# *Antiaris toxicaria var Africana (Moraceae):* PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL ACTIVITY OF THE ACQUEOUS ETHANOL EXTRACT


**BY**

**EHIGIATOR PHILIP NOSA**

**PHA1908486**

**COURSE CODE: PPJ 601**

**SUPERVISED BY EMMANUEL E. ODION (PhD)**

**DEPARTMENT OF PHARMACEUTICAL CHEMISTRY**

**FACULTY OF PHARMACY**

**UNIVERSITY OF BENIN**

**BENIN CITY, EDO STATE.**

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**A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.**

**SUPERVISED BY:**

**DR EMMANUEL E. ODION**

**NOVEMBER, 2025.**

## DECLARATION

I, EHIGIATOR PHILIP NOSA with matriculation number PHA1908486 hereby declare that this dissertation titled *Antiaris toxicaria* var Africana (Moraceae): PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL ACTIVITY OF THE ACQUEOUS ETHANOL EXTRACT is the original work carried out by me under the supervision of Dr Emmanuel E.Odion in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Edo State.

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**EHIGIATOR PHILIP NOSA** **DATE**

## CERTIFICATION

This is to certify that the project was carried out by EHIGIATOR PHILIP NOSA in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

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**EHIGIATOR PHILIP NOSA** **DATE**

**PROJECT STUDENT**

_____ _____

**DR EMMANUEL E. ODION** **DATE**

**PROJECT SUPERVISOR**

_____ _____

**DR VINCENT O. IMIEJE** **DATE**

**HEAD OF DEPARTMENT**

## DEDICATION

This project is dedicated to the almighty God, the giver of insight, wisdom knowledge and understanding, for granting us the wisdom, strength and provision to complete this project.

## ACKNOWLEDGEMENTS

I give thanks to God Almighty the honour and glory for His preservation and guidance throughout this journey.

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I am sincerely grateful to my parents, Mr and Mrs Ehigiator, for their unwavering love and support. To my siblings, Daniel Ehigiator, Courage Ehigiator, Esosa Ehigiator and Osahen Ehigiator, thank you for your encouragement and advice throughout my PharmD programme. Finally, I extend my gratitude to my friends, the Time Masters, GOFAMINT Students Fellowship and to all others who helped me in various ways. God bless you all.

## ABSTRACT

**Introduction**: *Antiaris toxicaria* is a plant found in tropical Africa, among other tropical regions, non-scientifical used of this plant include; for hunting, neurological complaints, skin infections, gastrointestinal complaints. So this incite the need for chemical characterisation and laboratory evaluation of the ethanolic bark fraction. This study profiles its phytochemicals, and antibacterial.

**Method:** The bark parts were collected, pulverized and extracted with 70% ethanol from which the ethanolic fraction were obtained and analyzed by HPLC and GC‑MS to identify non-volatile and volatile constituents found in the plant. Antibacterial activity was carried out against six clinical isolates (*Escherichia coli, Staphylococcus aureus, Enterobacter cloacae, Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cereus)* was assessed by Inhibitory Zone Diameter (IZD), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

**Results:** Chromatographic and spectrometric analyses revealed the presence of major constituents such as (Ephedrine),(Proanthocyanin compound ) and (Amphyllidine compound ) with (Ephedrine) occurring at the highest concentration (17.81 µg/Ml) for HPLC, while for GC-MS, constituents such as (n-Hexadecanoic acid), (d-Glucohexodialdose) and (2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-,cis-) with n-Hexadecanoic as the highest occurring (15.07 %). The extract produced moderate inhibition zones (14–20 mm) against the six clinical isolates. The findings of this study revealed that the 70% ethanolic extract of *Antiaris toxicaria* possesses measurable antibacterial activity against the tested microorganisms, particularly to Gram positive bacteria than that of Gram negative with MBC/MIC ratio to be $\geq 4$ for S. aureus, E. cloacae, E. coli, B. cereus depicting bacteriostatic and $\leq 4$ for P. aeruginosa and B. subtilis depicting bactericidal.

**Conclusion:** Th ethanolic bark exract of *Antiaris toxicaria* contains a complex mixture of alkaloids, phenolics, flavonoids, saponins constituents that together reveals measurable antibacterial effect.

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# CHAPTER ONE

## 1.0 INTRODUCTION

The harnessing of plants for medicinal use has in so much increased over the years considering its other uses such as aesthetic, source of food , source of building materials amongst others, and one of the major factor that has contributed to this is advancement of technology which has made it possible to properly refine the medicines in these plants as against the time of old where the whole plants are totally consumed, hence over the years so many plants have been placed under study for this course and one of those plants is the genus Antiaris (from the family of moraceae) .The genus is made up of just one species,which is *Antiaris toxicaria* (False Iroko). Hawthorne, (1995).

*Antiaris toxicaria* is a monoecious, It is a large tree, it has a wide distribution in tropical regions, occuring in Australia, tropical asia, tropical Africa, Indonesia, the Philippines, Tonga, and various pother tropical islands. Its seeds are spread by various birds and bats, although it has just one specie, it is made of five subspecies, the generic epithet Antiaris is derived from the Javanese name for it; ancar(5), while its english name may be called bark cloth tree, antiaris, false Iroko, false mvule or upas tree(7), in Edo it is called Ogiovu, Igbo (Ajiawu), Hausa (Farin loko).

## 1.1 Description

*Antiaris toxicaria* is a large tree, growing to 25-40m tall, with a trunk up to 40cm diameter, often buttressed at the base with pale grey bark. The trees have milky to watery latex 11, the leaves are elliptic to ovate, 7-9cm long and 3-6cm broad 12, Male flowers short stalked, they are discoid with many flowers, each flower with 2-7 tepals and 2-4 stamens, growing just below the leaves, while female flowers in disc or kidney-shaped heads to 3cm across. Ovary adnate to the perianth,1- locular with a single ovule and 2 styles the African trees bears larger fruit than Asian and Polynesia 4. The

edible fruit is red or purple drupe about 2cm in diameter, it is ellipsoid, dull and furry with a single seed with a swollen receptacle. The tree grows rapidly and attains maturity within 20 years.

### 1.2Botanical Classification of *Antiaris toxicaria*

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Rosales

Family: Moraceae

Tribe: Castilleae

Genus: Antiaris Lesch. (1810)

Species: A. toxicaria

### 1.3ETHNOMEDICINAL USES

Latex: The milky white latex is the most toxic part of the plant and was historically used as an arrow poison throughout Southeast Asia for hunting and warfare. When used medicinally, the latex's potency demands extreme caution, however case where it can be used are, Heart conditions; In very small, controlled doses, the latex has been used as a mild circulatory and cardiac stimulant, similar to the modern heart medicine digoxin. Skin ailments; In African traditions, the latex has been applied topically to address cuts, wounds, eczema, and leprosy.( Boer et al 1999)

Bark: The stem bark contains cardiac glycosides and flavonoids and is used for a variety of conditions. Fever and pain; The bark is used as a febrifuge (fever reducer) and anodyne (pain reliever). Hepatitis and dysentery; It has been traditionally used to treat hepatitis and as an antidysenteric for intestinal issues. Neurological disorders; In Ghana, stem bark extracts are traditionally used for neurological conditions such as epilepsy, pain, and limb tremors. A 2013 study found that an aqueous extract of the bark had anticonvulsant effects in animal models, likely by affecting the GABA system (Ilesanmi et al 2022)

Leaves: The leaves are rich in phenolic compounds and flavonoids, which contribute to their medicinal properties (Tjatjuk et al 2023). Astringent and febrifuge: Leaves, like the bark, are used as an astringent and febrifuge. Dysentery and leprosy; In some regions, leaves and bark are used to treat dysentery and leprosy. Antioxidant activity; Leaf extracts have demonstrated significant antioxidant activity, which may help protect against cellular damage. (Pier- Giorgio Pietta 2000)

Seeds: The seeds are also used medicinally, though with the same toxicity concerns as other parts of the plant. Dysentery; They are used for treating dysentery (Tjatjuk et al 2023).

**1.4 Pharmacological uses**

Pharmacological uses of *Antiaris toxicaria* among many are expressed in anticonvulsant activities ; The dried stem bark of *Antiaris toxicaria* distilled with water for five days has been seen to possess anticonvulsant activity in the similitude of diazepam which is due to the action of GABA system, using animal models such as male ICR mice and Sprague-Dawley rats.(De sarro et al., 2003) Antidepressant activities ; acqueous extract of *Antiaris toxicaria* has been seen to possesss antidepressant activity, as it was seen to increase mobility and decreased mobility periods significantly in both ths force swim test(FST) and the tail suspension test (TST) of animal models.(Agbaje EO et al). Muscle relaxants ; *Antiaris toxicaria* commonly known as upas tree, is a well known toxic plant that is widely distributed throughout Malaysian forests. The latex of *Antiaris*

*toxicaria* has been known for centuries that most poisoned darts used by indigenous people of Southeast Asia are prepared by concentration of latex harvested from *Antiaris toxicaria*. Prey wounded by such an arrow can rarely movemore than 100 meters. These poisons act as powerful muscle relaxants to paralyze the prey. (Philippe and Angenot). Antioxidant activities; The antioxidant activities of standard (ascorbic acid) and methanolic extract were increased with increasing concentration. The methanolic extract exhibited higher antioxidant activity than the standard (Pietta, 2000; Amarowicz and Pegg, 2008)

## 1.4 PHYTOCHEMICAL CONSTITUENTS OF *ANTIARIS TOXICARIA*

Phytoconstituents, or phytochemicals, are bioactive compounds naturally synthesized by plants as part of their metabolic processes. These secondary metabolites serve diverse ecological functions, including defense against herbivores, pathogens, and environmental stressors, while also mediating symbiotic relationships and facilitating plant-to-plant communication. From a pharmaceutical and ethnobotanical perspective, phytoconstituents represent a rich repository of structural diversity, encompassing alkaloids, glycosides, flavonoids, terpenoids, saponins, tannins, phenolic compounds, and steroids, among others. These compounds have long been recognized for their therapeutic potential, toxicological significance, and role in traditional medicine systems across cultures.

*Antiaris toxicaria* Lesch., commonly known as the upas tree or antiaris, is a tropical member of the Moraceae family with a notorious reputation in ethnobotany and toxicology. Indigenous to regions of Africa and Southeast Asia, this species has been historically employed in arrow poisons, traditional medicine, and ritualistic practices. The tree's latex and bark contain a complex array of phytoconstituents, most notably cardiac glycosides such as antiarin and α-antiarin, which exert potent cardiotoxic effects through inhibition of $Na^+/K^+$-ATPase. Beyond its toxicological profile, *Antiaris toxicaria* also harbors flavonoids, triterpenoids, phenolics, and other secondary metabolites

that contribute to its biological activities, including antimicrobial, anti-inflammatory, and cytotoxic properties.

The phytochemical investigation of *Antiaris toxicaria* is of considerable academic interest, not only for understanding the molecular basis of its toxicity but also for exploring potential therapeutic applications through careful isolation, characterization, and pharmacological evaluation of its constituents. This research endeavor seeks to comprehensively catalogue the phytoconstituents identified in *Antiaris toxicaria*, thereby contributing to the broader fields of natural product chemistry, pharmacognosy, and drug discovery.

