CHROMATOGRAPHIC ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF *Anthocleista vogelii* Planch.(Gentianaceae) ROOT BARK

BY

BERNICE OHIORENUAN IMOOBE

MAT NO: PHA1908521

SUPERVISED BY

Dr. EMMANUEL EIMIMODEBHEKI ODION

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

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this was successfully carried out by **BERNICE OHIORENUAN IMOOBE** in the department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

_____

_____ **DR. EMMANUEL E. ODION**

DATE

(PROJECT SUPERVISOR)

_____ _____

DR. VINCENT IMIEJE **DATE**

(HEAD OF DEPARTMENT)

_____ _____

BERNICE OHIORENUAN IMOOBE **DATE**

DEDICATION

This project work is dedicated to Almighty God for His mercies and faithfulness upon my life. It is also dedicated to my parents, Mr. and Mrs. Sylvester Imoobe, whose tireless sacrifices and belief in the power of education gave me the courage to pursue this path.

ACKNOWLEDGEMENT

My deepest gratitude goes to the Almighty God who kept me in good health, perfect peace and abundance throughout the period of my undergraduate study.

I am deeply grateful to my supervisor, Dr. Emmanuel E. Odion, for his patient guidance, critical insights, and unwavering support throughout this research

I extend sincere thanks to the Head of Department of Pharmaceutical Chemistry, Dr. Vincent Imieje, and my esteemed lecturers of the department of Pharmaceutical Chemistry, University of Benin, for their excellent teachings which shaped me for this study

I also appreciate Dr. Akpeh P. Kingsley of the Department of Biochemistry for his assistance with antioxidant analyses, Dr. David Okeke of Docchy Analytical Laboratory and environmental service LTD Awka for his assistance with HPLC analysis, Integrated research laboratory for assistance with GCMS analysis and Mr. Williams of the department of Pharmaceutical Microbiology for his assistance with the antimicrobial analysis.

I acknowledge also, Mrs Obi, Mr. Oviawe Thompson, Mr. Edionhon Charles Osagie and all the laboratory technicians of the department of Pharmaceutical Chemistry, University of Benin, for their assistance and support in carrying out this work

I appreciate Mr. Samson who aided in the collection of the plant.

I am forever grateful to my parents Mr and Mrs Sylvester Imoobe for their prayers, unwavering support and unconditional love.

My heartfelt gratitude goes to my siblings, Marvellous Imoobe and Peace Imoobe and friends; Imagbenikaro Osasenaga, Oni-Edigin Ekiomado, Idoro Raphael, Chukwunonyenim Solo-Okoh, Engr. Asibor Ehikalomen, Chukwuma Onyeka, Ighalo Ofure, Osaghae Jane, Aigbogho Uwadea, Bright Imoobe, Pastor Iwinosa Atohengbe, Pastor Emeka Mark, Dr. Daniel Ulaa, Joshua Aganbi, Dr. Ohis Igeleke and my project partner, Francess Ogedengbe for their moral support and impact throughout this project and my undergraduate journey.

TABLE OF CONTENTS

CERTIFICATION……ii

DEDICATION……iii

ACKNOWLEDEGEMENT……...iv

TABLE OF CONTENTS……v

ABSTRACT……...vi

CHAPTER ONE……1

1.0 INTRODUCTION……..……1
1.1 DEFINITION OF KEY TERMS…….…..…..2
1.2 LITERATURE REVIEW…….……..3
1.2.1 Overview of Medicinal Plant in Ethnopharmacology……4
1.2.2 Botanical Description and Taxonomy of *Anthocleista vogelii* Planch…….…..5
1.2.2.1 Morphological Characteristics……..…..5
1.2.2.2 Habitat and Distribution……..6
1.2.3 Phytochemical Constituent of Anthocleista Species……..…….7
1.2.4 Ethnobotanical and Traditional Uses of *A. vogelii* Root Bark……12
1.2.5 Closely Related Species and Comparative Context……13
1.2.6 Pharmacological Activities of *Anthocleista vogelii*……..14
1.2.6.1 Antioxidant Activity…….……..14
1.2.6.2 Antibacterial and Antifungal Activity……...…….14
1.2.6.3 Anti-inflammatory and Analgesic Activity……15
1.2.6.4 Ant diabetic and Hepatoprotective Activity……...15
1.2.6.5 Cytotoxic and Ant proliferative Potential……...15
1.2.7 Harvesting/Collection……16
1.3 CHROMATOGRAPHIC TECHNIQUES IN PHYTOCHEMICAL ANALYSIS.…17
1.4 ANTIOXIDANT ACTIVITY: MECHANISMS AND ASSAY METHODS……18
1.5 ANTIBACTERIAL ACTIVITY: MECHANISM AND EVALUATION METHODS……....20

1.5.1 Disc Diffusion and Broth Dilution Methods……..21
1.6 BIOLOGICAL ACTIVITIES (ANTIOXIDANT AND ANTIBACTERIAL)……..22
1.7 JUSTIFICATION OF STUDY……..23
1.8 AIM AND OBJECTIVES OF THE STUDY…….24

CHAPTER TWO……....26

2.0 MATERIALS AND METHODS……..26
2.1 COLLECTION AND IDENTIFICATION OF PLANT MATERIAL…….....26
2.2 EXTRACTION OF PLANT MATERIAL……....26
2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)……..26
2.3.1 Sample Preparation for High Performance Liquid Chromatography……..s26
2.3.2 High Performance Liquid Chromatography Procedure……...27
2.4 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)……..27
2.4.1 Preparation of Plant Extract for GC-MS Analysis……..27
2.4.2 GC-MS Analysis…….27
2.5 DETERMINATION OF *IN VITRO* ANTIOXIDANT ACTIVITY……28
2.5.1 Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity……....28
2.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay……28
2.5.3 Determination of the Scavenging Activity of ABTS Free radical……...29
2.5.4 Determination of Total Antioxidant Capacity…….....28
2.6 ANTIBACTERIAL ACTIVITY OF *Anthocleista vogelii* Planch……...29
2.6.1 Specimen Collection……29
2.6.2 Preparation of Test Bacteria…….....30
2.6.3 Antimicrobial Susceptibility Tests and Determination of IZD……..30
2.6.4 Determination of Minimum Inhibitory Concentration (MIC)…….....31
2.6.5 Determination of Minimum Bactericidal Concentration…….....31
2.6.6 Determination of the Mode of Action…….....32
2.7 STATISTICAL ANALYSIS METHOD…….....32

CHAPTER THREE…….....33

3.0 RESULTS……33
3.1 HIGH PERFORMANCE LIQUID CHRROMATOGRAPHY……...33
3.2 GAS CHROMATOGRAPHY-MAS SPECTROMETRY……..37

3.3 *IN VITRO* ANTIOXIDANT ACTIVITY……48
3.3.1 DPPH Radical Scavenging Activity……...48
3.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay……49
3.3.3 ABTS Radical Scavenging Activity……51
3.3.4 Total Antioxidant Capacity (TAC) Assay……...50
3.4 ANTI-MICROBIAL ASSAY……51
3.4.1 Determination of Zone of Inhibition……..51
3.4.2 Minimum Inhibitory Concentration…….....52
3.4.3 Minimum Bactericidal Concentration (MBC)…….....53
3.4.4 Mode of Antibacterial Activity……..54

CHAPTER FOUR…….55

4.0 DISCUSSION…….....55
4.1 PHYTOCHEMICAL CONSTITUENTS (HPLC & GC-MS FINDINGS)……..56
4.1.1 High-Performance Liquid Chromotagraphy (HPLC) Analysis……56
4.1.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis……59
4.1.3 Implications for Biological Activities……..60
4.2 *IN VITRO* ANTIOXIDANT ACTIVITY…….61
4.2.1 DPPH Radical Scavenging Assay……61
4.2.2 Ferric Reducing Antioxidant Power (FRAP) Assay……62
4.2.3 ABTS Radical Scavenging Activity……62
4.2.4 Total Antioxidant Capacity (TAC) Assay…….....63
4.3 ANTIMICROBIAL ASSAY…….....64

CONCLUSION…….....68

REFERENCES……69

APPENDICES…….80

LIST OF FIGURES

Figure 1: Pictorial representation of *Anthocleista vogelii* tree……..……..6

Figure 2: HPLC chromatogram of *Anthocleista vogelii* extract……33

Figure 3: GC-MS chromatogram of *Anthocleista vogelii* extract……..37

Figure 4: DPPH Analysis Bar Chart of *Anthocleista vogelii* root extract……..48

Figure 5: FRAP Analysis Bar Chart of *Anthocleista vogelii* root extract……..49

Figure 6: TAC Analysis Bar Chart of *Anthocleista vogelii* root extract…….50

Figure 7: ABTS Analysis Bar Chart of *Anthocleista vogelii* root extract……51

LIST OF TABLES

Table 1 Botanical Classification of *Anthocleista vogelii*……...7

Table 2 HPLC analysis of *Anthocleista vogelii* extract…….34

Table 3 GC-MS analysis of *Anthocleista vogelii* extract……..38

Table 4 Zones of Inhibition caused by *Anthocleista vogelii* root extract on selected bacteria……..52

Table 5 Minimum Inhibitory Concentration of *Anthocleista vogelii* extract……53

Table 6 Minimum Bactericidal Concentration of *Anthocleista vogelii* extract……54

ABSTRACT

Anthocleista vogelii Planch (Gentianaceae) root bark is employed in West African traditional medicine for managing infections and oxidative stress-related ailments. This study characterized the 70% ethanolic root bark extract using chromatographic techniques and evaluated its in-vitro antioxidant and antibacterial properties.

