial drugs.

3.2 ANTIMICROBIAL ACTVITY RESULT

Table 2; **Bacterial inhibitory zones produced by stem bark extract of *Antiaris toxicaria var. africana* and control drug (ciprofloxacin)**

Micro-organism	*Antiaris toxicaria var. africana* extract (15 mg/mL)	Control; Ciprofloxacin (1.6 µg /mL)
Staphylococcus aureus	19 mm	35 mm
Escherichia coli	20 mm	28 mm
Pseudomonas aeruginosa	19 mm	38 mm
Bacillus subtilis	18 mm	28 mm
Enterobacter cloacae	17 mm	40 mm
Bacillus cereus	No zone (0 mm)	38 mm

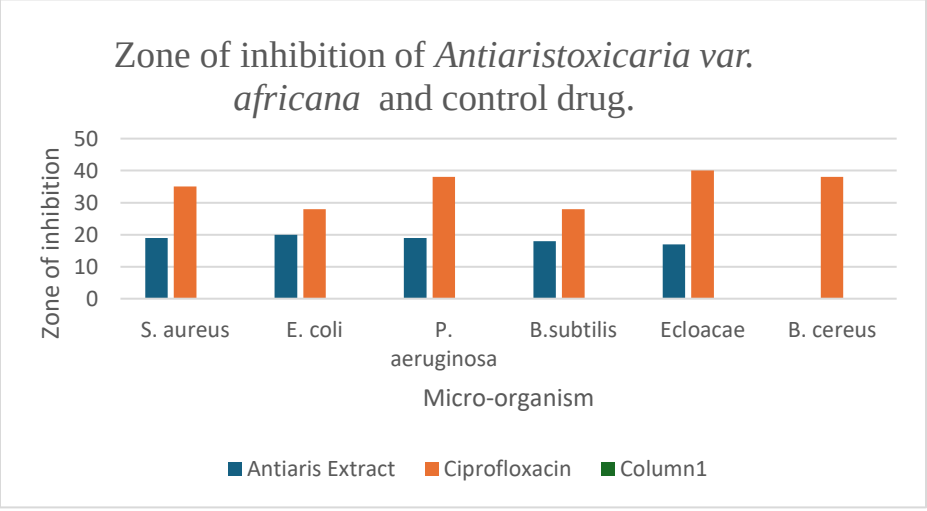


Fig 3.2: Zones of inhibition graph of *Antiaris toxicaria var. africana* and control drug.

The ethanolic stem bark extract of *Antiaris toxicaria var. africana* showed inhibitory action against a range of microorganisms, both Gram-positive and Gram-negative

when subjected to antimicrobial screening. As with *Bacillus cereus,* the extract showed no discernible activity, but it showed inhibitory zones of 17–20 mm against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Enterobacter cloacae.* On the other hand, ciprofloxacin, a common antibiotic, demonstrated consistently larger zones of inhibition (28–40 mm), demonstrating its increased potency. Considering the plant extract's crudeness, the observed inhibitory zones are significant even though the extract could not match the effectiveness of ciprofloxacin. *Antiaris toxicaria's* broad-spectrum potential is supported by its action against both *S. aureus* and *E. coli,* which lends credence to its ethnomedical application in infection management.

It is interesting to note that the lack of inhibition against *B. cereus* may indicate an innate resistance or that the extract's active phytochemical concentration was insufficient to have an impact. Due to variations in the permeability and composition of their cell walls, bacteria are frequently species-specific in their susceptibility to plant extracts. The bioactive substances found in the GC–MS analysis, such as fatty acids (e.g., hexadecanoic acid, methyl ester, and octadec-9-enoic acid) and phenolic derivatives (e.g., 2,4-di-tert-butylphenol), which are widely recognised for having antibacterial qualities., may be responsible for the study's moderate activity. The hypothesis that complex combinations in plant extracts may contribute to therapeutic benefits even when individual compounds are present in low amounts is supported by the likely synergistic actions of these chemicals.

In conclusion, the results support the traditional usage of *A. toxicaria var. africana* stem bark extract in the treatment of infectious disorders by showing that it has potential antibacterial activity.

The extract is not as effective as ciprofloxacin, but it is a good source of bioactive compounds that could be exploited as leads for the development of novel antimicrobial medications, especially in an era of growing antimicrobial resistance.