### 1.4.1 CARDIAC GLYCOSIDES (Cardenolides)

Cardiac glycosides are a class of naturally occurring organic compounds characterized by their potent effects on cardiac muscle contractility. These compounds consist of a steroid nucleus (aglycone or genin) attached to one or more sugar moieties (glycone). Based on their structural features, cardiac glycosides are primarily classified into two groups: cardenolides, which contain a five-membered unsaturated lactone ring, and bufadienolides, which possess a six-membered doubly unsaturated lactone ring at the C-17 position of the steroid nucleus (Prassas & Diamandis, 2008). Cardenolides represent the most extensively studied and clinically important subclass of cardiac glycosides. *Antiaris toxicaria* is particularly rich in cardiac glycosides, which are the primary toxic constituents

Major Cardiac Glycosides:The plant contains over 50 different cardiac glycosides including both novel compounds and known cardenolides with a steroidal aglycone linked at the 3β-OH group to one or more sugar moieties (Shi *et al*., 2010).

Isolated compounds include: Antiarosides A-I (compounds 1-9): Nine new cardiac glycosides with various substituents including unique 10-carboxy and 3α-hydroxy groups. Antiarotoxinin A: An

aglycone with 20-OH group; Antiaritoxiosides A-G and Antiarotoxinin B: Eight new cardiac glycosides with rare 10-hydroxy substituents. Antiarosides J-X: Fifteen new cardiac glycosides isolated from latex (Liu *et al*., 2013). Known Cardiac Glycosides: Major compounds include malayoside, α-antiarin, β-antiarin, convallatoxin, strophanthidin, strophalloside, glucostrophalloside, antiarigenin, periplogenin, cheiranthoside VII, strophanthidol, convallatoxol, cannogenol, antialloside, toxicarioside B, and periplorhamnoside.

Structure Features: General Features of Cardiac Glycosides;

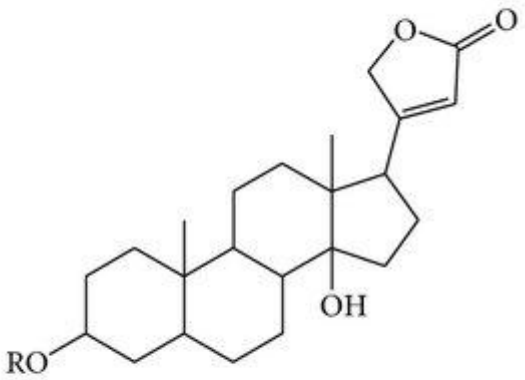
Steroidal C23 framework with 3β-sugar attachment, characteristic butenolactone ring, and various oxygenation patterns.

Unique features include rare 10-carboxy groups and 3α-hydroxy orientations not commonly found in other cardiac glycosides (Shi et al 2010)

Biosynthetic Pathway: A catabolic progression pathway has been proposed showing transformation from C-10 $CH_3$ → $CH_2OH$ → CHO → COOH → H, explaining the structural diversity of cardiac glycosides in this species (Shi et al 2014).

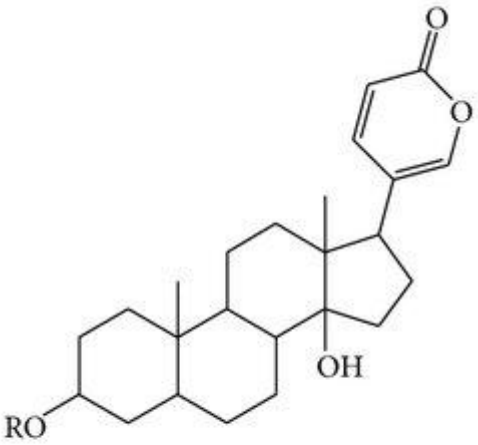
All contain a C23 steroid core with a characteristic α,β-unsaturated γ-butyrolactone ring at C-17. Sugar BVmoieties include rhamnose, glucose, antiarose, and allomethylose. Substitution patterns vary at C-3 (α or β orientation), C-5 (OH), C-10 ($CH_3$, $CH_2OH$, CHO, COOH, OH, or H), C-12 (OH),

and C-14 (OH)




Cardenolide

Digitoxin: R = digitoxose-digitoxose-digitoxose




Bufadienolide

Bufalin: R = H

### 1.4.2 COUMARINS

Coumarins represent a large family of naturally occurring phenolic compounds characterized by a benzopyrone structure consisting of a benzene ring fused to an α-pyrone ring (2H-1-benzopyran-2-one). First isolated in 1820 from *Dipteryx odorata* (Tonka bean), from which the name "coumarin" is derived from the French word "coumarou" (the native term for tonka bean), these compounds have attracted considerable scientific interest due to their diverse biological activities and widespread distribution in the plant kingdom (Murray et al., 1982). Coumarins are characterized by their distinctive sweet vanilla-like odor, particularly noticeable in freshly mown hay, which results from the release of coumarin from damaged plant tissues (Lake, 1999).

The basic coumarin nucleus can undergo extensive structural modifications through hydroxylation, alkoxylation, glycosylation, prenylation, and various other substitutions, resulting in over 1,300 naturally occurring coumarins identified to date (Venugopala *et al.,* 2013). These structural variations profoundly influence their physicochemical properties, biological activities, and pharmacological applications. Coumarins serve multiple ecological functions in plants, including defense against herbivores and pathogens, regulation of growth processes, and response to environmental stresses (Bourgaud *et al*., 2006). From *Antiaris toxicaria*, two new coumarin compounds and several known coumarins have been isolated from the trunk bark.

New Coumarins:

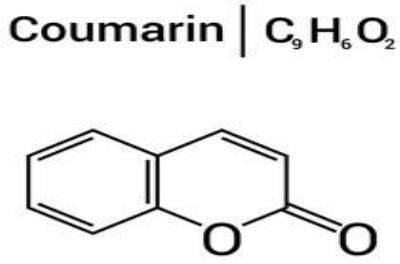
- Anticarin A: 8-hydroxy-7-methoxy-6-[2',3'-dihydroxyisopentyl]coumarin with molecular formula $C_{15}H_{18}O_6$
- Anticarin B: 4'-β-glucosyl-khellactone, a khellactone glycoside with molecular formula $C_{20}H_{24}O_{10}$ (Shi et al 2014)

Known Coumarins:

- (+)-Marmesin
- Decursinol
- (R)-Peucedanol
- Oxypeucedanin hydrate

Structure Features:

- Coumarins are benzopyrone derivatives with characteristic UV absorption at 316 and 258 nm (Shi et al 2010)
- Angular and linear dihydrofuranocoumarin and dihydropyranocoumarin structures




### 1.4.3 FLAVONOIDS

Flavonoids are one of the largest and most extensively studied groups of plant secondary metabolites, comprising over 10,000 structurally diverse compounds that share a common C6-C3-C6 carbon skeleton consisting of two aromatic rings (A and B) connected by a three-carbon bridge that typically forms a heterocyclic C ring (Panche et al., 2016). The name "flavonoid" derives from the Latin word "flavus," meaning yellow, reflecting the prominent role these compounds play as plant pigments. First isolated in 1930 by Nobel laureate Albert Szent-Györgyi, who initially termed them "vitamin P"

due to their perceived role in reducing capillary permeability, flavonoids have since been recognized as essential components of the human diet with significant health-promoting properties (Heim et al., 2002).

Flavonoids are ubiquitous in the plant kingdom, found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. They serve multiple functions in plants including UV protection, pigmentation to attract pollinators, defense against pathogens and herbivores, regulation of plant growth and development, and modulation of reactive oxygen species (ROS) during stress responses (Pietta, 2000). In human nutrition and health, flavonoids have attracted considerable attention due to their antioxidant, anti-inflammatory, anticancer, cardioprotective, and neuroprotective properties, making them subjects of intensive pharmaceutical and nutraceutical research (Hollman & Katan, 1999).

A. Prenylaurones

Antiarones A and B: The first examples of prenylaurones isolated from root bark (Hano et al 1990)

B. Prenylchalcones

Antiarones C, D, and E: Three new prenylchalcones from root bark (Hano et al 1990)

C. Prenylflavanones

Antiarones F, G, H, and I: Four new prenylflavanones from root bark

Antiarones K and L: New flavanones with 2,2-dimethylpyran ring systems

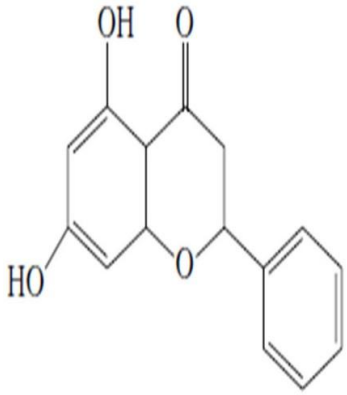
New Flavanones:

- Antiarone L: (2S)-3',5,8-trihydroxy-4'-methoxy-6,7-methylenedioxy-2'-prenylflavanone ($C_{21}H_{22}O_6$)

- Antiarone K: (2S)-2‴,2‴-dimethylpyrano-3',5,8-trihydroxy-2'-prenylflavanone ($C_{25}H_{26}O_6$)

Known Flavonoids:

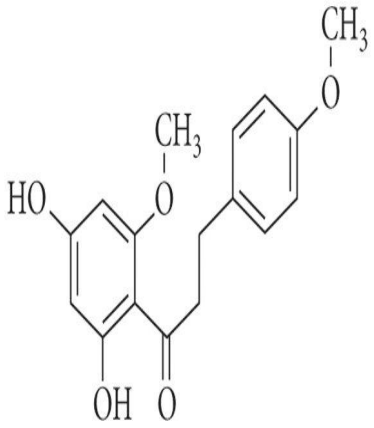
- (2S)-Pinocembrin




Chemical structure of Pinocembrin

### 1.4.4 DIHYDROCHALCONES

Several dihydrochalcone derivatives have been reported from the plant




Chemical structures of dihydrochalcone derivatives used in the study. Compound 1 (4′,6′-dihydroxy-2′,4-dimethoxy-5'-(2″-hydroxybenzyl) dihydrochalcone) (a) and compound 2 (calomelanone; 2′,6′-dihydroxy-4,4′-dimethoxydihydrochalcone) (b).

### 1.4.5 OTHER PHENOLIC COMPOUNDS

Various phenolic acids and derivatives including vanillic acid, p-hydroxybenzoic acid, vanillin, syringic acid, and hydroxymellein (Shi et al 2014)

Acetophenone Derivatives:

- 2,4,6-Trihydroxy-5-methoxyacetophenone
- 6-Acetoxy-2,4-dihydroxyacetophenone
- 3-Hydroxy-5-methoxybenzalacetophenone

## 1.5 Justification for the Study

1. Non-scientifical use of the plant: *Antiaris toxicaria* is used widely for treatment of infections, neurological complaints, gastrointestinal problems, management of convulsions, but controlled chemically proven data for the ethanolic aerial fraction are limited, making scientific validation necessary.
2. Incomplete chromatographic characterization: Existing reports note several phytochemicals in the species, but a systematic HPLC and GC- MS profile of the ethanolic bark extract is lacking, preventing reliable identification of which specific constituents cause various pharmacological effects.
3. The global rise in antibacterial resistance; thus infer the need for new antibacterial scaffolds; locally available medicinal plants such as *Antiaris toxicaria.*
4. Experimental linkage between compounds and effects: Correlating quantified phytochemicals with measured antibacterial will clarify which classes of compounds (for example flavonoids, alkaloids, saponins) are responsible for observed traditional benefits.

## 1.6 Aims

The aim of this study is to identify, and quantify phytochemical constituents and evaluate the antibacterial profile found in *Antiaris toxicaria*.