Root bark was collected in Akoko Edo, Edo State, Nigeria, authenticated (voucher UBH-A258), and extracted by cold maceration with 70% ethanol. High-performance liquid chromatography (HPLC) employed a C-12 column with water-acetonitrile gradient and UV detection at 280 nm. Gas chromatography-mass spectrometry (GC-MS) used a DB-5MS column with helium carrier gas and NIST library matching. Antioxidant activity was assessed via (2,2-diphenyl-1-picrylhydrazyl) DPPH, (2,2'-azino-bis-3-ethybenzothiazoline-6-sulfonic acid) ABTS, Ferric Reducing Antioxidant Power FRAP, and Total Antioxidant Capacity (TAC) assays. Antibacterial effects were tested by agar well diffusion and dilution methods against *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Enterobacter cloacae*.

HPLC identified 19 compounds, including ammodendrine (22.07 µg/mL), cyanogenic glycoside (20.19 µg/mL), and sparteine (15.98 µg/mL) with the higest concentrations. GC-MS detected 35 compounds, dominated by 2,8-decadiyne (18.0%), pentyl octanoate (9.7%), and elaidic acid (7.1%). The extract showed potent DPPH scavenging (91.92 % at 200 µg/ml), ABTS inhibition (93.24% at 200 µg/mL), FRAP (40.44 at 400 µg/ml), and TAC (89.15% at 1000 µg/ml). Inhibition zones ranged 13–18 mm; MICs were 25–100 mg/mL, with MBCs 100–200 mg/mL. The extract demonstrated bactericidal activity, particularly against *S.aureus*, *P. aeruginosa* and *E. clocae*. These results validate the ethnomedicinal use of *A. vogelii* root bark and highlight its potential as a natural source of antioxidants and antibacterials.

CHAPTER ONE

1.0 INTRODUCTION

Medicinal plants remain an important source of therapeutic agents and a starting point for drug discovery. Their secondary metabolites can act as antioxidants and as antibacterial compounds, which makes them relevant in efforts to address both oxidative-stress related illnesses and the growing problem of antimicrobial resistance (Newman and Cragg, 2020; WHO, 2023).

Anthocleista vogelii Planch (Gentianaceae) is widely used in West African traditional medicine. Local communities use various parts of the plant, including the root bark, to manage infections, fever and other ailments. Recent experimental work on *A. vogelii* and related *Anthocleista* species reports meaningful phytochemical content and biological activity, but most studies focus on leaves or stem bark rather than root bark (Ikpe *et al*., 2020; Ajayi *et al*., 2023).

Chromatographic tools such as (High Performance Liquid Chromatography) HPLC and Gas Chromatography-Mass Spectrometry (GC-MS) provide reliable chemical fingerprints that can be linked to antioxidant and antibacterial assays to clarify which constituents are responsible for observed effects (Bassey *et al*., 2023).

Given the limited, peer-reviewed data specifically addressing the root bark of *A. vogelii*, this study aims to apply chromatographic analysis, standard antioxidant assays and antibacterial testing to the 70% ethanolic root bark extract, with the intention of connecting chemical composition to biological activity and validating traditional use.

1.1 DEFINITION OF KEY TERMS

Antioxidants: Antioxidants are compounds that inhibit oxidation by neutralizing reactive oxygen species (ROS) and free radicals, thus preventing cellular damage and oxidative stress. They can be naturally derived from plants or synthetically produced (Kaur and Kapoor, 2021).

Antibacterial Agents: These are substances that inhibit the growth or kill bacteria by interfering with vital bacterial functions such as cell wall synthesis, protein formation, or nucleic acid replication (Yusuf *et al*., 2023). Plant-derived antibacterial agents are gaining renewed attention due to the rise of multidrug-resistant pathogens.

Chromatography: Chromatography is an analytical separation technique used to identify, isolate, and quantify components within a mixture based on their differential affinities between stationary and mobile phases. Common types include High-Performance Liquid Chromatography (HPLC), Thin-Layer Chromatography (TLC), and Gas Chromatography (GC) (Bassey *et al*., 2023).

Phytochemicals: Phytochemicals are bioactive, non-nutrient plant compounds produced through secondary metabolism. They include alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic acids, many of which possess pharmacological activities such as antioxidant, anti-inflammatory, and antimicrobial effects (Ogunyemi *et al*., 2022).

Root Bark Extract: The root bark extract refers to the concentrated preparation obtained from the powdered bark of the plant's root, typically extracted using solvents such as ethanol or methanol to isolate bioactive constituents (Ikpe *et al*., 2020).

Antioxidant Assay: An antioxidant assay is a laboratory test used to assess the ability of a substance to scavenge free radicals or reduce oxidized intermediates. Common assays include

the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test and the FRAP (Ferric Reducing Antioxidant Power) method (Kumar *et al*., 2022).

1.2 LITERATURE REVIEW

Medicinal plants continue to play a critical role in modern pharmacological research due to their vast array of bioactive compounds that serve as leads for drug discovery (Newman and Cragg, 2020). Globally, the World Health Organization estimates that more than 80% of the population in developing countries relies on traditional medicine for primary healthcare, mainly using plant-based preparations (WHO, 2023). The increasing prevalence of oxidative stress related disorders and antibiotic resistance has intensified interest in medicinal plants that possess antioxidant and antibacterial activities (Ogunyemi *et al*., 2022; Yusuf *et al*., 2023).

Anthocleista vogelii Planch (Gentianaceae), commonly known as the "Cabbage tree," forest fever tree, and locally known as apa oro, (Yoruba), kwari (Hausa), Ulemewuwu (Owan), Bini (Orimi) and Mpoto (Igbo) is one of such plants of ethnomedicinal importance. It has been traditionally employed in African herbal medicine for the treatment of malaria, diabetes, fever, gastrointestinal disorders, and infections (Ajayi *et al*., 2023). Scientific investigations have confirmed that different parts of the plant contain a diverse range of phytochemicals, including alkaloids, xanthones, iridoids, flavonoids, tannins, and phenolic acids, many of which are responsible for its pharmacological actions (Bassey *et al*., 2023).

Chromatographic and spectroscopic analyses have become indispensable in profiling plant-derived bioactive molecules, allowing researchers to establish structure activity relationships and validate ethnomedicinal claims (Kumar *et al*., 2022; Kaur and Kapoor, 2021). These analytical methods are essential for standardization, quality assurance, and the identification of potential drug candidates from natural sources.

The present review summarizes existing literature on the chromatographic profiling, antioxidant and antibacterial activities of *A. vogelii* root bark. It also identifies research gaps that justify the present study, especially in the quantitative characterization of its bioactive compounds using modern chromatographic techniques.

1.2.1 Overview of Medicinal Plants in Ethnopharmacology

Medicinal plants have been the foundation of traditional medicine systems for centuries and continue to serve as an indispensable source of therapeutic agents. Ethnopharmacology, which studies the bioactive principles of plants used in indigenous medicine, provides an important link between traditional knowledge and modern scientific validation (Owolabi *et al*., 2021). Approximately 60% of currently available drugs are derived, directly or indirectly, from natural products, with a significant proportion originating from plants (Newman and Cragg, 2020).

The World Health Organization recognizes the relevance of medicinal plants in healthcare delivery, particularly in developing regions where over 80% of the population depends on herbal remedies for primary health care (WHO, 2023). This reliance is largely due to accessibility, affordability, and the perceived safety of plant-based therapies when compared with synthetic drugs (Kumari *et al*., 2022). Ethnopharmacological research is therefore essential to scientifically validate traditional claims, identify bioactive compounds, and ensure the safety and efficacy of herbal preparations (Yusuf *et al*., 2023).

Medicinal plants possess a diverse array of secondary metabolites such as alkaloids, flavonoids, terpenoids, phenolics, and glycosides, which are responsible for their pharmacological actions. These compounds exhibit antioxidant anti-inflammatory antimicrobial antidiabetic and anticancer properties (Ogunyemi *et al*., 2022). The discovery of such phytochemicals has led to the development of several modern drugs including

quinine, artemisinin, and paclitaxel all of which originated from ethnopharmacological studies (Kaur and Kapoor, 2021).