3.3 RESULT OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Table 3; Phyto-constituents from HPLC analysis of the DCM fraction of *Antiaris toxicaria var. africana*

S/N	Compounds	Retention time	Area	Height	Concentration (µg/mL)
1.	Kaempferol	0.226	1567.7301	71.377	1.8214
2.	Steroid	2.223	6781.4592	212.138	4.6952
3.	Proanthocyanidin	3.950	8155.3112	255.541	13.9885
4.	Anthocyanin	6.893	4481.9072	140.323	5.7657
5.	Narigenin	10.593	4332.5197	135.220	5.5688
6.	Dihydrocytisine	13.300	4913.1862	154.220	6.3206
7.	Cyanogenic glycoside	15.783	12807.2396	395.766	8.9749
8.	Aphyllidine	19.573	12950.5678	358.644	0.8215
9.	Ammodendrine	22.293	4750.4508	148.987	2.3273
10	Tannin	26.000	6794.9023	212.169	3.6986
11	Flavonones	28.566	5789.7791	180.678	9.9310
12	Cardiac glycoside	29.490	4450.4074	139.936	5.5250
13	Flavone	34.176	9150.8176	195.436	11.3604
14	Ribalinidine	37.260	6555.3447	204.731	8.1357
15	Spartein	38.326	9314.0338	293.230	11.5595
16	Phytate	39.590	4172.2344	135.004	5.1781
17	Oxalate	40.930	3158.2206	104.042	1.1912
18	Epihedrine	42.090	5702.9629	183.801	7.0800
19	Sapogenin	42.943	6412.2832	203.007	7.9606