## 1.7 Specific objectives

1. To prepare and fractionate the ethanolic extract of the bark parts of *Antiaris toxicaria* using standard extraction and column fractionation techniques.
2. To identify and quantify phytoconstituents in the ethanolic fraction by High Performance Liquid Chromatography (HPLC).
3. To profile volatile and semi-volatile constituents of the ethanolic fraction using Gas Chromatography–Mass Spectrometry (GC- MS).
4. To determine the antibacterial activity of the ethanolic extract against selected clinical bacterial isolates.
5. To establish the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), as well as the ratio (MBC/MIC) of the ethanolic extract for each tested bacteria.

# CHAPTER TWO

## 2.0 Materials and Methods

### 2.1 Materials

**The Equipment and solvents used for this study encompassed various components. They include;** standard ciprofloxacin, sterile Mueller-Hinton nutrient agar, stock solutions of Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. A swab stick, test tubes, a test tube holder, test tube rack, transparent measuring ruler, tripod stand, universal bottles, a water bath, and analytical weighing balance,10 mm cork borer, 20 % Tween 80, absolute ethanol, acetone, beakers, Bunsen burner, cotton wool, disinfectant ( soap, detergent), a disinfectant jar, distilled water, foil paper, forceps, a freezer, , glass funnel, glass stirrer, an inoculating loop, lighter, maceration jar, measuring cylinder, micropipette, mortar, paper tape, pestle, Petri dish, plant extract, portable autoclave, porcelain dish, anhydrous pyridine, Potassium hydroxide, retort stand, rotatory evaporator, sample holder, separatory funnel, silica gel (mesh size 60–120).

### 2.2 Sample Collection, Identification and Preparation

The stem bark of *Antiaris toxicora* was collected on the 21st March, 2025 from the tree located in front of the Faculty of Pharmacy (New building), Ugbowo Campus of the University of Benin with latitude and longitude of 6° 20'N, 6° 58'N and 5° 35'E, 5° 41'E respectively. It was identified and authenticated by Prof E.I. Aigbokhan of Plant Biology and Biotechnology Department, and herbarium number EIA02063 was issued following the deposition of its parts in the herbarium for future references.

The bark was air dried for 14 days under shade, pulverized with electrical milling machine to fine powder and macerated with 70 % ethanol for three days. The solvent was carefully decanted and

passed through filtered paper of size 1. The filtrate was concentrated in-vacuum at 45$^{o}$C and the extract obtained was stored at 4$^{o}$C until used.

### 2.2 Preparation of Sample for HPLC analysis

To 0.20 g of the extract was added 15 mL of ethanol and 10 mL of 50 % m/v potassium hydroxide (KOH). These were maintained in a water bath at 60$^{o}$C for 3 min before washing in a separation funnel with 20 mL of ethanol, 10 mL of cold and hot water each and 3 mL of hexane. The combined extracts were bulked together and washed thrice with 10 mL of 10 %v/v aqueous ethanol and the wash was evaporated on a dish (evaporating) and solubilized in 1000 µL of pyridine, with 200 µL was taken for HPLC analysis.

#### 2.2.1 HPLC Analysis

Method previously described by Kaisoon *et al*., 2011 and recently described by Odion and co-workers 2025 was used in the HPLC analysis. High performance liquid chromatography (HPLC) analysis was performed using Shimadzu LC-10AD dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. The analysis was performed using a C-12 normal phase column (Phenomenex, Gemini 5 µ, 200 mm length × 4.8 mm internal diameter). The mobile phase consisted of acetic acid-acidified deionized water (pH 2.8) as solvent A and acetonitrile as solvent B at a flow rate of 0.8 mL/min. The column was equilibrated with 5% solvent B for 20 min after each injection of samples. The column temperature was set to 38°C and the injection volume was 20 µL. The wavelengths were set to 280 nm for the detection of phenolics, Phenolic compound identification and quantification were performed by comparing respective retention times and peak areas with pure standard compounds utilizing the method of external standards to construct calibration curve. Gradient elution was executed 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 (Odion *et al*., 2025)

### 2.3 Gas chromatography-mass spectrometric analysis of extract

This analysis was carried out by hyphenated GC-MS with triple axis detector and a 10 µL syringe auto injector, the GC system (7890A) is an Agilent USA model, while the MS is an inert MSD 5675C. Capillary column from Agilent (19091-433HP-5Ms) treated with 5% phenyl methylsilox and measuring 30 m (length), 0.2 µm (internal diameter), 250 µm (thickness) was utilized for the chromatographic separation while Helium was the carrier gas. Ion source and interface temperatures were set at 250ºC and 300ºC, 16.2 psi as pressure and 1:50 split ratio with 1 µl injector in split mode, while 280ºC was the injection temperature. Temperature of the column was started at 50ºC for 2 min and adjusted at the rate of 20ºC/min to 100ºC. This was elevated to 250ºC at 20ºC/min and maintained for 5 min. While acquiring data, software from the manufacturer was utilized to control the system and compounds qualification was achieved by mass spectra comparison with standard from National Institute of Standard and Technology 11 library (Odion *et al*., 2025).

### 2.4 Antibacterial Activity of Antiaris toxicora

#### 2.4.1 Specimen Collection

Microorganisms used in this study were selected bacterial isolates obtained from the University of Benin Teaching Hospital, Benin City, Edo state, Nigeria. They are: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Enterobacter cloacae* and *Bacillus cereus*.

### 2.4.2 Preparation of Test Bacteria

All test bacterial isolates were maintained in 20 % glycerol broth and frozen. Prior to use, test microorganisms were sub-cultured from stock into sterile nutrient agar plates and were incubated overnight at 37°C. After incubation, bacterial colonies from the overnight plates of each microorganism were suspended in sterile broth for 12 hours and adjusted to 0.5 McFarland standard to give an inoculum size of approximately 10 CFU/mL for each bacteria.

### 2.4.3 Antimicrobial Susceptibility Tests and Determination of Inhibitory Zone Diameter (IZD)

An antimicrobial susceptibility test was performed to evaluate the antibacterial activity of the 70 % Ethanolic fraction of *Antiaris toxicaria* using the agar well diffusion method. Six different bacterial isolates were selected, and each was assigned to a separate sterile Petri dish containing 30 mL of Mueller-Hinton agar. After the agar solidified, the plates were dried in a hot air oven at 40°C for approximately 5 minutes to remove surface moisture. A standardized bacterial suspension of each bacterial isolate containing approximately 10 colony-forming units per milliliter (CFU/mL) was prepared, and a sterile inoculating wire loop was used to streak each agar plate with its corresponding bacterial isolate. The wire loop was sterilized by flaming after each use to prevent cross-contamination. Two wells were bored into each agar plate using a sterile 10mm corkborer and the base of the well were sealed with 0.02 mL Mueller-Hinton agar. A concentration of 200 mg/mL Ethanolic fraction of *Antiaris toxicaria* was prepared and 0.2 ml of the stock solution was introduced into one of the well created in each petri dish for each isolate. A solution of 1.6µg/ml of standard ciprofloxacin was also prepared to be used as the Control for the analysis and 0.2 ml of the standard solution was introduced into the second well created in each petri dish. The plates were incubated at 37°C for 24 hours and the growth of the organism in each petri dish were observed to assess its resistance or susceptibility to 200mg/ml of the Ethanolic fraction of *Antiaris toxicaria* for all

bacterial isolate. The zones of inhibition around the wells were measured in millimeters to determine the antimicrobial efficacy of the extract against each bacterial strain (Odion *et al*., 2025)

### 2.4.4 Determination of minimum inhibitory concentration (MIC)

Agar dilution method of Afoyan and Meyer (1997) was used in this study for the determination of the Minimum Inhibitory Concentration (MIC) of the ethanolic fraction of *Antiaris toxicaria*. Four sterile petri dishes were used and labelled 25mg, 12.5mg 6.25mg and 3.125mg. A twofold serial dilution of the test ethanolic fraction was prepared using 20% Tween 80 as the diluent to give concentrations of 31.25mg, 62.5mg, 125mg and 250mg. 1mL of the prepared 62.5mg of the 70% Ethanolic fraction of *Antiaris toxicaria* was introduced into a universal bottle containing 9mL of Meuller-Hinton molten agar and the mixture was swayed gently to ensure homogeneity. The mixture was poured into the petri dish labelled 31.25mg. The agar was allowed to solidify and the plates were dried in a hot air oven at 40C for approximately 5 minutes to remove surface moisture. The same procedure was repeated for 62.5mg, 125mg and 250mg of the test fraction. Using a marker, the base of each Petri dish was divided into six sections and these sections in each plate were labelled to represent each bacterial isolate used for the analysis. From the standardized bacterial suspension of each bacterial isolate containing approximately $10^7$ colony-forming units per milliliter (CFU/mL) prepared, a sterile inoculating wire loop was used to streak each section on each agar plate with its corresponding bacterial isolate. The wire loop was sterilized by flaming after each use to prevent cross-contamination. The plates were incubated at 37°C for 24 hours. After the incubation, the plates were visually examined for growths on the inoculated spots and the lowest concentration of 70% Ethanolic fraction of *Antiaris toxicaria* that inhibits growth was considered as the Minimum Inhibitory Concentration (MIC) (Odion *et al*., 2025)

### 2.4.5 Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration was determined using agar plate method. It was determined from the agar dilution of the MIC tests by sub-culturing into freshly prepared agar plates that did not contain any test extract (*Antiaris toxicaria*). The dilution plates were then incubated at 37°C for 24 hours. After incubation, the plates were visually examined for growths in the inoculated spots. The lowest concentration of the extract that showed no growth was considered as the Minimum Bactericidal Concentration. (Odion *et al*., 2025)

### 2.4.6 Ratio of MBC/MIC

The ratio of minimum bactericidal concentration (MBC) to minimum inhibitory concentration (MBC) was calculated for all the test bacterial (MBC/MIC), to get values and compare to the standard value of inference(<4), whether bactericidal or bacteriostatic.

## CHAPTER THREE

### 3.0 Result

### 3.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY(HPLC)

High performance liquid chromatography is a method of phytochemical analysis that indicates retention time, peak area, and identified compounds with varying concentration calculated. A total of 19 peaks with 19 bioactive constituent was seen (Figure 1), retention time ranged from 0.253 min to 44.170 min, and base on the concentration of the constituents spartein (10.8538 µg/mL), anthocyanin (12.5867 µg/mL), tannin (12.8602 µg/mL), aphyllidine (15.2809 µg/mL), proanthocyanidine (15.7960 µg/mL) and Ephedrine (17.8061 µg/mL) were most prominent (Table 1).