In contemporary pharmacognosy, ethnopharmacological knowledge guides the selection of plants for bioassay-guided fractionation and chemical profiling. Advanced analytical techniques such as chromatographic separation, spectroscopic characterization, and molecular docking have made it possible to isolate and identify active constituents responsible for biological effects (Kumar *et al*., 2022). This integration of traditional medicine and modern science underscores the continuing importance of medicinal plants in addressing global health challenges, including oxidative stress, microbial resistance, and metabolic disorders (Ajayi *et al*., 2023).

1.2.2 Botanical Description and Taxonomy of *Anthocleista vogelii* Planch.

Anthocleista vogelii Planch, a member of the family Gentianaceae, is a medium to large-sized tropical tree widely distributed in the humid forests of West and Central Africa. The genus *Anthocleista* comprises about 15–20 species, many of which are recognized for their medicinal and ecological importance (Bassey *et al*., 2023)

1.2.2.1 Morphological Characteristics

The plant is typically a deciduous tree that can reach heights of 15–30 metres with a straight, cylindrical trunk and a dense crown of large, opposite leaves. The leaves are simple, glabrous, and oblong, with entire margins and prominent midribs, sometimes exceeding 30 cm in length (Ikpe *et al*., 2020). The flowers are large, fragrant, and white to pale yellow, arranged in terminal panicles. Each flower possesses a tubular corolla and numerous stamens, characteristic of the Gentianaceae family (Ajayi *et al*., 2023).

The fruit is a smooth, ovoid capsule containing numerous small, brown seeds embedded in pulp. The root bark is thick and fibrous, brown on the outer surface, and yellowish inside. It emits a bitter taste, attributed to its rich composition of alkaloids and iridoid glycosides (Ikpe *et al*., 2020; Bassey *et al*., 2023).

1.2.2.2 Habitat and Distribution

Anthocleista vogelii thrives in tropical rainforest zones and secondary forests, often growing near riverbanks and swampy areas. It prefers moist, well-drained soils and is native to West and Central Africa, occurring in countries such as Nigeria, Cameroon, Ghana, Sierra Leone, and the Democratic Republic of Congo (Bassey *et al*., 2023). In Nigeria, it is commonly found in the southern rainforest belt where it forms part of the understorey vegetation.

Figure 1 Pictorial representation of *Anthocleista vogelii* tree

Table1: Botanical Classification of *Anthocleista vogelii*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magniolopsida
Order	Gentianales
Family	Gentianaceae
Genus	Anthocleista
Species	*A. vogelii*

(Source: Bassey *et al*., 2023; Ikpe *et al*., 2020)

1.2.3 Phytochemical Constituents of Anthocleista Species

Plant secondary metabolites (phytochemicals) are non-nutritive compounds synthesized by plants that serve defence and signalling functions and frequently underlie the biological activity of medicinal plants. Major classes include alkaloids, phenolics (and flavonoids), tannins and saponins; these classes are commonly associated with antioxidant and antimicrobial properties and thus are central to evaluating the pharmacological potential of *Anthocleista vogelii* root-bark (Chen *et al*., 2023; Aryal *et al*., 2022).

Phytochemical profiling of *A. vogelii* reveals a rich spectrum of secondary metabolites that may underlie its antioxidant and antibacterial effects. In one recent study, the root extract of *A. vogelii* was shown to contain alkaloids, tannins, phenolics, flavonoids, glycosides, terpenoids and saponins. The total phenolic content was reported as 24.87 ± 2.12 mg GAE/g and total flavonoid content as 16.18 ± 0.54 mg QE/g, with an IC_{50} of 14.08 µg/mL for DPPH radical scavenging. (Aiwonegbe *et al*., 2025)

Another GC-MS based investigation of A. vogelii (leaf material) identified major volatile/semi-volatile components including tridecane (≈21.61 %) and 2,6-dimethyl-undecane

(≈24.36 %) alongside alkaloids, tannins, alcohols, aldehydes, terpenes, ketones and phenols (Bassey *et al*., 2023). Although this work used leaf tissue, the chemical classes detected suggest that similar constituents may be present in the root bark as well.

These phytochemical analyses support the view that *A. vogelii* root bark possesses multiple classes of compounds especially phenolics and flavonoids that likely contribute to its bioactivity. However, direct chromatographic quantitation of pure compounds in the root bark remains limited in published literature, reinforcing the need for detailed HPLC and GC-MS profiling of the root bark in the present study.

Alkaloids

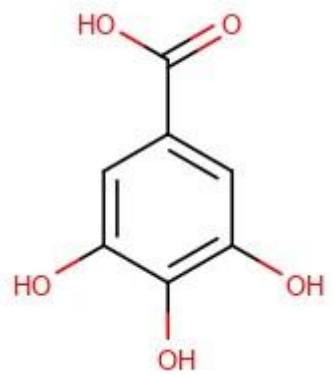
Alkaloids are nitrogenous, often heterocyclic compounds biosynthesised from amino-acid precursors. They display diverse pharmacological actions (antimicrobial, analgesic, antimalarial and cytotoxic) and are widely studied in drug discovery for their potent bioactivities (Aryal *et al*., 2022).

Several phytochemical screenings of *A. vogelii* (roots, stem-bark, leaves) report alkaloids among detected constituents (Ikpe *et al*., 2020; Nzor *et al*., 2024) These findings indicate that alkaloids are present in the species and likely occur in the root-bark investigated in this project (Ikpe *et al*., 2020). An example is reserpine, reserpine is an indole alkaloid known for its antihypertensive and antimicrobial properties. Though not exclusive to *Anthocleista vogelii*, its presence in related Gentianaceae species has been documented. Reserpine acts by inhibiting bacterial efflux pumps, which enhances the intracellular concentration of antibiotics and other antimicrobial agents, thereby increasing their efficacy (Singh *et al*., 2024). This mechanism is particularly relevant in combating multidrug-resistant bacteria.

Phenolics

Phenolic compounds bear one or more hydroxylated aromatic rings and are principal contributors to plant antioxidant activity because they can donate electrons or hydrogen atoms to neutralize free radicals and chelate pro-oxidant metals (Chen *et al*., 2023). Phenolics also contribute to antimicrobial and anti-inflammatory effects.

Quantitative and qualitative analyses of *A. vogelii* extracts identify phenolics as an abundant class. Ikpe *et al*. (2020) reported notable total phenolic content in root extracts, while Nzor *et al*. (2024) and Bassey *et al*. (2024) support these findings across other plant parts, indicating phenolics are major contributors to the species' antioxidant potential. Gallic acid is a potent phenolic compound found in *Anthocleista vogelii*. It acts by donating hydrogen atoms to reactive oxygen species and chelating transition metal ions, thereby reducing oxidative stress. Gallic acid also disrupts microbial membranes and inhibits key enzymes involved in bacterial metabolism (Hadidi *et al*., 2025; Singh *et al*., 2019). These mechanisms support its antioxidant and antimicrobial efficacy.

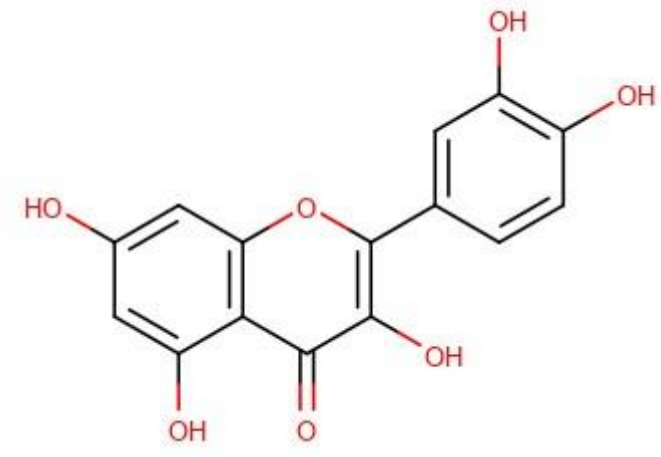


Gallic acid (3,4,5-trihydroxybenzoic acid)

Flavonoids

Flavonoids are a large subclass of polyphenols (C6–C3–C6) with established antioxidant, antimicrobial and enzyme-modulating activities (Chen *et al*., 2023). They neutralize radicals, chelate metals and can disrupt microbial processes

Flavonoids were detected both qualitatively and quantitatively in *A. vogelii* root and leaf extracts (Ikpe *et al*., 2020; Bassey *et al*., 2023). Their presence helps explain the radical-scavenging and antibacterial activity observed in crude extracts. Quercetin is a widely distributed flavonoid found in many medicinal plants, including *Anthocleista vogelii*. It exhibits strong antioxidant activity by scavenging reactive oxygen species and inhibiting lipid peroxidation. Quercetin also modulates inflammatory pathways such as NF-κB and MAPK, reducing the expression of pro-inflammatory cytokines (Akaahan *et al*., 2025; Alharbi *et al*., 2025). These actions make it effective in managing oxidative stress and inflammation.