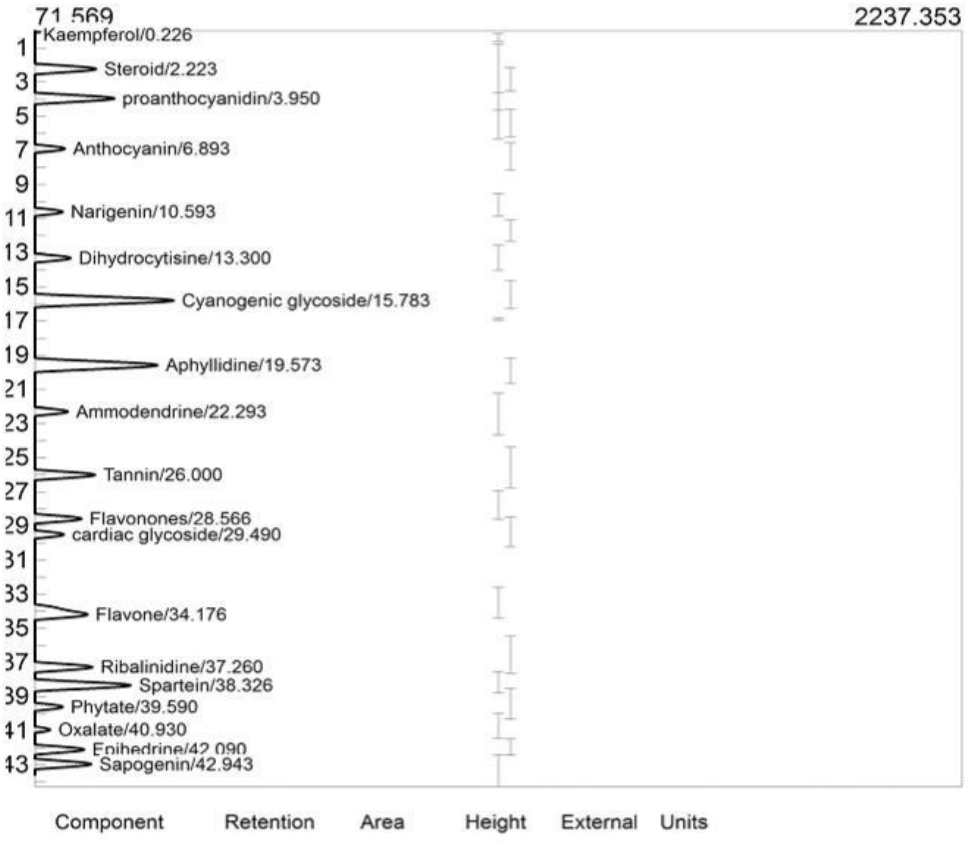


Fig: 3.3 HPLC graph of the DCM fraction of Antiaris toxicaria var. Africana

Antiaris toxicaria var. africana's ethanolic stem bark extract's HPLC profile showed a wide range of phytochemicals, with the most prevalent compounds being proanthocyanidin (13.99 µg/mL), flavone (11.36 µg/mL), and sparteine (11.56 µg/mL). Flavonoids such as kaempferol, narigenin, anthocyanin, and proanthocyanidin are known to have antibacterial and antioxidant properties, therefore their high concentration is particularly significant. For example, recent research have shown that kaempferol exhibits antibacterial activity against fungus and bacteria in plant extracts. (Periferakis *et al*., 2022) Furthermore, by compromising the integrity of the membrane and scavenging free radicals, phenolic substances like proanthocyanidins have been demonstrated to inhibit bacterial growth.

This is in line with the extract's elevated proanthocyanidin levels, which most likely support the antibacterial action seen. The identification of sapogenins, cardiac glycosides, and other secondary

metabolites enhances the extract's potential for therapeutic use. Though they are more frequently described in terms of cardiotonic effects, cardiac glycosides can also have cytotoxic or antibacterial actions in some situations, which may be a subtle factor in the extract's reported bioactivity. The HPLC results generally show good agreement with the GC-MS results and the antimicrobial testing, indicating that the stem bark of *A. toxicaria var. africana* contains chemicals that can suppress bacterial infections.

These findings lend credence to the plant's ethnomedical application with a strong scientific basis and encourage further study, such as safety evaluation, MIC/MBC computations, and purification of the most potent compounds.

RECOMMENDATION FOR FURTHER STUDY

1. **Isolation and characterisation of active compounds**: Future studies should concentrate on identifying and separating the particular phytochemicals that exhibit antimicrobial activity, then employing sophisticated analytical methods to clarify their structures.
2. **Toxicological and safety evaluation**: To determine if the extracts or compounds are suitable for clinical use, thorough toxicity and safety evaluations are required.
3. **Mechanism of action studies**: By examining the molecular processes that underlie the antibacterial activity, the pharmacological pathways involved will become more clear.
4. **Broader antimicrobial screening**: More research should examine the extract’s ability to fight 4. against a wider range of pathogens, including resistant bacterial strains, clinical isolates, and fungi.
5. Formulation and drug development studies: Efforts should be made to explore novel delivery systems, such as nanoparticles or herbal-based formulations, to improve bioavailability and therapeutic efficacy.

CONCLUSUON

According to HPLC analysis, it shows that the stem bark extract of *Antiaris toxicaria var. africana* contains a variety of phytochemicals, such as flavonoids, alkaloids, cardiac glycosides, tannins, steroids, and other secondary metabolites, as this study showed. Many of the bioactive qualities of these substances are closely linked to their antibacterial ability. The antimicrobial assay also showed that the extract had inhibitory activity against a variety of bacterial species, but it was generally less effective than ciprofloxacin, the conventional medication, indicating that while the extract possesses notable antibacterial potential, it may require higher concentrations or further purification to achieve comparable efficacy to standard antibiotics. The extract's antimicrobial spectrum was selective, as evidenced by its notable ineffectiveness against *Bacillus cereus.*

These results back up *Antiaris toxicaria var. africana's* traditional use in the treatment of infectious disorders and confirm its ethnomedical significance. The extract's relatively low potency, despite its encouraging bioactivity, emphasises the need for more research to maximise its medicinal potential.

REFERENCES

Dias, D. A., Urban, S., and Roessner, U. (2012). A historical overview of natural products in drug discovery. Metabolites, 2(2), 303–336. https://doi.org/10.3390/metabo2020303

Divekar, P. A., Narayana, S., Divekar, B. A., Kumar, R., Gadratagi, B. G., Ray, A., Singh, A. K., Rani, V., Singh, V., Singh, A. K., Kumar, A., Singh, R. P., Meena, R. S., and Behera, T. K. (2022). Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. International Journal of Molecular Sciences, 23(5), 2690. https://doi.org/10.3390/ijms23052690