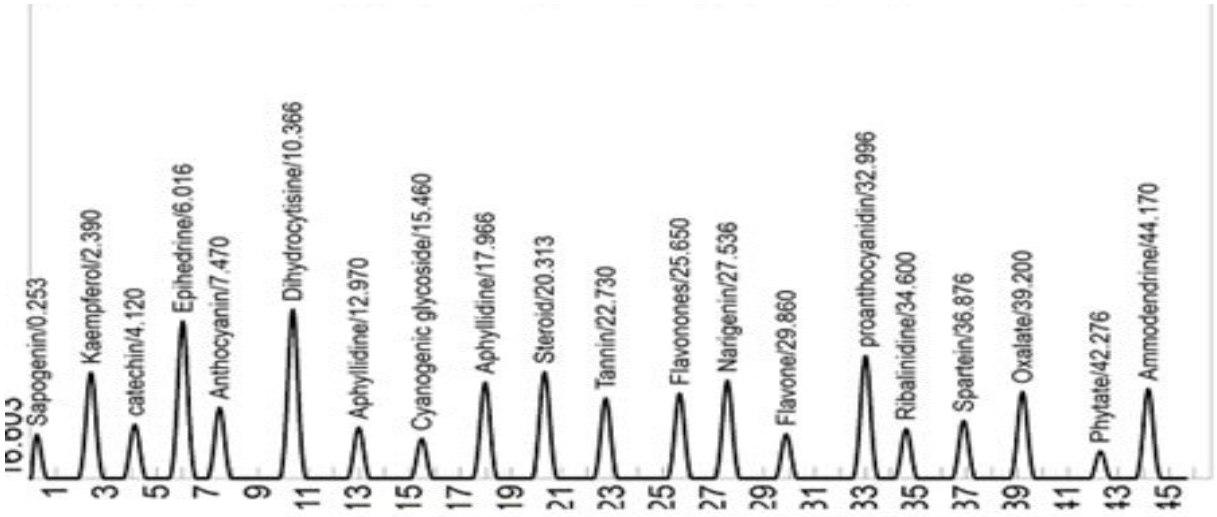


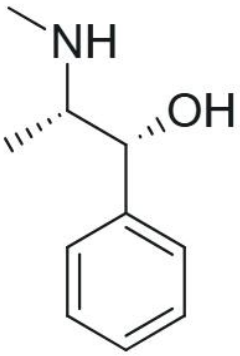

Figure 1 HPLC chromatogram of the stem bark of *Antiaris toxicaria var african*

HPLC analysis was carried out to separate, identify, and quantify the primary constituents in the sample. The method utilized a reversed-phase column with appropriate mobile phase composition optimized for the target analytes. Detection was performed using [UV-Vis/DAD/FLD] detection at specific wavelengths corresponding to the maximum absorbance of the compounds of interest. Retention times, peak areas, and peak heights were recorded for each detected component. Calibration curves were prepared using authentic standards to enable accurate quantification, and results are expressed as concentration (mg/L or μg/mL) or percentage composition

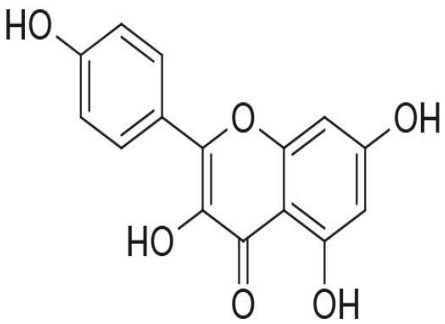
Table 1 HPLC analysis of the stem bark of *Antiaris toxicora var africana*

| S/N | Phytochemicals | Retention Time (min) | Area ($m^2$) | Concentration (µg/mL) |
|---|---|---|---|---|
| 1 | Sapogenin | 0.253 | 4097.1544 | 3.2072 |
| 2 | Kaempferol | 2.390 | 12411.2552 | 8.5931 |
| 3 | Catechin | 4.120 | 6535.5572 | 7.8694 |
| 4 | Ephedrine | 6.016 | 18197.8156 | 17.8061 |
| 5 | Anthocyanin | 7.470 | 8449.9404 | 12.5867 |
| 6 | Dihydrocystine | 10.366 | 19612.9266 | 8.8247 |
| 7 | Cyanoglycoside | 15.460 | 4973.5534 | 1.4404 |
| 8 | Aphyllidine | 17.966 | 11346.0970 | 15.2809 |
| 9 | Steroid | 20.313 | 12767.3954 | 5.8931 |
| 10 | Tannin | 22.730 | 9578.7656 | 12.8602 |
| 11 | Flavonones | 25.650 | 10085.2201 | 4.8545 |
| 12 | Narigenin | 27.536 | 11533.7678 | 5.0642 |
| 13 | Flavone | 29.860 | 5482.1426 | 2.4076 |
| 14 | Proanthocyanidin | 32.996 | 14329.5937 | 15.7960 |
| 15 | Ribalinidine | 34.600 | 6053.9620 | 5.6351 |
| 16 | Spartein | 36.876 | 6994.1588 | 10.8538 |
| 17 | Oxalate | 39.200 | 10237.5643 | 3.6541 |
| 18 | Phytate | 42.276 | 3511.2838 | 2.4305 |
| 19 | Ammodendrine | 44.170 | 10547.4784 | 2.5825 |

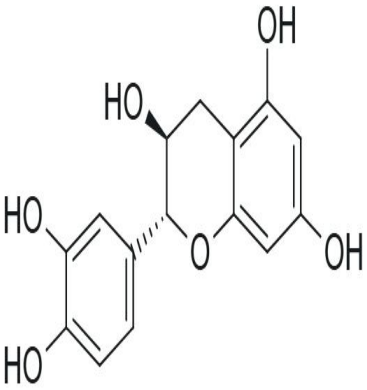
**STRUCTURAL REPRESENTATION**




EPHEDRINE




KAEMPFEROL

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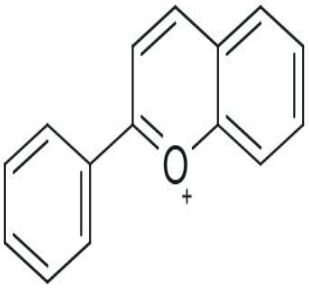


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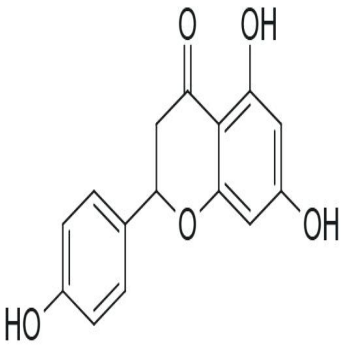
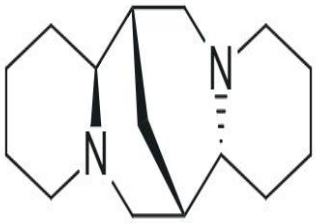
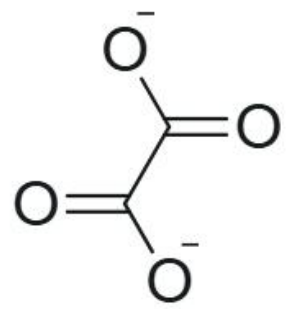
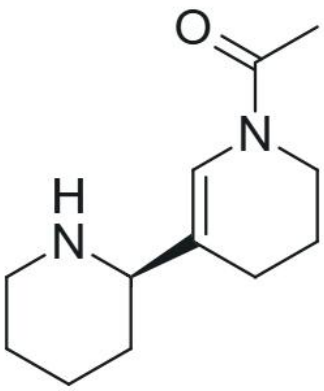


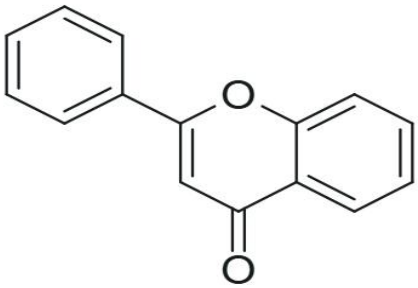

NARIGENIN




SPARTEIN




OXALATE AMMODENDRINE FLAVONE

**3.2 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)**

Gas chromatography-mass spectrometry (GC-MS) analysis was performed which resulted in revealing about 17 bioactive compounds present in the *Antiaris toxicaria* extract. These compounds were identified based on their retention times, molecular weight and various area percentages.

Some major compounds seen in the result include Acetaldehyde N-Formyl-N Methyl hydrazone(9.68%), 2-furanmethanol,5-ethenyltetrahydro-alpha,alpha 5-trimethyl-cis(11.30%), Pentafluoropropionic acid and undecyl ester(3.61%), Ethyl alpha,d-glucopyranoside(6.16%), d-glucohexodialdose(11.48%), Methyl-beta-D-thiogalactoside(4.07%), Hexadecanoic acid,methyl ester(4.68%), n-Hexadecanoic acid(15.07%), Bis(2-ethylhexyl)pthalate(4.41%), Trans-13-octadecanoic(8.01%), Octadecanoic acid(4.29%) as seen in the results below;

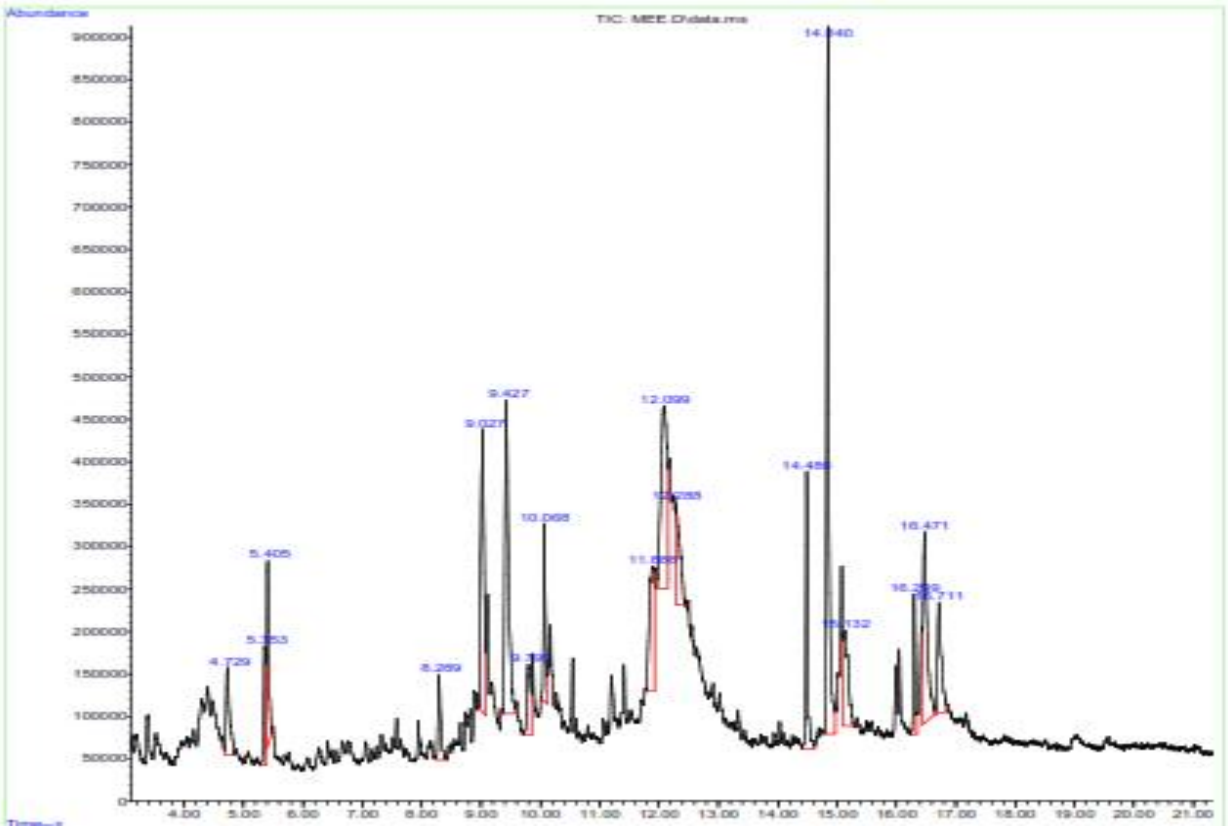


Figure 2 Gas chromatogram of the 70 % ethanol extract of stem bark of *Antiaris toxicaria* var africana

GC-MS analysis was carried out for the identification and quantification of volatile and semi-volatile organic compounds. The sample was prepared according to standard protocols and injected into the GC system equipped with a capillary column. Mass spectrometric detection in electron ionization (EI) mode provided structural information through fragmentation patterns. Compound identification was achieved by comparing retention indices and mass spectra with those of authentic standards. Quantification was performed using either external standard calibration or internal standard methods, with results reported as relative abundance (%) or absolute concentration.