Quercetin; 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one

Tannins

Tannins are high-molecular-weight polyphenols classified as hydrolysable or condensed; they bind proteins and membranes and frequently exert antimicrobial and antioxidant effects (Kováč *et al*., 2022).

Tannins are consistently reported in *A. vogelii* phytochemical screens (Ikpe *et al*., 2020; Nzor *et al*., 2024). Their protein-precipitating and cell-surface effects plausibly contribute to the species' antibacterial actions.

Saponins

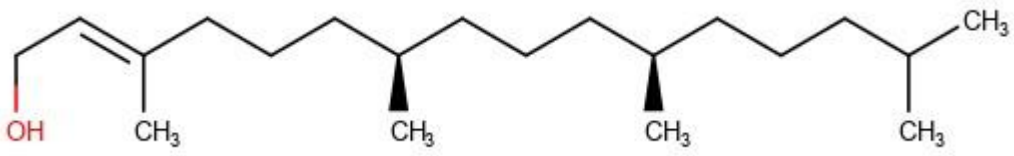
Saponins are glycosides containing triterpenoid or steroid aglycones linked to sugars, notable for surfactant properties and membrane-activity that can produce antimicrobial effects (Chen *et al*., 2023).

Saponins have been recorded in root, stem and leaf extracts of *A. vogelii* (Ikpe *et al*., 2020; Bassey *et al*., 2023). Their amphipathic nature may underlie membrane-disruptive antibacterial activity seen in experimental assays

Volatile Constituents

Volatile compounds in *Anthocleista vogelii* root bark have been identified through GC-MS analysis. These include terpenoids, fatty acid esters, and aromatic hydrocarbons. Terpenoids such as phytol exhibit antimicrobial and antioxidant properties by disrupting microbial membranes and scavenging free radicals. Fatty acid esters like hexadecanoic acid methyl ester and 9,12-octadecadienoic acid methyl ester contribute to anti-inflammatory and antioxidant effects by modulating lipid metabolism and inhibiting lipid peroxidation. Aromatic hydrocarbons, though less abundant, integrate into microbial membranes and alter their fluidity, leading to impaired cellular function. The synergy between volatile and non-

volatile constituents enhances the overall pharmacological efficacy of the plant (Bassey *et al.*, 2025). Phytol is a diterpene alcohol identified in the volatile profile of *Anthocleista vogelii* through GC-MS analysis. It exhibits antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation. Phytol also disrupts microbial membranes, leading to increased permeability and cell death (Aiwonegbe *et al*., 2025; Edet *et al*., 2018). These properties enhance the pharmacological potential of the plant.



Phytol ((E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol)

1.2.4 Ethnobotanical and Traditional Uses of *A. vogelii* Root Bark

Anthocleista vogeli Planch (Gentianaceae) is an indigenous African medicinal plant with long-standing ethnobotanical importance. It is widely distributed across tropical Africa, particularly in Nigeria, Ghana, and Cameroon, where it is used by traditional healers to treat a range of ailments. Although most scientific reports focus on the stem or leaf extracts, traditional accounts and community surveys highlight the root bark as a valued part of the plant used for various therapeutic purposes (Ikpe *et al*., 2020).

The plant is typically prepared as aqueous or ethanolic decoctions, infusions, or topical formulations depending on the disease condition and local traditions (Aiwonegbe *et al*., 2025).

Decoctions of the root bark are administered orally to relieve fever and malaria symptoms, Infusions are taken to manage stomach discomfort, intestinal worms, and constipation, crushed root bark paste is applied to skin infections, ulcers, and abscesses, reflecting the plant's observed antibacterial and antioxidant properties. Root preparations are used by some

local healers for managing diabetes and hypertension, likely due to the presence of flavonoids and alkaloids with vasorelaxant and glucose-lowering effects. The root bark is also consumed in small doses as a strength-enhancing or "body-cleansing" herbal drink in some communities.

1.2.5 Closely Related Species and Comparative Context

The genus *Anthocleista* (family Gentianaceae) consists of about 15–20 species distributed across tropical Africa and Madagascar, several of which have established roles in traditional African medicine. Among these, *Anthocleista djalonensis*, *Anthocleista grandiflora*, and *Anthocleista nobilis* are the species most closely related to *A. vogelii* and share overlapping ethnomedicinal applications (Ogu *et al*., 2022).

Phytochemical studies across *Anthocleista* species reveal consistent patterns in secondary metabolite profiles. These species are rich in iridoid glycosides**,** flavonoids**,** alkaloids**,** saponins**,** and tannins, which contribute to their broad pharmacological activities (Ofor *et al*., 2023). Comparative analyses indicate that *A. djalonensis* and *A. vogelii* exhibit similar antioxidant and antimicrobial capacities, though differences in metabolite concentration may account for variations in potency. For example, methanolic extracts of *A. djalonensis* have shown higher total phenolic content than comparable extracts of *A. vogelii*, while *A. grandiflora* tends to contain more terpenoids and steroids (Ekpo *et al*., 2021).

Biological evaluations of related *Anthocleista* species support their therapeutic potential against infectious and oxidative stress-related diseases. Studies on *A. djalonensis* leaf and stem bark have demonstrated antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, similar to that reported for *A. vogelii* root bark (Uchendu *et al*., 2024). Likewise, *A. grandiflora* extracts have shown anti-inflammatory and hepatoprotective effects in rodent models, confirming that pharmacological activities within the genus are broadly conserved (Egbuna *et al*., 2022).

This taxonomic and chemical resemblance suggests that *A. vogelii*, while distinct, shares a core phytochemical signature with its congeners, supporting the ethnopharmacological rationale for its traditional use and motivating comparative chromatographic studies to identify unique or shared bioactive compounds across the genus.

1.2.6 Pharmacological Activities of *Anthocleista vogelii*

The pharmacological relevance of *Anthocleista vogelii* has been increasingly validated by contemporary studies exploring its antioxidant**,** antimicrobial**,** anti-inflammatory**,** antidiabetic**,** and hepatoprotective activities.

1.2.6.1 Antioxidant Activity

Multiple investigations report that *A. vogelii* extracts demonstrate substantial free-radical scavenging capacity, especially in DPPH and ABTS assays. The methanolic root extract showed concentration-dependent inhibition of oxidative radicals comparable to standard antioxidants such as ascorbic acid (Ikpe *et al*., 2020). These findings support its ethnomedicinal use in treating oxidative stress-related conditions such as chronic infections and inflammatory diseases.

1.2.6.2. Antibacterial and Antifungal Activity

Several studies have shown that both the stem and root extracts of *A. vogelii* inhibit a broad spectrum of pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans* (Uchendu *et al*., 2024). The observed antibacterial activity is believed to result from the synergistic effect of alkaloids, tannins, and saponins, which disrupt microbial cell walls and inhibit enzymatic activity (Ofor *et al*., 2023).

1.2.6.3. Anti-inflammatory and Analgesic Effects

Preclinical studies using rat models have demonstrated that ethanolic extracts of *A. vogelii* significantly reduce carrageenan-induced paw edema and acetic acid-induced writhing, indicating both anti-inflammatory and analgesic properties (Egbuna *et al*., 2022). These effects are consistent with the presence of flavonoids and terpenoids, compounds known for their cyclooxygenase-inhibiting activity.

1.2.6.4. Antidiabetic and Hepatoprotective Activities

Aqueous and methanolic extracts of *A. vogelii* have shown glucose-lowering effects in streptozotocin-induced diabetic rats, suggesting insulin-sensitizing or pancreatic β-cell protective actions (Akah *et al*., 2021). Additionally, the hepatoprotective effect demonstrated by decreased serum ALT, AST, and ALP levels indicates that *A. vogelii* may protect hepatic tissue from oxidative or toxic damage (Ezeorah *et al*., 2023).

1.2.6.5. Cytotoxic and Antiproliferative Potential

Emerging data also suggest that extracts of *A. vogelii* may exert cytotoxic effects against selected cancer cell lines. In vitro studies have shown moderate inhibition of HeLa and MCF-7 cells, although these findings remain preliminary and require further chromatographic isolation and characterization of the active constituents (Bassey *et al*., 2023).

Overall, the available literature confirms that *A. vogelii* possesses diverse pharmacological properties supporting its traditional use. However, many reports rely on crude extracts; thus, comprehensive chromatographic profiling and in vivo validation are crucial to isolate bioactive molecules and elucidate their mechanisms.