Djerdjouri, A., Abbad, M., Boumrah, Y., Malik, S., Makhzoum, A., and Lakhdar, K. (2024). Tapping the potential of Calotropisprocera hairy roots for cardiac glycosides production and their identification using UHPLC/QTOF-MS. 3 Biotech, 14(9), 199. https://doi.org/10.1007/s13205-024-04035-1

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., and Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. Journal of experimental pharmacology, 15, 51–62. https://doi.org/10.2147/JEP.S379805

Evbuomwan, I. O., Stephen Adeyemi, O., and Oluba, O. M. (2023). Indigenous medicinal plants used in folk medicine for malaria treatment in Kwara State, Nigeria: an ethnobotanical study. BMC complementary medicine and therapies, 23(1), 324. https://doi.org/10.1186/s12906-023-04131-4

Gutiérrez-Grijalva, E.P., López-Martínez, L.X., Contreras-Angulo, L.A., Elizalde-Romero, C.A., Heredia, J.B. (2020). Plant alkaloids: structures and bioactive properties. In: Swamy, M. (eds) Plant-derived Bioactives. Springer, Singapore. https://doi.org/10.1007/978-981-15-2361-8_5

Huang, J., Li, X., Zhang, Q. and Chen, L., 2025. A high-quality chromosome-level genome assembly of Antiaris toxicaria. BMC Genomic Data, 26(1), p.45. Available at: https://doi.org/10.1186/s12863-025-01309-2

Huang, Q., Liu, X., Zhao, G., Hu, T., and Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Animal nutrition (Zhongguo xu mu shou yi xue hui), 4(2), 137–150. https://doi.org/10.1016/j.aninu.2017.09.004

Huang, W., Wang, Y., Tian, W., Cui, X., Tu, P., Li, J., Shi, S., and Liu, X. (2022). Biosynthesis investigations of terpenoid, alkaloid, and flavonoid antimicrobial agents derived from medicinal plants. antibiotics (Basel, Switzerland), 11(10), 1380. https://doi.org/10.3390/antibiotics11101380

Nath, A., Anusree, S., Navis, S. and Prasobh, G.R., 2020. Herbal temedies for cardiovascular diseases. Int. J. Pharm. Sci. Rev. Res., 64(1), pp.133-138. Available at: https://globalresearchonline.net/journalcontents/v64-1/25.pdf

O'Neill, J. (2016). Tackling drug-resistant infections globally: Final report and recommendations. Review on antimicrobial resistance. UK Government and wellcome trust. https://www.scirp.org/reference/referencespapers?referenceid=2618400

Omotayo B. Ilesanmi, Afolabi C. Akinmoladun, Christianah A. Elusiyan, Ifedayo V. Ogungbe, Tiwalade A. Olugbade, and M. Tolulope Olaleye. Neuroprotective flavonoids of the leaf of *Antiaris africana Englea* against cyanide toxicity, Journal of Ethnopharmacology,Volume 282,2022,114592,ISSN 0378-8741. https://doi.org/10.1016/j.jep.2021.114592.

Periferakis, A., Periferakis, K., Badarau, I.A., Petran, E.M., Popa, D.C., Caruntu, A., Costache, R.S., Scheau, C., Caruntu, C. and Costache, D.O., 2022. Kaempferol: Antimicrobial properties, sources, clinical, and traditional applications. International Journal of Molecular Sciences, 23(23), https://doi.org/10.3390/ijms232315054

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., Alshahrani, M. Y., and Islam, M. R. (2022). Flavonoids: A bioactive compound from medicinal plants and its therapeutic applications. BioMed Research International, 2022, 5445291. https://doi.org/10.1155/2022/5445291

Royal Botanic Gardens, Kew, 2024. Antiaris toxicaria (J.F.Gmel.) Lesch. Plants of the world online. Available at: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:850341-1

Rudrapal, M., Khairnar, S. J., Khan, J., Bin Dukhyil, A., Ansari, M. A., Alomary, M. N., Alshabrmi, F. M., Palai, S., Deb, P. K., and Devi, R. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. Frontiers in Pharmacology, 13, Article 806470. https://doi.org/10.3389/fphar.2022.806470

Sathishkumar, M., Sneha, K., Won, S. W., Cho, C. W., Kim, S., and Yun, Y. S. (2009). Cinnamon zeylanicum bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloids and surfaces. B, Biointerfaces, 73(2), 332–338. https://doi.org/10.1016/j.colsurfb.2009.06.005