Table 2 GC-MS analysis of 70 % ethanol extract of *Antiaris toxicaria* var africana

| S/N | Compounds | RT (min) | % Area | Qual |
|---|---|---|---|---|
| 1 | Benzeneacetaldehyde | 4.729 | 3.08 | 64 |

| | | | | |
|---|---|---|---|---|
| 2 | Phenol, 2-methoxy- | 5.353 | 2.76 | 94 |
| 3 | Benzoic acid, methyl ester | 5.405 | 3.55 | 92 |
| 4 | 2-Methoxy-4-vinylphenol | 8.289 | 2.32 | 90 |
| 5 | Acetaldehyde N-formyl-N-methylhydrazone | 9.027 | 9.68 | 52 |
| 6 | 2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-,cis- | 9.427 | 11.30 | 37 |
| 7 | trans-Cinnamic acid | 9.799 | 2.79 | 87 |
| 8 | Pentafluoropropionic acid, undecyl ester | 10.068 | 3.61 | 81 |
| 9 | Ethyl .alpha.-d-glucopyranoside | 11.888 | 6.16 | 78 |
| 10 | d-Glucohexodialdose | 12.099 | 11.48 | 47 |
| 11 | Methyl-.beta.-D-thiogalactoside | 12.288 | 4.07 | 58 |
| 12 | Hexadecanoic acid, methyl ester | 14.486 | 4.68 | 99 |
| 13 | n-Hexadecanoic acid | 14.480 | 15.07 | 99 |
| 14 | Bis(2-ethylhexyl) phthalate | 15.132 | 4.41 | 87 |
| 15 | Heptadecanoic acid, 16-methyl-, methyl ester | 16.299 | 2.75 | 97 |
| 16 | trans-13-Octadecenoic acid | 16.471 | 8.01 | 99 |
| 17 | Octadecanoic acid | 16.711 | 4.29 | 93 |

Where ; RT is Retention Time, and Qual is Qualitative analysis

## 3.3 ANTIBACTERIAL ACTIVITY RESULT

The antibacterial activity of the test extract (40 mg) was evaluated against six bacterial species using the agar well diffusion method, with Tween-80 serving as the negative control and ciprofloxacin (1.6 mg/mL) as the positive control.

The extract produced measurable zones of inhibition against *Staphylococcus aureus* (20 mm), *Enterobacter cloacae* (15 mm), *Bacillus subtilis* (14 mm), and *Pseudomonas aeruginosa* (17 mm), while *Escherichia coli* and *Bacillus cereus* showed no inhibition. Tween-80 exhibited no antibacterial effect on any of the test organisms. In contrast, ciprofloxacin demonstrated strong inhibitory activity across all species, with zones ranging from 28 mm to 40 mm.

These results indicate that the extract possesses selective antibacterial properties, with greater efficacy against certain Gram-positive and Gram-negative bacteria, though its activity is markedly lower than that of the standard antibiotic.

### 3.3.1 Inhibitory Zone Diameter

The inhibitory zone diameter represents a quantitative measure used in antimicrobial susceptibility testing to evaluate the effectiveness of antimicrobial agents against specific microorganisms. When an antimicrobial substance is applied to an agar plate inoculated with a bacterial culture, it diffuses outward from the point of application, creating a concentration gradient. In regions where the antimicrobial concentration exceeds the threshold required to inhibit microbial growth, a clear zone forms around the antimicrobial source. This clear area, devoid of visible bacterial growth, is termed the zone of inhibition.

The diameter of this zone, measured in millimeters, correlates inversely with the minimum inhibitory concentration (MIC) of the antimicrobial agent. A larger inhibitory zone typically indicates greater susceptibility of the test organism to the antimicrobial compound, while smaller zones suggest

reduced susceptibility or resistance. This measurement principle forms the basis of the widely employed disk diffusion method, also known as the Kirby-Bauer test, which provides a standardized approach for assessing antimicrobial efficacy in clinical and research settings. The inhibitory zone diameter of Hydroethanolic extract of *Antiaris toxicaria* is seen below;

Table 3, Inhibitory zone diameter of 70% Ethanolic extract of *Antiaris toxicaria* var africana

| Bacteria | Extract (40 mg) | Tween-80 | Ciprofloxacin (1.6mg/mL) |
|---|---|---|---|
| *Escherichia coli* | 0 | 0 | 28 |
| *Staphylococcus aureus* | 20 | 0 | 35 |
| *Enterobacter cloacae* | 15 | 0 | 40 |
| *Bacillus subtilis* | 14 | 0 | 28 |
| *Pseudomonas aeruginosa* | 17 | 0 | 38 |
| *Bacillus cereus* | 0 | 0 | 38 |

The antibacterial activity of 40mg/ml hydroethanolic extract of *Antiaris toxicaria* was compared to 1.6mg/ml ciprofloxacin(control) using a paired t-test. On analysis, a p-value of 0.00546, indicating significant difference (p,0.001) in the antibacterial effect between the samples under analysis. This suggest that although that of the control seems to higher and more potent, the hydroethanolic extract fraction exhibits potent antibacterial activity.

### 3.3.2MINIMUM INHIBITORY CONCENTRATION

Minimum inhibitory concentration (MIC) assay was carried out to determine the lowest concentration of *Antiaris toxicaria* extract that is capable of inhibiting visible growth of selected bacteria cultures. . Four concentrations (25, 12.5, 6.25, and 3.125 mg/mL) were tested.

At 25 mg/mL, complete growth inhibition was observed for all organisms tested. At 12.5 mg/mL, inhibition persisted only for *Staphylococcus aureus*, *Bacillus subtilis*, and *pseudomonas aeruginosa*, further persistent inhibition was seen in the 6.25 mg/mL for isolate *staphylococcus aureus* and *pseudomonas aeruginosa*, and then for the 3.125 mg/mL isolate, it exhibited no inhibition to any of the tested bacteria except *staphylococcus aureus*.

These findings indicate that the extract has broad-spectrum antibacterial activity at higher concentrations, with *Staphylococcus aureus*, *Bacillus subtilis*, and *pseudomonas aeruginosa* showing greater susceptibility compared to the other isolates.

Table 4, Minimum inhibitory concentration of 70% Ethanolic extract of *Antiaris toxicaria* var africana

| Microorganism | Concentrations (mg/mL) | | | |
|---|---|---|---|---|
| | 25 | 12.5 | 6.25 | 3.125 |
| *Escherichia coli* | No Growth | Growth | Growth | Growth |
| *Staphylococcus aureus* | No Growth | No Growth | No Growth | NoGrowth |
| *Enterobacter cloacae* | No Growth | Growth | Growth | Growth |
| *Bacillus subtilis* | No Growth | No Growth | Growth | Growth |
| *Bacillus cereus* | No Growth | Growth | Growth | Growth |
| *Pseudomonas aeruginosa* | No Growth | No Growth | No Growth | Growth |

### 3.3.3 MINIMUM BACTERIAL CONCENTRATION

Minimum bactericidal concentration (MBC) assay was carried out on *Antiaris toxicaria* to determine the lowest concentration of the test substance capable of killing the selected bacteria isolated cultures. Concentrations evaluated include 10 mg/mL and 20 mg/mL. At 10mg/ml, visible bacterial effect was seen across all organisms, while at 20 mg/mL, microorganism such as S*taphylococcus aureus, Bacillus subtillis* and *Pseudomonas aeruginosa* was completely killed with bacterial effect seen for *Escherichia coli*, *Enterobacter clocae* and *Bacillus cereus.*

Table 5, Minimum bactericidal concentration of 70% Ethanolic extract of Antiaris toxicaria var africana

| | Concentration(mg/mL) | |
|---|---|---|
| | 10 | 20 |
| *Escherichia coli* | Growth | Growth |
| *Staphylococcus aureus* | Growth | No Growth |
| *Enterobacter cloacae* | Growth | Growth |
| *Bacillus subtilis* | Growth | No Growth |
| *Pseudomonas aeruginosa* | Growth | No Growth |
| *Bacillus cereus* | Growth | Growth |

# CHAPTER FOUR

## 4.0 DISCUSSION

High performance liquid chromatography (HPLC) analysis was performed using Shimadzu LC-10AD dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/VIS detector. The analysis was performed using a C-12 normal phase column (Phenomenex, Gemini 5 µ, 200 mm length × 4.8 mm internal diameter). The mobile phase consisted of acetic acid-acidified deionized water (pH 2.8) as solvent A and acetonitrile as solvent B at a flow rate of 0.8 mL/min. The column was equilibrated with 5% solvent B for 20 min after each injection of samples. The column temperature was set to 38°C and the injection volume was 20 µL. The wavelengths were set to 280 nm for the detection of phenolics, Phenolic compound identification and quantification were performed by comparing respective retention times and peak areas with pure standard compounds utilizing the method of external standards to construct calibration curve. Gradient elution was executed 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.

Table 1 shown above indicate an HPLC result of *Antiaris toxicora*. The HPLC analysis revealed nineteen compounds, revealing a diverse secondary metabolite profile, they include; cyanoglycosides (1.4404µg/ml),flavone (2.4076µg/ml), phytate (2.4305µg/ml), ammoderine (2.5825µg/ml), sapogenin (3.2072µg/ml), oxalate(3.6541µg/ml), flavonones (4.8545µg/ml), narigenin (5.0642µg/ml), ribalinidine (5.6351µg/ml), steroid (5.8931µg/ml), catechin (7.8694µg/ml), kaempferol(8.5931µg/ml), dihydrocystein(8.8247µg/ml), spartein (10.8583µg/ml), anthocyanin (12.5867µg/ml), tanin (12.8602µg/ml), amphyllidine (15.2809µg/ml), proanthocyanin (15.7960µg/ml), ephedrine (17.8061µg/ml) respectively. For usage, cyanoglycosides are seen to possess plant defense mechanism against pathogen and herbivores (McConville *et al* 2021), phytate

also known as Myo-inositol hexakisphosphate, it inhibits crystallization of calcium in urine(potential kidney stones) (Silalahi J. *et al* 2002), flavone as well as other flavonoids which were found to be present e.g Kaempferol, Narigenin, Catechin, Anthocyanin, Flavonones are seen to possess a number of medicinal benefits, including anticancer, antioxidant, antiinflammatory , antidiabetic, anti tumour and antiviral properties (Chiow *et al* 2015, Kuma *et al* 2013). Alkaloids such as ephedrine, aphyllidine, sparteine, ammodendrine, ribalindine are seen to possess the following properties including antioxidants, antibacterial activity, antihyperglycemic activity, treatments of chronic kidney, Alzheimer's and parkinson's disease (Chu *et al* 2024). Sapogenins posess medicinal benefits such as antiparasitic activity, antimicrobial and antiinflammatory activity Dinesh Kumar et al, Tanins are seen to have the following effects including antidiarrheal activity, anti haemorrhagic, anti-inflammatory (Tijani *et al* 2024).In details, we have the Glycosidic Compounds, under which are Cyanogenic Glycosides; The detection of cyanogycoside at 1.44 µg/mL is noteworthy from a toxicological perspective. Cyanogenic glycosides are plant secondary metabolites that release hydrogen cyanide upon enzymatic hydrolysis, serving as a chemical defense mechanism against herbivores (Gleadow & Møller, 2014). While the concentration detected is relatively low, the presence of these compounds necessitates careful consideration in the preparation and dosing of traditional medicines derived from this plant. Improper processing or excessive consumption could lead to cyanide poisoning, manifesting as symptoms ranging from headache and dizziness to more severe outcomes including seizures and respiratory failure (Bolarinwa *et al*., 2016). Traditional preparation methods such as prolonged boiling or fermentation may reduce cyanogenic glycoside content, highlighting the importance of indigenous knowledge in safe medicinal plant utilization. Also present are Alkaloid Components, under which are Ephedrine; It emerged as the most abundant compound in the extract with a concentration of 17.81 µg/mL. This sympathomimetic alkaloid is well-documented for its bronchodilator, decongestant, and central nervous system stimulant properties (Devlin & Drummond, 2014). The presence of ephedrine in to *Antiaris toxicaria* is

particularly significant as it may contribute to the plant's traditional use in respiratory ailments. Ephedrine acts primarily through indirect sympathomimetic activity by promoting the release of norepinephrine from sympathetic neurons, resulting in α and β-adrenergic receptor stimulation (Magkos & Kavouras, 2005). However, the relatively high concentration detected warrants caution regarding dosage in traditional preparations, as ephedrine toxicity can manifest as hypertension, tachycardia, and in severe cases, cardiac arrhythmias (Haller & Benowitz, 2000).