1.2.7 Harvesting/Collection

The proper harvesting and collection of medicinal plant materials are critical steps that influence the quality, purity, and potency of phytochemical constituents. For *Anthocleista vogelii* Planch (Gentianaceae), standard ethnopharmacological practice emphasizes the collection of the root bark during the dry season, typically between December and March, when secondary metabolite concentration is at its peak due to reduced water content (Nnamani *et al*., 2022).

Plant identification and authentication are essential before collection to ensure the correct species is obtained, as *A. vogelii* shares morphological similarities with other *Anthocleista* species. Verification is commonly carried out by a qualified taxonomist or botanist at a recognized herbarium where a voucher specimen is deposited for reference (Okafor *et al*., 2023).

Harvesting is generally performed using clean, non-corrosive tools to avoid contamination. The root bark is carefully peeled to minimize damage to the main plant and ensure regeneration. After collection, the samples are washed to remove adhering soil particles**,** air-dried under shade to preserve thermolabile compounds, and then pulverized into fine powder using a sterile mechanical grinder (Aiwonegbe *et al*., 2025). Proper drying conditions are vital, as direct sunlight or excessive heat can degrade volatile phytochemicals and phenolic compounds responsible for antioxidant and antibacterial activity.

Environmental and ethical considerations are also critical. Sustainable harvesting practices such as partial bark removal and collection from mature plants are recommended to prevent overexploitation and ensure biodiversity conservation (Egbuna *et al*., 2022). These steps collectively preserve the plant's phytochemical integrity and ensure reproducibility in subsequent chromatographic and pharmacological analyses.

1.3 CHROMATOGRAPHIC TECHNIQUES IN PHYTOCHEMICAL ANALYSIS

Chromatography remains a central analytical technique in phytochemical research, allowing the isolation, identification, and quantification of bioactive compounds in complex plant matrices. In studying *Anthocleista vogelii* and other medicinal plants, chromatographic methods such as Thin Layer Chromatography (TLC)**,** High-Performance Liquid Chromatography (HPLC)**,** Gas Chromatography–Mass Spectrometry (GC–MS)**,** and Liquid Chromatography–Mass Spectrometry (LC–MS) are routinely employed (Okorie *et al*., 2023).

1.3.1 Thin Layer Chromatography (TLC)

TLC is widely used as a preliminary qualitative method for detecting classes of phytochemicals such as flavonoids, alkaloids, and terpenoids. Compounds are separated based on differential adsorption to a stationary phase and mobility in a solvent system, producing characteristic *Rf* values (Singh *et al*., 2020). In *A. vogelii* extracts, TLC fingerprints provide a rapid means of confirming the presence of flavonoids and phenolics before more detailed analysis.

1.3.2 High-Performance Liquid Chromatography (HPLC)

HPLC provides both qualitative and quantitative resolution of non-volatile compounds. It is often coupled with UV–Vis or diode-array detectors for accurate determination of phenolic acids, flavonoids, and iridoid glycosides. Studies on African medicinal plants have successfully profiled chlorogenic acid, quercetin, and kaempferol derivatives using reversed-phase C18 columns and gradient elution systems (Ojo *et al*., 2024). The method's reproducibility and precision make it suitable for standardizing herbal formulations derived from *A. vogelii* root bark.

1.3.3 Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS is the preferred technique for analyzing volatile and semi-volatile constituents such as terpenes, fatty acids, and alcohols. The technique combines chromatographic separation with mass-spectrometric detection, enabling compound identification via spectral libraries (Bassey *et al*., 2023). GC–MS analysis of *A. vogelii* extracts has identified compounds including tridecane, 2,6-dimethyl-undecane, and hexadecanoic acid, confirming the plant's complex chemical profile.

1.3.4 Liquid Chromatography–Mass Spectrometry (LC–MS)

LC–MS provides higher sensitivity and allows detection of thermolabile, non-volatile compounds that cannot be analyzed by GC–MS. This technique is increasingly employed for metabolomic profiling and dereplication in plant research. LC–MS enables accurate mass determination and structural elucidation of flavonoid glycosides and iridoids found in *A. vogelii* (Nguyen *et al*., 2022).

1.3.5 Relevance to Current Study

In the chromatographic analysis of *A. vogelii* root bark, these methods complement each other TLC aids rapid screenings, HPLC ensures quantification, while GC-MS and LC-MS provide structural insights. Employing multiple chromatographic techniques enhance accuracy, reproducibility, and the discovery of novel compounds that may underpin the plant's antioxidant and antibacterial properties.

1.4 ANTIOXIDANT ACTIVITY: MECHANISMS AND ASSAY METHODS

Antioxidants neutralize reactive oxygen species (ROS) and other free radicals, limiting oxidative damage to proteins, lipids and nucleic acids. Two broad molecular mechanisms account for most antioxidant behaviour in plant extracts.

1.4.1 Hydrogen Atom Transfer (HAT): In this mechanism an antioxidant donates a hydrogen atom (a proton plus an electron) to a radical, converting it to a more stable, non-reactive species. HAT reactions are fast and are commonly measured by assays that monitor the disappearance of a radical species over time. Assays that reflect HAT activity provide information about direct radical-quenching potential (Kumar *et al*., 2022).

1.4.2 Single Electron Transfer (SET): Here the antioxidant transfers a single electron to reduce an oxidant, often changing colour in the process. SET reactions are the basis of many colorimetric assays used in phytochemistry because they produce reproducible absorbance changes. Plant phenolics and flavonoids can act by either mechanism depending on conditions such as solvent, pH or the nature of the radical (Lobo *et al*., 2022).

Some antioxidants combine HAT and SET behaviour. The choice of assay therefore affects the apparent potency of an extract. For example, a compound that performs well in an SET assay may score differently in a HAT-based test, so multiple complementary assays improve reliability (Kumar *et al*., 2022; Nguyen *et al*., 2022).

Common antioxidant assays and what they measure;

1.4.3 DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay uses a stable nitrogen radical that changes from purple to yellow when reduced. It is simple, rapid and suitable for crude extracts. Results are usually reported as IC_{50}, the concentration that scavenges 50% of the radical. DPPH primarily reflects H-donation and electron transfer capacity under the assay conditions, and it is widely used as a screening tool for plant extracts (Ikpe *et al*., 2020).

1.4.4 ABTS (2,2'-azino-bis-3-ethybenzothiazoline-6-sulfonic acid) Radical Cation Decolourization Assay

The ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay produces a blue-green radical cation that is reduced by antioxidants. ABTS is compatible with both hydrophilic and lipophilic antioxidants and often provides a broader estimate of activity than DPPH. Values are frequently expressed relative to Trolox equivalents (Lobo *et al*., 2022).

1.4.5 Ferric Reducing Antioxidant Power (FRAP)

FRAP measures the ability of an antioxidant to reduce Fe3+ to Fe2+ in an acidic medium, forming a coloured ferrous-TPTZ complex. It is an SET-based assay and gives a quantitative measure of reducing power, often reported as μmol Fe2+ equivalents per gram. FRAP correlates well with phenolic content in many plant studies but can miss antioxidants that act only by HAT (Nguyen *et al*., 2022).

1.5 ANTIBACTERIAL ACTIVITY: MECHANISMS AND EVALUATION METHODS

Antibacterial activity describes the ability of a substance to inhibit or destroy bacterial cells. This property is particularly relevant in drug discovery, as bacteria continue to evolve resistance to conventional antibiotics. Medicinal plants have been recognized as valuable sources of bioactive metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, which demonstrate notable antibacterial effects (Al-Rimawi *et al*., 2024).

These bioactive compounds act through diverse mechanisms, including disruption of bacterial cell membranes, inhibition of DNA or protein synthesis, interference with cell wall formation, and impairment of essential enzyme systems (Baranova *et al*., 2023). Because plant extracts often contain multiple active constituents, they can produce synergistic

antibacterial effects that reduce the likelihood of microbial resistance compared with single-target synthetic antibiotics (Barba-Ostria *et al*., 2022).

The evaluation of antibacterial activity is usually carried out in vitro through standardized microbiological methods. The most common include agar diffusion techniques (disc or well diffusion) for measuring inhibition zones, and broth dilution assays for determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Gonzalez-Pastor *et al*., 2023). Advanced methods such as time-kill kinetics, micro dilution assays, and biofilm inhibition tests are increasingly applied to achieve a more comprehensive understanding of antibacterial potency (Silva *et al*., 2023).

Combining these methods with chromatographic and phytochemical analyses helps establish correlations between specific compounds and their antibacterial effects. Such integrated evaluation is essential for validating the therapeutic significance of medicinal plants and developing new antibacterial agents from natural sources (Bader *et al*., 2023).