Shi, L. S., Kuo, S. C., Sun, H. D., Morris-Natschke, S. L., Lee, K. H., and Wu, T. S. (2014). Cytotoxic cardiac glycosides and *coumarins* from *Antiaris toxicaria*. Bioorganic & medicinal chemistry, 22(6), 1889–1898. https://doi.org/10.1016/j.bmc.2014.01.052

Singh, M., Khatoon, S., Singh, S., Kumar, V., Rawat, A. K., and Mehrotra, S. (2010). Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. Pharmacognosy research, 2(4), 254–257. https://doi.org/10.4103/0974-8490.69127

Subiono, T., Sadarudin, and Tavip, M.A., 2023. Qualitative and quantitative phytochemicals of leaves, bark and roots of *Antiaris toxicaria* Lesch., a promising natural medicinal plant and source of pesticides. Plant Science Today, 10(1), pp.5-10. Available at: https://doi.org/10.14719/pst.1896

Thiam, K., Zhao, M., Marchioni, E., Muller, C. D., Diop, Y. M., Julien-David, D., and Emhemmed, F. (2022). Cytotoxic and pro-apoptotic effects of leaves extract of *Antiaris africana* Engler (*Moraceae*). Molecules (Basel, Switzerland), 27(22), 7723. https://doi.org/10.3390/molecules27227723

Timilsena, Y. P., Phosanam, A., and Stockmann, R. (2023). Perspectives on Saponins: Food functionality and applications. International journal of molecular sciences, 24(17), 13538. https://doi.org/10.3390/ijms241713538

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H., and Jaremko, M. (2020). Important Flavonoids and their role as a therapeutic agent. Molecules (Basel, Switzerland), 25(22), 5243. https://doi.org/10.3390/molecules25225243

WHO (2019). WHO global report on traditional and complementary medicine 2019. Geneva: World Health Organization. https://iris.who.int/handle/10665/312342

World Agroforestry Centre. (2025) *Antiaris toxicaria* Lesch: Tree species database entry. Available at: https://apps.worldagroforestry.org/usefultrees/pdflib/Antiaris_toxicaria_ETH.pdf