Quinolizidine and Other Alkaloids; Aphyllidine, detected at 15.28 µg/mL, represents the quinolizidine alkaloid class. These tetracyclic compounds are known for their diverse biological activities, including antimicrobial, anti-inflammatory, and cytotoxic properties (Wink & Botschen, 2015). The presence of other alkaloids including Ribalinidine (5.64 µg/mL), Spartein (10.85 µg/mL), and Ammodendrine (2.58 µg/mL) further emphasizes the alkaloid-rich nature of this species. Spartein, in particular, has documented antiarrhythmic properties and has been investigated as a potential therapeutic agent for cardiac dysrhythmias (Kohl *et al*., 2005). The synergistic or antagonistic interactions among these alkaloids may influence the overall pharmacological profile of traditional preparations.

Flavonoid and Polyphenolic Compounds, examples present in the sample include, Proanthocyanidins and Anthocyanins; Proanthocyanidin was identified at a concentration of 15.80 µg/mL, representing one of the most abundant phytochemicals in the extract. These oligomeric and polymeric flavonoids, also known as condensed tannins, are renowned for their potent antioxidant capacity, which often exceeds that of vitamins C and E (Bagchi *et al*., 2000). Proanthocyanidins exhibit multiple pharmacological activities including cardioprotective effects through improvement of endothelial function, anti-inflammatory activity via inhibition of NF-κB signaling, and antimicrobial properties against various pathogenic organisms (Rue *et al*., 2017). The detection of anthocyanins at 12.59 µg/mL complements the antioxidant profile, as these water-soluble pigments have demonstrated protective effects against oxidative stress-mediated diseases, including cardiovascular disorders,

diabetes, and certain cancers (Khoo *et al*., 2017). Other flavonoids present include, Flavonoid Glycosides and Aglycones; The presence of kaempferol (8.59 µg/mL), catechin (7.87 µg/mL), naringenin (5.06 µg/mL), flavonones (4.85 µg/mL), and flavone (2.41 µg/mL) demonstrates a rich flavonoid diversity. Kaempferol, a flavonol widely distributed in edible plants, has been extensively studied for its anti-inflammatory, antioxidant, and anticancer properties through modulation of various signaling pathways including PI3K/Akt and MAPK cascades (Imran *et al*., 2019). Catechin, a flavan-3-ol abundant in tea and various medicinal plants, exhibits neuroprotective, cardioprotective, and antidiabetic effects through multiple mechanisms including free radical scavenging and metal chelation (Khan *et al*., 2014). Naringenin, predominantly found in citrus fruits, has demonstrated promising antioxidant, anti-inflammatory, and lipid-lowering effects, with potential applications in metabolic syndrome management (Salehi *et al*., 2019).

Tannins; Hydrolyzable or condensed tannins were detected at 12.86 µg/mL. These polyphenolic compounds are characterized by their ability to precipitate proteins and possess astringent properties (Chung *et al*., 1998). Beyond their traditional use in treating diarrhea and dysentery through protein precipitation, tannins exhibit antimicrobial activity against bacteria, fungi, and viruses, potentially through multiple mechanisms including iron deprivation, enzyme inhibition, and cell membrane disruption (Buzzini *et al*., 2008). The antimicrobial properties of tannins may partially explain the traditional use of *Antiaris toxicaria* in wound healing and infection management. There was also the presence of Steroidal and Triterpenoid Compounds such as Sapogenins and Steroids; Sapogenin (3.21 µg/mL) and steroid (5.89 µg/mL) were identified in the extract. Sapogenins are the aglycone portions of saponins and can be either steroidal or triterpenoid in nature. These compounds have attracted considerable pharmaceutical interest due to their diverse biological activities including anticancer, antifungal, and immunomodulatory properties (Vincken *et al*., 2007). Steroidal compounds in plants often exhibit hormonal or hormone-modulating activities and may contribute to anti-inflammatory effects through various mechanisms (Mahato *et al*., 1988). The cardiotoxic

glycosides historically associated with *Antiaris* species are typically cardiac glycosides with steroidal structures, suggesting that the steroid fraction warrants detailed characterization for potential cardioactive compounds.Other metabolites include, Oxalates and Phytates; Oxalate (3.65 µg/mL) and phytate (2.43 µg/mL) were identified as anti-nutritional compounds in the extract. Oxalic acid and its salts can complex with essential minerals such as calcium, iron, and magnesium, reducing their bioavailability and potentially contributing to kidney stone formation in susceptible individuals (Noonan & Savage, 1999). Phytic acid (inositol hexaphosphate) similarly chelates divalent minerals, limiting their absorption in the gastrointestinal tract (Schlemmer *et al*., 2009). However, both compounds have demonstrated paradoxical beneficial effects in various studies. Phytate, for instance, exhibits antioxidant properties and has been investigated for its potential anticancer effects through inhibition of cell proliferation and angiogenesis (Vucenik & Shamsuddin, 2003).

### 4.1 Toxicological Considerations

While the therapeutic potential is evident, the presence of ephedrine at relatively high concentrations and cyanogenic glycosides necessitates careful toxicological evaluation. *Antiaris toxicaria* has a well-documented history of use as an arrow poison, with the toxic principle traditionally attributed to cardiac glycosides similar to those found in *Strophanthus* and *Acokanthera* species (Neuwinger, 1996). The cardiac glycosides inhibit $Na^+/K^+$-ATPase, leading to increased intracellular calcium concentrations and enhanced myocardial contractility, which can progress to fatal arrhythmias at toxic doses (Prassas & Diamandis, 2008).

The therapeutic index of preparations containing multiple bioactive compounds requires careful determination through systematic toxicological studies. Traditional preparation methods, dosing regimens, and route of administration significantly influence both efficacy and safety profiles. The discrepancy between toxic effects observed with arrow poison preparations (typically using latex or

concentrated extracts) and the apparent safety of properly prepared traditional medicines highlights the critical importance of preparation methodology and dosage control.

Table 2, seen above show the GC-MS reading of the bark of plant *Antiaris toxicaria*, and thirteen compounds were identified which amounted to 89.39%, representing a different chemical profile compared to the HPLC analysis. This complementary analytical approach captures compounds that are typically missed by liquid chromatography, providing a more complete picture of the plant's phytochemical makeup, they include; Benzeneacetaldehyde (3.08 %), Benzoic acid, methyl ester(3.55 %), Acetaldehyde N-Formyl-N Methyl hydrazine (9.68 %), 2-furanmethanol,5-ethenyltetrahydro-alpha,alpha 5-trimethyl-cis (11.30 %), Pentafluoropropionic acid and undecyl ester(3.61 %), Ethyl alpha,d-glucopyranoside (6.16 %), d-glucohexodialdose (11.48%), Methyl-beta-D-thiogalactoside(4.07 %), Hexadecanoic acid, methyl ester(4.68 %), n-Hexadecanoic acid (15.07 %), Bis(2-ethylhexyl)pthalate(4.41 %), Trans-13-octadecanoic(8.01%), Octadecanoic acid (4.29 %). The compounds identified have been reported to possess different biological activities, Benzeneacetaldehyde also known as phenylacetaldehyde is known to have nematocidal effect (Yates *et al* 2024, Birhanu *et al* 2024), Benzoic acid methyl ester has antibacterial and antifungal activity ( Olmo *et al*., 2017) Acetaldehyde N-formyl-N Methyl hydrazone which belongs to the family of hydrazones,has anticonvulsants activity (Ragavendran *et al*., 2007) 2-furanmethanol has anticancer activity (Kumar *et al*., 2022) and also antimalaria and anti microbial activty , Octadecanoic acid has antioxidant activity, while both octadecanoic and hexadecanoic acid have been reported to possess antibacterial activity (El-Sayed O.H *et al*., 2015) , Bis(2-ethylhexyl)pthalate has antibacteria and larvicidal activity (Zhao Y.S *et al*., 2021) Ethyl alpha,d-glucopyranoside has been reported to have antioxidant and hypoglycemic activity (Wang *et al*., 2015). In details, Fatty acids (Saturated and Unsaturated Fatty Acids); The most striking finding from this GC-MS analysis is the dominance of fatty acids, which collectively represent nearly 35 % of the total extract composition. n-Hexadecanoic acid (palmitic acid) emerged as the single most abundant compound at 15.07 %

relative area, followed by trans-13-octadecenoic acid at 8.01 %, and octadecanoic acid (stearic acid) at 4.29 %. We also detected hexadecanoic acid methyl ester (4.68 %) and heptadecanoic acid 16-methyl ester (2.75 %).

Finding these fatty acids in such high concentrations is not entirely surprising—they're common components of plant cell membranes and storage lipids (Gunstone *et al*., 2007). However, their abundance in medicinal plant extracts is often overlooked, even though they contribute significantly to biological activity. Palmitic acid, despite being a saturated fat, has shown anti-inflammatory properties in certain contexts and plays important roles in cell signaling (Carta *et al*., 2017). The presence of unsaturated fatty acids like trans-13-octadecenoic acid is particularly interesting because unsaturated fats generally exhibit better antioxidant and anti-inflammatory activities than their saturated counterparts (Das, 2006).

Research has demonstrated that fatty acids from medicinal plants can possess antimicrobial activity against various pathogens, including bacteria, fungi, and even some viruses (Kabara *et al*., 1972; Yff *et al*., 2002). They work by disrupting microbial cell membranes, which makes it harder for pathogens to develop resistance compared to conventional antibiotics. This mechanism might partially explain why *Antiaris toxicaria* preparations have been used traditionally for treating infections and promoting wound healing.

The methyl esters of fatty acids we detected (hexadecanoic acid methyl ester and heptadecanoic acid 16-methyl ester) are particularly worth noting. These compounds can form either naturally in the plant or during the extraction process with ethanol. They tend to be more volatile than the parent fatty acids, which is why they show up nicely in GC-MS analysis. Fatty acid esters have been investigated for various biological activities, including anticancer and antimicrobial effects (Harada *et al*., 2002).