1.5.1 Disc Diffusion and Broth Dilution Methods

The agar diffusion and broth dilution assays are among the most widely used techniques for evaluating the antibacterial activity of plant extracts and natural compounds. These methods provide complementary information on the qualitative and quantitative effectiveness of a test sample against different bacterial strains (Silva *et al*., 2023).

In agar diffusion methods, such as disc diffusion or well diffusion, a known concentration of the extract is applied to agar plates previously inoculated with the target microorganism. After incubation, the diameter of the inhibition zone around each sample indicates the degree of antibacterial activity (Gonzalez-Pastor *et al*., 2023). While simple and reproducible, this method mainly provides comparative rather than absolute potency data, as factors like compound solubility and diffusion rate can influence results (Barba-Ostria *et al*., 2022).

The broth dilution method, which includes both macro and micro dilution variants, offers a more quantitative measure by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. These are defined respectively as the lowest concentrations capable of inhibiting visible bacterial growth and killing the microorganism (Al-Rimawi *et al*., 2024). Broth dilution assays are often preferred for standardizing plant extract activity since they allow for statistical analysis and comparison across bacterial species (Baranova *et al*., 2023).

Recent methodological improvements have incorporated microplate readers and resazurin-based colorimetric indicators, which enhance accuracy and reduce experimental variability (Bader *et al*., 2023). Combining both agar diffusion and broth dilution tests ensures comprehensive evaluation of antimicrobial potential and supports the validation of bioactive plant-derived compounds for therapeutic use.

1.6 BIOLOGICAL ACTIVITIES (ANTIOXIDANT AND ANTIBACTERIAL)

Medicinal plants possess diverse biological activities arising from their rich content of bioactive compounds such as flavonoids, alkaloids, phenolic acids, tannins, and terpenoids. Among the most studied properties are their antioxidant and antibacterial potentials, which play key roles in disease prevention and therapy. The antioxidant capacity of these plants is mainly attributed to phenolic and flavonoid compounds that scavenge reactive oxygen species, chelate pro-oxidant metals, and stabilize free radicals, thereby protecting cellular macromolecules from oxidative stress (Muscolo *et al*., 2024). Such activity is important in mitigating chronic and degenerative diseases linked to oxidative damage and in maintaining general metabolic balance (Aliyu-Amoo and Isa, 2023).

In addition, plant-derived compounds exhibit significant antibacterial effects. Their mechanisms of action include disrupting microbial cell walls and membranes, interfering

with DNA synthesis, inhibiting vital metabolic enzymes, and preventing protein formation (Baranova *et al*., 2023). This broad spectrum of antibacterial mechanisms, often enhanced by the synergistic action of multiple constituents within plant extracts, makes them promising alternatives to synthetic antibiotics, especially in the face of rising resistance (Bader *et al*., 2023).

The interplay between antioxidant and antibacterial activities often contributes to the overall therapeutic potential of medicinal plants. Antioxidants can indirectly support antibacterial efficacy by maintaining host cell integrity and reducing oxidative stress that may otherwise impair immune responses (Silva *et al*., 2023). As a result, the study of both activities not only provides a scientific foundation for validating traditional remedies but also highlights their potential for pharmaceutical development. This dual biological effect underlines the importance of plants such as *Anthocleista vogelii,* which are valued for their medicinal use in managing infections and oxidative stress-related disorders (Barba-Ostria *et al*., 2022).

1.7 JUSTIFICATION OF STUDY

While some studies have explored the phytochemistry of *A. vogelii*, few have focused specifically on its root bark using chromatographic methods. This research addresses that gap, providing a comprehensive analysis of its antioxidant and antibacterial potential.

It bridges the gap between traditional ethnopharmacological claims and modern scientific validation. The findings are expected to contribute to the growing body of evidence supporting the pharmacognostic importance of *Anthocleista vogelii*, establish its antioxidant and antibacterial potential, and provide a foundation for the isolation and development of novel therapeutic agents from its root extracts.

Because *A. vogelii* root bark is expected to contain a range of secondary metabolites from polar phenolics to moderately non-polar compounds 70 % ethanol strikes a middle ground,

strong enough to solubilize non-polar constituents and still capable of pulling in hydrophilic ones. This ensures the extract is broadly representative of the root's bioactive profile and maximizes the chances of detecting antioxidant and antibacterial activity effectively.

1.8 AIM AND OBJECTIVES OF THE STUDY

The aim of this study is to evaluate the chromatographic profile, antioxidant, and antibacterial potential of the root bark of *Anthocleista vogelii* Planch (Gentianaceae) in order to scientifically validate its traditional medicinal use and identify possible bioactive compounds with therapeutic potential.

The specific objectives are to;

Collect and identify the leaves and root bark of *Anthocleista vogelii* Planch (Gentianaceae) from its natural habitat and authenticate it by a qualified taxonomist in a recognized herbarium to ensure botanical accuracy.

Prepare the plant material (root bark) through appropriate cleaning, drying, and pulverization procedures to obtain uniform particle size suitable for extraction.

Carry out extraction of the bioactive constituents from the powdered root bark using suitable solvent (70% ethanol) through standard extraction method (maceration).

Conduct chromatographic analysis (using High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry) of the hydro-ethanolic extract of *A. vogelii* root bark to identify and characterize the phytochemical constituents.

Evaluate the antioxidant activity of the root bark extract using standard assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), Ferric Reducing Antioxidant Power (FRAP) methods, ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assay and Total Antioxidant Capacity (TAC).

Assess the antibacterial activity of the root bark extract against selected bacterial strains through disc diffusion and broth dilution methods.

Correlate the chromatographic and bioassay results to establish relationships between phytochemical constituents and observed biological activities.

Provide a scientific basis for the traditional use of *A. vogelii* root bark and identify potential lead compounds for further pharmacological development.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The leaves of the plant of were collected from an uncultivated land in Akoko Edo, Edo state for identification and authentication in July, 2025. It was identified and authenticated by Prof. H.A. Akinnibosun, a taxonomist in the Department of Plant Biology and Biotechnology, as *Anthocleista vogelii*, with voucher number University of Benin and Herbarium specimen (UBH-A258) and was deposited in the Department's herbarium.

2.2 EXTRACTION OF PLANT MATERIAL

The roots of the plant which is the part of interest were initially washed thoroughly under running tap water to remove any impurities after collection. They were then cut into small pieces and left to air dry for 14 days to prevent loss of thermolabile constituents that may be present in the plant. Once dried, the roots were pulverized into a fine powder using a mechanical grinder and stored in an airtight container to maintain their quality and potency.

The powdered root weighing 400g was placed in a jar and was extracted by maceration using 70% ethanol (2x2.5L) for 72 hours. The jar was shaken continuously for at least two (2) hours on the first day of extraction. After 72 hours the extract was decanted and filtered using filter paper of size 1. The filtrate was then evaporated using a rotary evaporator at 60℃ to reduce the filtrate after which it was placed in a beaker and concentrated to dryness using a water bath at 60℃.

2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

2.3.1 Sample Preparation for High-Performance Liquid Chromatography

A portion of the hydro-ethanolic fraction of *Anthocleista vogelii* weighing 0.2 g was placed in a test tube, followed by the addition of 15 mL of ethanol and 10 mL of 50% (w/v) potassium

hydroxide. The mixture was heated in a water bath at 60 °C for 3 hours to allow the reaction to proceed. After heating, the content of the test tube was transferred into a separatory funnel. The tube was rinsed sequentially with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water, and 3 mL of hexane, and each rinse was added to the funnel. The combined extracts were washed three times with 10 mL of 10% (v/v) aqueous ethanol. The solvent was then evaporated, and the residue was dissolved in 1000 µL of pyridine. A 200 µL portion of this solution was placed into an autosampler vial for chromatographic analysis (Odion *et al*., 2024)..

2.3.2 High-Performance Liquid Chromatography Procedure.

The High performance liquid chromatography analysis of the ethanol extract of *Anthocleista vogelii* Planch was performed using Shimadzu LC- 10AD dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. The separation was achieved on a C-12 normal phase column (Phenomenex, Gemini 5 µ, 200 mm length × 4.8 mm internal diameters) for the analysis. The Mobile phase consisted of solvent A and B, Solvent A (deionized water acidified acetic acid to pH 2.8), and solvent B (acetonitrile) with a flow rate of 0.8 mL/min. Following each injection, the column was equilibrated with 5% solvent B for 20 minutes. The column temperature was maintained at 38 °C, the injection volume was 20 µL, and detection was carried out at 280 nm. Standard compounds were identified and quantified by comparing their retention times and peak areas with those of pure reference standards, using calibration curves prepared from external standards (Odion *et al*., 2024).