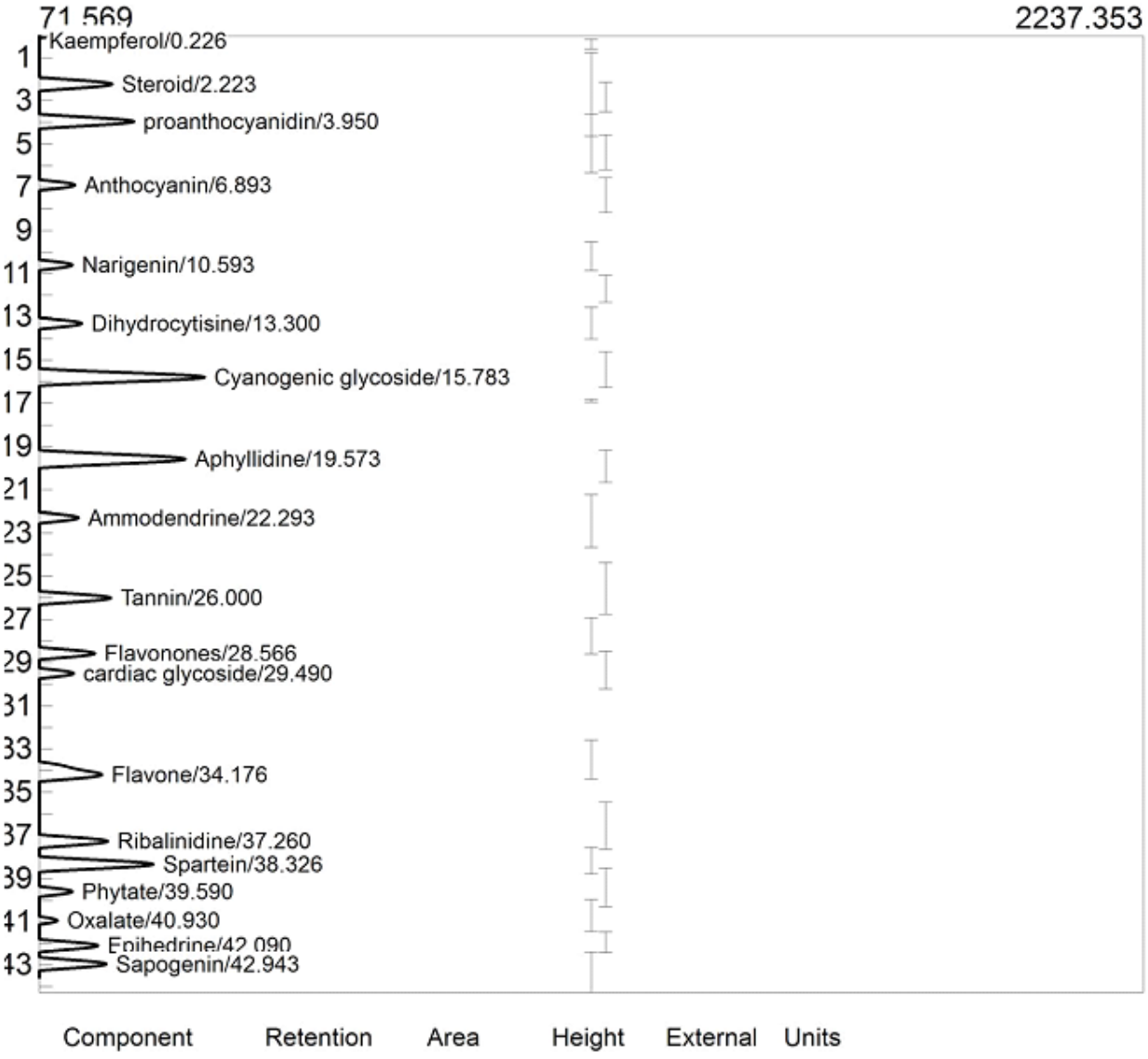
World Agroforestry, 2025. *Antiaris toxicaria* species profile. Available at: https://apps.worldagroforestry.org/treedb2/speciesprofile.php?Spid=1782

Yeshi, K., Crayn, D., Ritmejerytė, E., and Wangchuk, P. (2022). Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. Molecules (Basel, Switzerland), 27(1), 313. https://doi.org/10.3390/molecules27010313

Zhang, J., Onakpoya, I. J., Posadzki, P., and Eddouks, M. (2015). The safety of herbal medicine: from prejudice to evidence. Evidence-based complementary and alternative medicine : eCAM, 2015, 316706. https://doi.org/10.1155/2015/316706

APPENDIX A: HPLC RESULT

Client: Rita
Collected: 09 July 2025
Description: UV
Column: RESTEK 15METER MXT-1
Carrier: Acetic acid AT 5PSI
Data file: Phytochemical
Sample: Rita extract
Comment: Type your comment here



Kaempferol	0.226	1567.7301	71.377	1.8214	ug/ml
Steroid	2.223	6781.4592	212.138	4.6952	ug/ml
proanthocyanidin	3.950	8155.3112	255.541	13.9885	ug/ml
Anthocyanin	6.893	4481.9072	140.323	5.7657	ug/ml
Narigenin	10.593	4332.5197	135.220	5.5688	ug/ml
Dihydrocytisine	13.300	4913.1862	154.031	6.3206	ug/ml
Cyanogenic glycoside	15.783	12807.2396	395.766	8.9749	ug/ml
Aphyllidine	19.573	12590.5678	358.644	0.8215	ug/ml
Ammodendrine	22.293	4750.4508	148.987	2.3273	ug/ml
Tannin	26.000	6794.9023	212.169	3.6986	ug/ml
Flavonones	28.566	5789.7791	180.678	9.9310	ug/ml
cardiac glycoside	29.490	4450.4074	139.936	5.5250	ug/ml
Flavone	34.176	9150.8176	195.436	11.3604	ug/ml
Ribalinidine	37.260	6555.3447	204.731	8.1357	ug/ml
Spartein	38.326	9314.0338	293.230	11.5595	ug/ml
Phytate	39.590	4172.2344	135.004	5.1781	ug/ml
Oxalate	40.930	3158.2206	104.042	1.1912	ug/ml
Epihedrine	42.090	5702.9629	183.801	7.0800	ug/ml
Sapogenin	42.943	6412.2832	203.007	7.9606	ug/ml
		121881.3578		121.9041	