Sugar derivatives; We identified several carbohydrate-derived compounds that give us insight into the plant's metabolic processes. Ethyl α-D-glucopyranoside showed up at 6.16 % relative area, d-glucohexodialdose at 11.48 %, and methyl-β-D-thiogalactoside at 4.07 %. Together, these sugar derivatives account for over 20 % of the extract.

d-Glucohexodialdose is an oxidized form of glucose that forms when sugars undergo oxidative degradation. Its presence at relatively high levels (11.48 %) might indicate oxidative processes occurring either in the living plant tissue or during extraction and analysis (Martins *et al*., 2001). This compound and other sugar oxidation products can contribute to the overall antioxidant capacity of plant extracts through various mechanisms.

Ethyl α-D-glucopyranoside is essentially a glucose molecule bound to an ethanol molecule—a glycoside that likely formed during the ethanol extraction process rather than existing naturally in the plant. While this might sound like an extraction artifact, such compounds can actually enhance the bioavailability of the parent sugars and potentially contribute to biological activities (Matsuura, 2002).

The presence of methyl-β-D-thiogalactoside is particularly intriguing. This is a thioglycoside, meaning it contains sulfur in its structure. Thioglycosides are less common than regular glycosides and can exhibit unique biological properties. Some thioglycosides have shown antimicrobial and anticancer activities in laboratory studies (Zeng *et al*., 2008). The sulfur-containing nature of this compound might also contribute to antioxidant activity through different mechanisms than the phenolic compounds we found in the HPLC analysis.

Aromatic Compounds (Phenolic Compounds); Several aromatic compounds with phenolic structures were identified, including phenol 2-methoxy- (2.76 %), 2-methoxy-4-vinylphenol (2.32 %), and trans-cinnamic acid (2.79 %). While these percentages might seem small compared to the fatty acids, these aromatic compounds often pack a powerful pharmacological effect.

Phenol 2-methoxy-, also known as guaiacol, is a phenolic compound with antiseptic and analgesic properties that has been used medicinally for over a century (Babushok *et al*., 2011). It's also a major component of smoke, which is why it's sometimes associated with traditional smoking or heating preparation methods. Guaiacol exhibits antimicrobial activity and can act as an expectorant, helping to loosen mucus in respiratory conditions—aligning well with traditional uses of *Antiaris toxicaria* for breathing problems.

2-Methoxy-4-vinylphenol, also called 4-vinylguaiacol, is another phenolic compound that contributes to aromatic properties. Beyond its pleasant smell (it has a clove-like aroma), this compound has demonstrated antioxidant and antimicrobial activities in various studies (Callemien & Collin, 2010). It's also been investigated for potential anti-inflammatory effects through inhibition of inflammatory mediators.

Trans-cinnamic acid is a particularly well-studied phenolic compound found in cinnamon and many other medicinal plants. It has shown a broad spectrum of biological activities including antimicrobial, antifungal, antiviral, antioxidant, and anticancer properties (Guzman, 2014). Trans-cinnamic acid can inhibit bacterial growth by disrupting cell wall synthesis and interfering with essential enzymes. It also exhibits anti-inflammatory effects by reducing the production of inflammatory cytokines and mediators (Sharma, 2011). The presence of this compound adds to the overall therapeutic potential of *Antiaris toxicaria* extracts.

Aldehydes and Related Compounds; Benzeneacetaldehyde appeared at 3.08 % relative area. This aromatic aldehyde has a characteristic floral, hyacinth-like odor and possesses antimicrobial properties. Aldehydes in general can react with proteins and nucleic acids in microbial cells, leading to their death or growth inhibition (Sherry, 1971). However, aldehydes can also be somewhat reactive in biological systems, which is something to keep in mind when considering safety profiles.

Benzoic acid methyl ester (3.55 %) is the methyl ester of benzoic acid, a well-known preservative and antimicrobial agent. Benzoic acid and its derivatives have been used for decades in food preservation and pharmaceutical formulations due to their ability to inhibit the growth of bacteria, yeasts, and molds (Chipley, 2005). The ester form may be more lipophilic than benzoic acid itself, potentially allowing better penetration into microbial cell membranes.

Other compounds; Phthalate Ester Detection; One compound that deserves special attention is bis(2-ethylhexyl) phthalate (DEHP), detected at 4.41 % relative area. This finding requires careful interpretation because phthalates are commonly used as plasticizers in various materials including plastic containers, tubing, and packaging materials (Net *et al*., 2015).

There are two possible explanations for this detection: either DEHP is genuinely present in the plant tissue (plants can absorb phthalates from contaminated soil or water), or it's a contaminant introduced during sample collection, storage, or analysis through contact with plastic materials. Given that phthalates are ubiquitous environmental contaminants and known endocrine disruptors with potential health concerns (Heudorf *et al*., 2007), this finding warrants further investigation.

If DEHP is truly present in the plant at these levels, it raises safety concerns for traditional preparations. However, before drawing conclusions, we should verify this finding with careful attention to minimizing contamination during sample handling—using glass containers instead of plastic throughout the process. The detection of phthalates is unfortunately common in plant extract analyses and often reflects laboratory contamination rather than genuine plant constituents (Fasano *et al*., 2012).

Nitrogen-Containing Compounds; Acetaldehyde N-formyl-N-methylhydrazone showed up at a surprisingly high 9.68 % relative area. This nitrogen-containing compound is less commonly reported in plant extracts. Hydrazones are a class of organic compounds that have attracted interest

for their diverse biological activities, including antimicrobial, anti-inflammatory, anticonvulsant, and anticancer properties (Rollas & Küçükgüzel, 2007).

However, there's a caveat here too. Some hydrazones can form as artifacts during extraction or analysis, particularly when aldehydes react with nitrogen-containing compounds under certain conditions. The relatively low match quality (52 %) suggests some uncertainty in the compound identification, so confirmation with additional analytical techniques would be prudent before drawing firm conclusions about its presence and significance.

Furan Derivative, the compound 2-furanmethanol, 5-ethenyltetrahydro-α,α,5-trimethyl-, cis- was detected at 11.30 % relative area, making it the second most abundant compound after palmitic acid. This is a furan derivative with a complex structure. Furan compounds are heterocyclic organic compounds that can form during thermal processing of foods and plant materials (Perez Locas & Yaylayan, 2004).The relatively low match quality (37 %) for this compound raises questions about the confidence of this identification. Furan derivatives can exhibit various biological activities, but they can also raise toxicological concerns depending on their structure. Some furan compounds have shown antimicrobial and antioxidant properties, while others have demonstrated genotoxic or hepatotoxic effects in high doses (Kellert *et al*., 2008). Given the uncertainty in identification and the relatively high percentage, this compound deserves focused investigation with complementary analytical techniques.

Fluorinated Compound; the detection of pentafluoropropionic acid undecyl ester (3.61 %) is quite unusual and frankly surprising. Fluorinated compounds are extremely rare in nature—fluorine is one of the least incorporated elements in biological systems, with only a handful of naturally occurring organofluorine compounds known (O'Hagan & Harper, 1999).

This finding almost certainly represents either a misidentification by the mass spectrometry library matching algorithm or contamination from laboratory sources. Pentafluoropropionic acid and its

derivatives are sometimes used in analytical chemistry for derivatization procedures that make compounds more volatile and easier to detect by GC-MS. If this compound is real and not an artifact, it would be quite remarkable and worthy of detailed investigation. However, the more likely explanation is analytical interference or contamination, especially given that this type of compound is not characteristic of plant secondary metabolism.

### 4.2 Comparing GC-MS and HPLC Results

When we compare the GC-MS results with the earlier HPLC analysis, we're looking at two complementary but different chemical landscapes. HPLC excels at analyzing polar, thermally labile, and high molecular weight compounds—which is why we detected alkaloids, flavonoids, tannins, and glycosides in that analysis. GC-MS, on the other hand, is better suited for volatile and semi-volatile compounds that can be vaporized without decomposition—hence the predominance of fatty acids, simple phenolics, and smaller molecules in this analysis.

Interestingly, there's very little overlap between the two compound lists, which isn't unusual. The phenolic compounds detected by GC-MS (guaiacol derivatives and cinnamic acid) complement the larger flavonoid and tannin profiles from HPLC—they're all part of the phenylpropanoid biosynthetic pathway that plants use to produce aromatic compounds (Vogt, 2010). Together, these compounds likely work synergistically to provide antioxidant and antimicrobial effects.

The absence of alkaloids in the GC-MS profile doesn't mean they're not there—it's more likely that compounds like ephedrine and the quinolizidine alkaloids detected by HPLC are either not volatile enough for GC analysis or decompose at the high temperatures required for gas chromatography. This underscores why using multiple analytical techniques is crucial for comprehensive phytochemical characterization.

### 4.3 Antibacterial Activity

The present study evaluated the antibacterial potential of *Antiaris toxicaria* stem bark extract against six clinically relevant bacterial pathogens using disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. The results demonstrate significant antibacterial activity with notable variation in susceptibility patterns among the tested organisms.

### 4.3.1 Disk Diffusion Assay (Inhibitory Zone Diameter)

The disk diffusion assay revealed that *Antiaris toxicaria* extract (40 mg) exhibited varying degrees of antibacterial activity against four of the six tested organisms. *Staphylococcus aureus* demonstrated the highest susceptibility with an inhibition zone diameter of 20 mm, followed by *Pseudomonas aeruginosa* (17 mm), *Enterobacter cloacae* (15 mm), and *Bacillus subtilis* (14 mm). Conversely, *Escherichia coli* and *Bacillus cereus* showed complete resistance with no observable zones of inhibition.

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, inhibition zones greater than 15 mm are generally considered indicative of susceptibility for most antimicrobial agents (CLSI, 2020). The zone diameters observed for *S. aureus*, *P. aeruginosa*, and *E. cloacae* suggest moderate to good antibacterial activity. These findings align with previous studies on *Antiaris* species, which have documented broad-spectrum antimicrobial properties attributed to their rich phytochemical composition, including cardiac glycosides, flavonoids, and terpenoids (Kuete *et al*., 2011; Omogbai *et al*., 2013).

The superior activity against *S. aureus* is particularly noteworthy given the clinical significance of this organism as a leading cause of nosocomial infections and its increasing resistance to conventional antibiotics (Tong *et al*., 2015). Plant extracts with anti-staphylococcal properties represent valuable leads for novel antimicrobial agent development (Cowan, 1999). The observed activity against *P. aeruginosa*, a notoriously drug-resistant Gram-negative pathogen, further underscores the therapeutic potential of this extract (Breidenstein *et al*., 2011).

The absence of inhibition zones for *E. coli* and *B. cereus* in the disk diffusion assay, despite demonstrable activity in the MIC assay (discussed below), suggests possible limitations in extract diffusion through the agar medium. This phenomenon has been reported for various plant extracts and may be attributed to the lipophilic nature of bioactive compounds, high molecular weight

constituents, or poor solubility in aqueous environments (Balouiri *et al*., 2016; Ncube *et al*., 2008). The use of different solvents or incorporation of permeabilizing agents might enhance diffusion and improve correlation between disk diffusion and broth dilution methods (Eloff, 1998).

The positive control, ciprofloxacin (1.6 mg/mL), produced inhibition zones ranging from 28 to 40 mm across all tested organisms, confirming the viability and susceptibility of the bacterial cultures. The negative control, Tween-80, showed no antimicrobial activity, validating that any observed inhibition was solely attributable to the plant extract rather than the solvent system (Aligiannis *et al*., 2001).