2.4 GAS CHROMATOGRAPHY-MAS SPECTROMETRY (GC-MS)

2.4.1 Preparation of Plant Extract for GC-MS Analysis

To prepare the extract of *A. vogelii* for GC-MS evaluation, 50 mg of the extract was weighed into a 100 ml beaker. Ten millilitres of a 1:1 mixture of hexane and dichloromethane were added, and the solution was thoroughly homogenized. The resulting mixture was purified by passing it through a column packed with 100–200 mm mesh silica gel and 3 g of anhydrous sodium sulfate, which had been conditioned with hexane to form a slurry. A 1 µL portion of the cleaned extract was then introduced into the injection port of a Gas Chromatograph (Agilent 6890N) equipped with an auto sampler and linked to an Agilent Mass Spectrometric detector (Odion *et al*., 2024).

2.4.2 GC-MS Analysis

A 1 µL portion of the purified crude extract was introduced in pulsed split-less mode onto a DB-5MS fused silica capillary column (30 m × 0.25 mm × 0.15 µm film thickness). Helium served as the carrier gas, with the head pressure regulated at 20 psi to maintain a constant flow rate of 1 mL/min. The column oven was programmed to start at 55 °C, held for 0.4 min, then ramped to 200 °C at 25 °C per minute, followed by an increase to 280 °C at 8 °C per minute, and finally raised to 300 °C at 25 °C per minute, where it was held for 2 minutes. Compound identification was achieved by comparing retention times (Odion *et al*., 2024).

2.5 DETERMINATION OF *IN VITRO* ANTIOXIDANT ACTIVITY

The antioxidant activity of the ethanolic fraction of *Anthocleista vogelii* was evaluated in vitro using four assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Total Antioxidant Capacity (TAC).

2.5.1 Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the extract against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). Briefly, 0.5ml of 0.3mM DPPH solution in methanol was added to 2ml of various concentrations (0.2-1.0mg/mL) of the extracts. The reaction tubes were shaken and incubated for 15min at room temperature in the dark; absorbance read at 517nm. All tests were performed in triplicate. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5mL of 0.3mM DPPH and 2mL methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_1) / (A_0)] \times 100$,

Where; A_0 was the absorbance of DPPH radical + Methanol.

A_1 was the absorbance of DPPH radical + sample extract or standard.

2.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). To 1.5ml of freshly prepared FRAP solution (25 ml of 300 mM) acetate buffer pH 3.6, 2.5ml of 10mM 2,4,6 – tripyridyl-triazine (TPTZ) in 40mM HCl, and 2.5ml of 20 mM Ferric chloride ($FeCl_3.6H_2O$) solution was added to 1ml of the extracts at concentrations of 100-600uM. The reaction mixtures were incubated at 37^0c for 30mins and the increase in absorbance at 593nm was measured. $FeSO_4$ was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mM.Fe(ų/g) of the extract) for the extracts were then extrapolated from the standard curve.

2.5.4 Determination of the Scavenging Activity of $ABTS^{\cdot+}$ Free Radical

The ABTS reagent was prepared according to the method of Pellegrini *et al*. (1999). To determine the scavenging activity, 0.9 ml of ABTS reagent was mixed with 0.1 ml of the sample and the absorbance was measured at734 nm after 6 min of incubation at room temperature. The $ABTS^{+}$ scavenging effect (%) was calculated as:

$$\text{Scavenging activity for } ABTS^{\cdot+} (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\ \%$$

2.5.4 Determination of Total Antioxidant Capacity

Total antioxidant activity was estimated by phosphomolybdenum assay (Prieto *et al*., 1999). The method is based on the reduction of molybdenum (1v) to molybdenum (v) by the extract and the subsequent formation of a green phosphate/molybdenum (v) complex at acid pH. Three millilitres (3mL) of the extracts (1mg/ml) was added to 1ml molybdate reagent solution. These tubes were incubated at 95℃ for 90min. After incubation, the tubes were normalized to room temperature for 20-30minutes and the absorbance of the reaction mixture was measured at 695nm. Ascorbic acid was used as the standard.

2.6 ANTIBACTERIAL ACTIVITY OF *Anthocleista vogelii* Planch

2.6.1 Specimen Collection

The microorganisms employed in this study were bacterial isolates obtained from the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. The bacterial isolates used included: *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus,* and *Enterobacter cloacae*.

2.6.2 Preparation of Test Bacteria

All test microorganisms were preserved in 20% glycerol Tryptic Soy Broth and stored frozen until required. Prior to use, they were sub-cultured from stock onto sterile nutrient agar plates

and incubated at 37 °C overnight. Following incubation, identical colonies from the plates were transferred into sterile broth and grown for 12 hours. The cultures were then adjusted to a 0.5 McFarland standard, corresponding to an inoculum density of approximately 10^5cfu/mL. This suspension was further diluted 1:100 to obtain a final inoculum concentration of about 10^6cfu/mL.

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

An antimicrobial susceptibility assay was carried out to evaluate the antibacterial activity of the hydro-ethanolic extract of *Anthocleista vogelii* using the agar well diffusion method (Perez *et al.,* 1990). A stock solution of the extract was prepared at a concentration of 200 mg/mL. Mueller Hinton agar was prepared according to the manufacturer's instructions, sterilized at 121 °C for 15 minutes in an autoclave, and allowed to cool. Portions of 30 mL were dispensed into six sterile Petri dishes and left to solidify. Once set, the agar plates were dried in a hot-air oven at 50 °C for 5 minutes to remove excess surface moisture, and each plate was properly labelled.

A standardized suspension of each bacterial isolate was prepared in 1 mL of sterile water, and the isolates were streaked onto the respective agar plates using a sterile inoculating loop. Wells of 8 mm diameter were bored into the agar with a sterile cork borer, and the bases were sealed with molten Mueller Hinton agar. Into each well, 0.2 mL of the plant extract was dispensed using a calibrated micropipette. The plates were allowed to stand for 30 minutes before incubation at 37 °C for 24 hours. Following incubation, the diameters of the inhibition zones were measured with a meter rule, and the results were recorded.

2.6.4 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent (such as an antibiotic, antifungal, or bacteriostatic drug) that prevents visible microbial growth after overnight incubation. The MIC of the hydr-ethanolic extract of *Anthocleista vogelii* was determined using the agar dilution method described by Afolayan and Meyer (1997).

Five sterile Petri dishes were prepared and labelled with the extract concentrations: 200 mg, 100 mg, 50 mg, 25 mg, and 12.5 mg. Agar medium was prepared according to the manufacturer's instructions and maintained in a water bath at 50 °C. The stock solution of the extract was filtered and incorporated into molten agar at appropriate volumes to achieve the desired concentrations (12.5–200 mg). For example, 1 mL of the 12.5 mg extract solution was added to 9 mL of molten Mueller Hinton agar, mixed gently, and poured into the Petri dish labelled 12.5 mg. The same procedure was followed for the remaining concentrations. Plates were then dried in a hot-air oven at 50 °C for 5 minutes.

Each plate was divided into four sections with a marker, and each section was labeled to represent three test microorganisms and one control. Using a sterile inoculating loop, the corresponding isolates were streaked onto the designated sections. Plates were incubated at 37 °C for 24 hours, after which growth was assessed and recorded.

2.6.5. Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) is defined as the lowest concentration of an antibacterial agent required to kill a specific bacterium. To determine the MBC of the hydro-ethanolic extract, plates showing no visible growth during the MIC assay were swabbed and streaked onto fresh Mueller Hinton agar plates containing extract concentrations of 200 mg,

100 mg, and 50 mg. The plates were then incubated at 37 °C for 24 hours, after which the MBC was determined.

2.6.6 Determination of the Mode of Action

The bacteriostatic or bactericidal nature of the hydro-ethanolic aerial fraction was determined by calculating the MBC/MIC from the MIC and MBC values obtained The MBC/MIC ratio was calculated by dividing the MBC (mg/ml) by the MIC (mg/ml) for bacteria whose MBC and MIC values were obtained. Values of MBC/MIC ≤ 4 were interpreted as bactericidal and MBC/MIC ≥ 4 were interpreted as bacteriostatic (Ishak *et al*., 2025; Tankeshwar, 2023.

2.7 STATISTICAL ANALYSIS METHOD

For the antimicrobial activity, a statistical comparison using an independent samples t-test (assuming equal variances) was conducted in Microsoft excel to determine whether the difference in the zones of inhibition between 200mg/ml hydro-ethanolic fraction of *Anthocleista vogelii* root extract and 0.5 mg/ml Ciprofloxacin was statistically significant.

.

CHAPTER THREE

3.0 RESULTS

3.1 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Phytochemical profiling of the root bark extract was performed using chromatographic separation, with retention times, peak areas and concentrations recorded for each detected compound. Figure 2 shows the different peaks for the various phytochemicals seen.