APPENDIX B: GC-MS RESULT

B Library Search Report

Data Path : C:\Users\Admin\Documents\7072025\

Data File : RIA.D

Acq On : 07 Jul 2025 14:54

Operator
: NIMR
Sample
: RIA

Misc :

ALS Vial : 16 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L
Minimum Quality: 0

Unknown Spectrum: Apex

Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	5.422	7.39	D:\MassHunter\Library\NIST14.L Benzoic acid, methyl ester	16700	000093-58-3	95
			Benzoic acid, methyl ester	16699	000093-58-3	94
			Benzoic acid, methyl ester	16698	000093-58-3	94

2 10.543 18.86 D:\MassHunter\Library\NIST14.L
2,4-Di-tert-butylphenol 70632 000096-76-4
91

2,4-Di-tert-butylphenol 70633 000096-76-4
91 Phenol, 2,5-bis(1,1-dimethylethyl) 70651
005875-45-6 90

3 11.298 3.15 D:\MassHunter\Library\NIST14.L
7-Hexadecene, (Z)- 87839 035507-09-6
97

2-Tetradecene, (E)- 61855 035953-54-9
93 Cyclohexadecane 87836 000295-
65-8 91

4 12.328 4.44 D:\MassHunter\Library\NIST14.L
Cyclododecane 38264 000294-62-2
91

2-Propenoic acid, pentadecyl ester 142112
043080-23-5 91 2-Propenoic acid, tridecyl ester
115357 003076-04-8 90

5 13.273 5.48 D:\MassHunter\Library\NIST14.L
9-Octadecene, (E)- 113637 007206-25-9
95

3-Octadecene, (E)- 113638 007206-19-1
94 1-Nonadecene 126870 018435-
45-5 94

6 14.491 14.35 D:\MassHunter\Library\NIST14.L
Hexadecanoic acid, methyl ester 130822 000112-
39-0 98 Pentadecanoic acid, 14-methyl-, me
130841 005129-60-2 97 thyl ester

Hexadecanoic acid, methyl ester 130813 000112-39-0 97

7 14.846 17.69 D:\MassHunter\Library\NIST14.L
n-Hexadecanoic acid 117418 000057-10-3 99 n-Hexadecanoic acid 117419 000057-10-3 99 n-Hexadecanoic acid 117416 000057-10-3 93

8 15.064 6.39 D:\MassHunter\Library\NIST14.L
1-Nonadecene 126870 018435-45-5 94 Trifluoroacetic acid, pentadecyl e 182086 959010-23-2 91 ster

1-Octadecene 113633 000112-88-9 91

9 16.048 5.02 D:\MassHunter\Library\NIST14.L
6-Octadecenoic acid, methyl ester, 155752 002777-58-4 99 (Z)-

9-Octadecenoic acid (Z)-, methyl e 155750 000112-62-9 99 ster

8-Octadecenoic acid, methyl ester 155719 002345-29-1 99

10 16.294 6.66 D:\MassHunter\Library\NIST14.L
Methyl stearate 157879 000112-61-8 99

Methyl stearate 157884 000112-61-8 98 Methyl stearate 157883 000112-61-8 98

B Library Search Report

Data Path : C:\Users\Admin\Documents\7072025\

Data File : RIA.D

Acq On : 07 Jul 2025 14:54

Operator
: NIMR
Sample
: RIA

Misc :

ALS Vial : 16 Sample Multiplier: 1

Search Libraries: D:\MassHunter\Library\NIST14.L
Minimum Quality: 0

Unknown Spectrum: Apex

Integration Events: RTE Integrator - lscint.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
11	16.471	6.64	D:\MassHunter\Library\NIST14.L			
			Octadec-9-enoic acid	142076	1000190-13-7	98
			E-15-Heptadecenal	113605	1000130-97-9	91
			Oleic Acid	142071	000112-80-1	90
12	17.032	3.94	D:\MassHunter\Library\NIST14.L			

	1-Docosene	167463	001599-67-3	99
	1-Nonadecene	126870	018435-45-5	94
	Pentacos-1-ene	205943	016980-85-1	93