**4 3.2 Minimum Inhibitory Concentration (MIC)**

The MIC assay provided more quantitative insight into the antibacterial potency of *Antiaris toxicaria* extract. At the highest concentration tested (25 mg/mL), complete growth inhibition was observed for all six bacterial species, demonstrating broad-spectrum activity. However, substantial differences in susceptibility emerged at lower concentrations.

*Staphylococcus aureus* exhibited exceptional sensitivity with growth inhibition maintained at concentrations as low as 3.125 mg/mL, suggesting an MIC of ≤3.125 mg/mL. This remarkable potency against *S. aureus* corroborates the disk diffusion results and is consistent with ethnomedicinal applications of *Antiaris* species for treating skin and wound infections, conditions often associated with staphylococcal colonization (Omogbai *et al*., 2013; Burkill, 1985).

*Pseudomonas aeruginosa* demonstrated the second-highest susceptibility with sustained inhibition at 6.25 mg/mL (MIC = 6.25 mg/mL), while *Bacillus subtilis* required 12.5 mg/mL for complete growth suppression (MIC = 12.5 mg/mL). The enhanced activity against Gram-positive bacteria compared to most Gram-negative organisms reflects a common pattern observed with many plant-derived antimicrobials (Cowan, 1999; Nazzaro *et al*., 2013). This preferential activity is typically attributed

to structural differences in bacterial cell walls; Gram-positive bacteria possess a relatively simple peptidoglycan layer that is more permeable to lipophilic compounds, whereas Gram-negative bacteria have an additional outer membrane containing lipopolysaccharides that acts as a permeability barrier (Nikaido, 2003; Hemaiswarya *et al*., 2008).

*Escherichia coli*, *Enterobacter cloacae*, and *Bacillus cereus* all required the maximum tested concentration (25 mg/mL) for growth inhibition, indicating lower susceptibility. This reduced effectiveness against Gram-negative pathogens is consistent with findings reported for various *Antiaris* extracts and other medicinal plant preparations (Kuete *et al*., 2011; Tadesse *et al*., 2005). The intrinsic resistance mechanisms of Gram-negative bacteria, including efflux pumps and reduced outer membrane permeability, likely contribute to these higher MIC values (Pagès *et al*., 2008).

According to classification criteria proposed by Aligiannis *et al*. (2001) and Kuete (2010), plant extracts with MIC values below 100 µg/mL are considered to have strong antimicrobial activity, those between 100-500 µg/mL have moderate activity, between 500-1000 µg/mL have weak activity, and above 1000 µg/mL are considered inactive. By these standards, the *Antiaris toxicaria* extract demonstrates moderate to weak antibacterial activity for most organisms (MIC 3.125-25 mg/mL = 3125-25,000 µg/mL), with the notable exception of *S. aureus*, which approaches the moderate activity range. However, it should be noted that these classification criteria were originally developed for purified compounds and isolated fractions; crude extracts typically exhibit higher MIC values due to the dilution effect of inactive constituents (Rios & Recio, 2005).

The broad-spectrum activity at 25 mg/mL suggests the presence of multiple bioactive compounds with complementary or synergistic antimicrobial mechanisms. Previous phytochemical investigations of *Antiaris toxicaria* have identified numerous bioactive metabolites, including cardiac glycosides (such as α-antiarin and β-antiarin), flavonoids, saponins, tannins, and alkaloids (Omogbai *et al*., 2013; Neuwinger, 1996). These compound classes are well-documented for their antimicrobial

properties through various mechanisms, including membrane disruption, enzyme inhibition, and interference with nucleic acid synthesis (Cushnie & Lamb, 2005; Cowan, 1999).

### 4.3.3 Minimum Bactericidal Concentration (MBC)

The MBC assay distinguished between bacteriostatic and bactericidal effects of the extract. At 10 mg/mL, all tested organisms showed visible growth upon subculturing, indicating that this concentration was insufficient to kill the bacteria despite inhibiting growth in some cases (as demonstrated by the MIC results). At 20 mg/mL *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Bacillus subtilis* were completely killed (no growth observed), while *Enterobacter cloacae*, *Escherichia coli*, and *Bacillus cereus* remained viable.

The ratio of MBC to MIC is commonly used to characterize antimicrobial action: ratios ≤4 indicate bactericidal activity, while ratios >4 suggest bacteriostatic effects (Levison & Levison, 2009; Pankey & Sabath, 2004). For *S. aureus*, with an MIC of ≤3.125 mg/mL and MBC of 20 mg/mL, the MBC/MIC ratio is ≥6.4, technically suggesting bacteriostatic activity. However, this interpretation should be considered cautiously given that the actual MIC may be lower than 3.125 mg/mL (the lowest concentration tested). For *P. aeruginosa* (both with MIC =6.25 mg/mL and MBC = 20 mg/mL), the MBC/MIC ratios are <4, which appears bactericidal crediting P. kemila & C. Krishnaveni 2016. For *B. subtilis* (both with MIC=12.5mg/ml and MBC=20mg/ml), the MBC/MIC ratios are <4 depicting bactericidal.

For *E. cloacae* and *B. cereus*, which remained viable at 20 mg/mL despite having MIC values of 25, 25, and 6.25 mg/mL respectively, the extract appears to exert primarily bacteriostatic effects. Bacteriostatic agents inhibit bacterial growth and reproduction without directly killing the cells, typically through interference with essential metabolic processes such as protein synthesis, DNA replication, or folic acid metabolism (Pankey & Sabath, 2004). The clinical relevance of bacteriostatic versus bactericidal activity depends on the infection site and host immune status;

bactericidal agents are generally preferred for severe infections, immunocompromised patients, and infections in immune-privileged sites (Levison & Levison, 2009).

The incomplete bactericidal activity observed at 20 mg/mL for half of the tested organisms may reflect several factors. First, crude plant extracts contain complex mixtures of compounds with varying antimicrobial potencies, and the concentration of individual active constituents may be insufficient for bactericidal effects (Rios & Recio, 2005). Second, some bacterial species possess more robust survival mechanisms, including stress response systems and DNA repair machinery, which may enable them to recover from extract-induced damage (Poole, 2012). Third, the formation of persister cells—metabolically dormant bacterial subpopulations that exhibit tolerance to antimicrobial agents—could explain the survival of some bacteria at concentrations exceeding the MIC (Lewis, 2010).

# CHAPTER FIVE

## 5.0 Conclusions

The current study successfully quantified the relative abundance of bioactive compounds in bark extract of *Antiaris toxicaria* using HPLC and GC–MS techniques. Chromatographic and spectrometric analyses revealed the presence of major constituents such as (Ephedrine),(Proanthocyanin compound ) and (Amphyllidine compound ) with (Ephedrine) occurring at the highest concentration for HPLC, while for GC-MS, constituents such as (n-Hexadecanoic acid), (d-Glucohexodialdose) and (2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-,cis-) with n-Hexadecanoic as the highest occuring. Correlation of chemical profiles with pharmacological assays indicated that fractions or extracts rich in (Ephedrine and n-Hexadecanoic)exhibits strong activityg as antioxidant, anti-inflammatory, antimicrobial effects. Overall, the results highlight the potential of *Antiaris toxicaria* as a source of pharmacologically active compounds and provide a scientific basis for its further use.

HPLC and GC–MS proved to be reliable tools for qualitative and quantitative characterization of plant constituents, offering valuable markers for future standardization and quality control of herbal formulations, also analysing the toxicity of *Antiaris toxicaria* when taken for medicinal, and by the level of Antiaris compound present which is known to be a poisonous glycoside, for large usage or extensive usage, measures should be taken to alleviate the poison or should be avoided.

The findings of this study revealed that the 70% ethanolic extract of *Antiaris toxicaria* possesses measurable antibacterial activity against the tested microorganisms, particularly to Gram positive bacteria than that of Gram negative. The extract demonstrated a dose-dependent inhibitory effect, with greater zones of inhibition observed at higher concentrations. This suggests that the bioactive constituents extracted with 70% ethanol contribute significantly to the plant's antimicrobial potential

## 5.1 Recommendations

Further studies involving purification and characterization of the active components are recommended to better understand the mechanisms of action and to explore possible pharmaceutical applications.

Further experiments like Antioxidant tests should be carried out on *Antiaris toxicaria* to harness more knowledge that can be applied to both traditional and academic settings.

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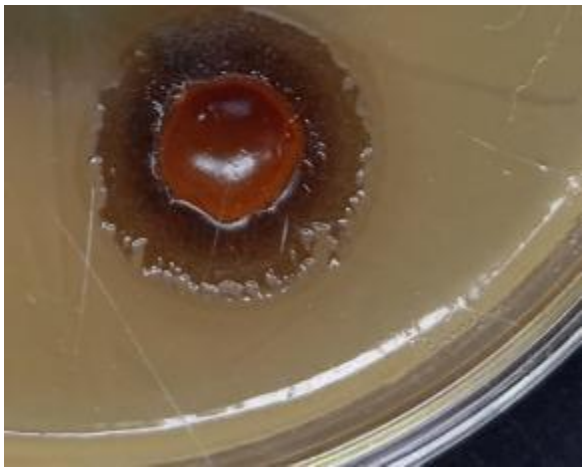
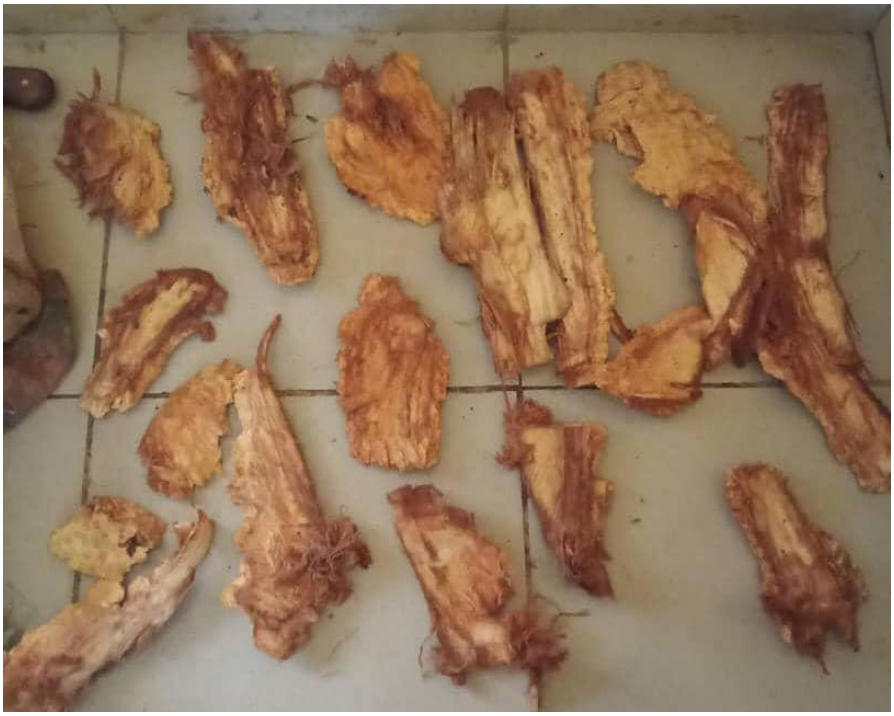
# APPENDIX



Antibacterial activity against *Bacillus subtilis*



Bark of Antiaris toxicaria under shade drying

Antiaris toxicaria tree