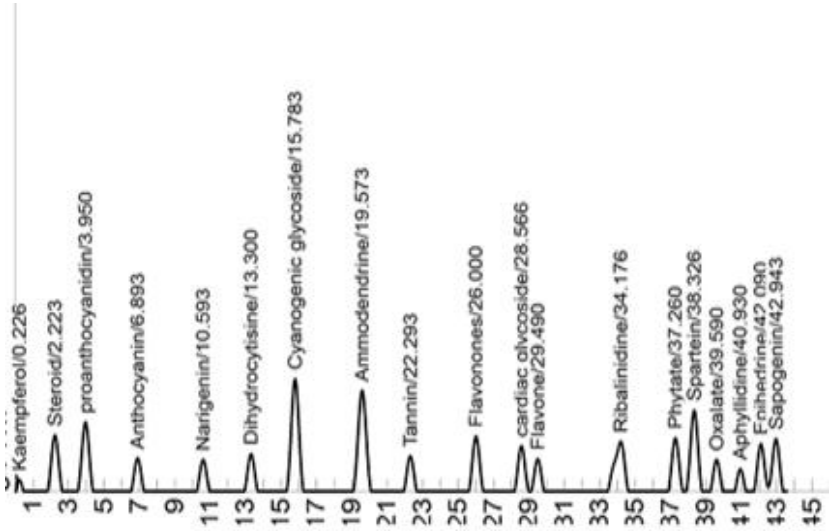


Figure 2 HPLC chromatogram of *Anthocleista vogelii* extract

Table 2 shows the result of HPLC profiling of *Anthocleista vogelii* extract. A total of 19 bioactive constituents were identified, representing various phytochemical classes such as alkaloids, flavonoids, glycosides, steroids, tannins/phenolics and chelating compounds. The retention times ranged from 0.226 min (Kaempferol) to 42.943 min (Sapogenin), with concentrations spanning from 0.0000µg/mL (Rablinidine) to 22.0694µg/mL (Ammodendrine). Among the compounds, Ammodendrine (22.0694 µg/mL), Cyanogenic glycoside (20.1936 µg/mL), and Sparteine (15.9760 µg/mL) were detected in relatively high concentrations, whereas Rablinidine (0.0000µg/mL), Anthocyanin (2.0696 µg/mL), and Oxalate (1.3133 µg/mL) were present in trace amounts. The chemical diversity and relative

abundance of these metabolites indicate that the extract harbors multiple bioactive agents, which may act synergistically to produce its biological effects.

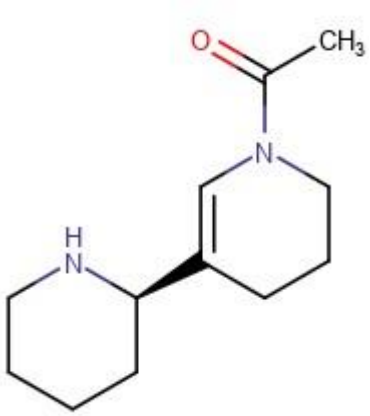
Table 2 HPLC analysis of *Anthocleista vogelii* extract

S/N	Compound	Retention Time	Area	Concentration (µg/mL)
1	Kaempferol	0.226	1569.6014	3.3343
2	Steroids	2.223	6781.4592	8.2903
3	Proanthocyanidine	2.950	8155.3112	10.4914
4	Anthocyanin	6.893	4483.8064	2.0696
5	Narigenin	10.593	4333.2764	7.4327
6	Dihydrocystine	13.300	4913.7781	5.9167
7	Cyanogenic glycoside	15.783	12807.2396	20.1936
8	Ammodendrine	19.573	12593.4877	22.0694
9	Tannins	22.293	4751.2560	12.9619
10	Flavonones	26.000	6798.6578	8.7424
11	Cardiac glycosides	28.566	5791.1226	4.0095
12	Flavones	29.490	4450.4840	5.7253
13	Rablinidine	34.176	9156.1380	0.0000
14	Phytate	37.260	6555.3447	8.8109
15	Spartein	38.326	9314.0338	15.9760
16	Oxalate	39.590	4172.2344	1.3133
17	Aphyllidine	40.930	3158.2206	4.2430
18	Ephedrine	42.090	5701.2744	3.9473
19	Sapogenin	42.943	6410.6442	10.9960

The chemical structures of some of the phytochemicals identified by HPLC profiling are shown below;

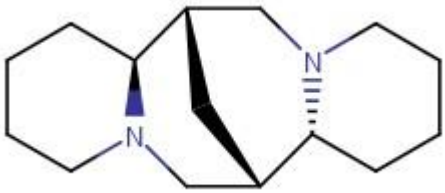
1. Alkaloids

a. Ammondendrine



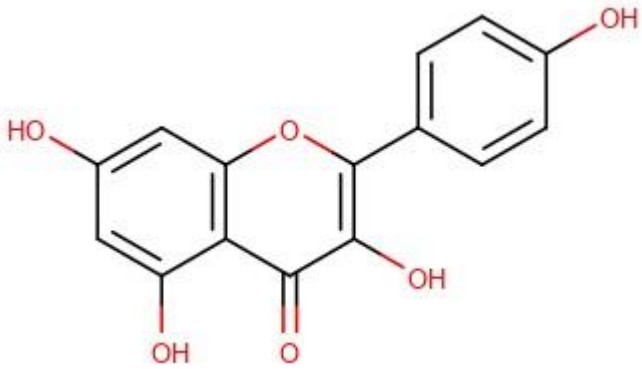
b. Sparteine

((1S,2R,9S,10S)-7,15-diazatetracyclo[7.7.1.02,7.010,15]heptadecane)

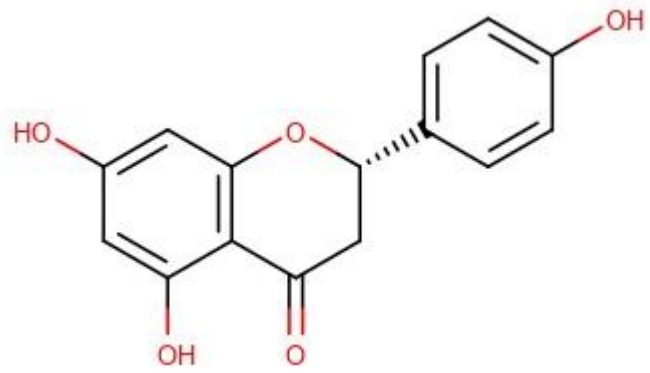


2. Flavonoids

a. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one)

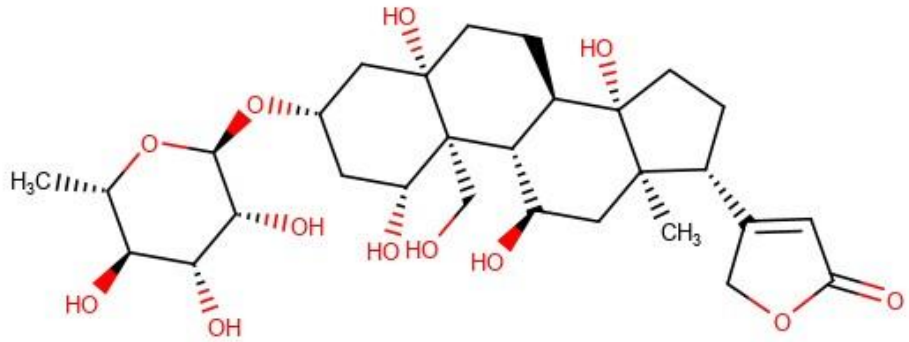


b. Naringenin ((2S)-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydrochromen-4-one)



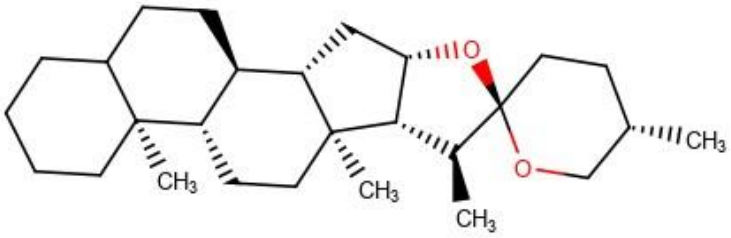
3. Glycosides

a. Cardiac glycosides



4. Saponins/Related compounds

a. Sapogenin



3.2 GAS CHROMATOGRAPHY-MAS SPECTROSCOPY

Gas Chromatography–Mass Spectrometry (GC–MS) profiling of the crude extract revealed thirty-five distinct compounds within a retention time range of 3.45–18.67 min. Their identification was achieved by matching the obtained chromatographic spectra with those in the NIST 14 reference library. The metabolite spectrum encompassed fatty acids and their esters, hydrocarbons, heterocyclic derivatives, carbohydrate-related molecules, and ether-linked lipids. The chromatogram is seen in Figure 3.

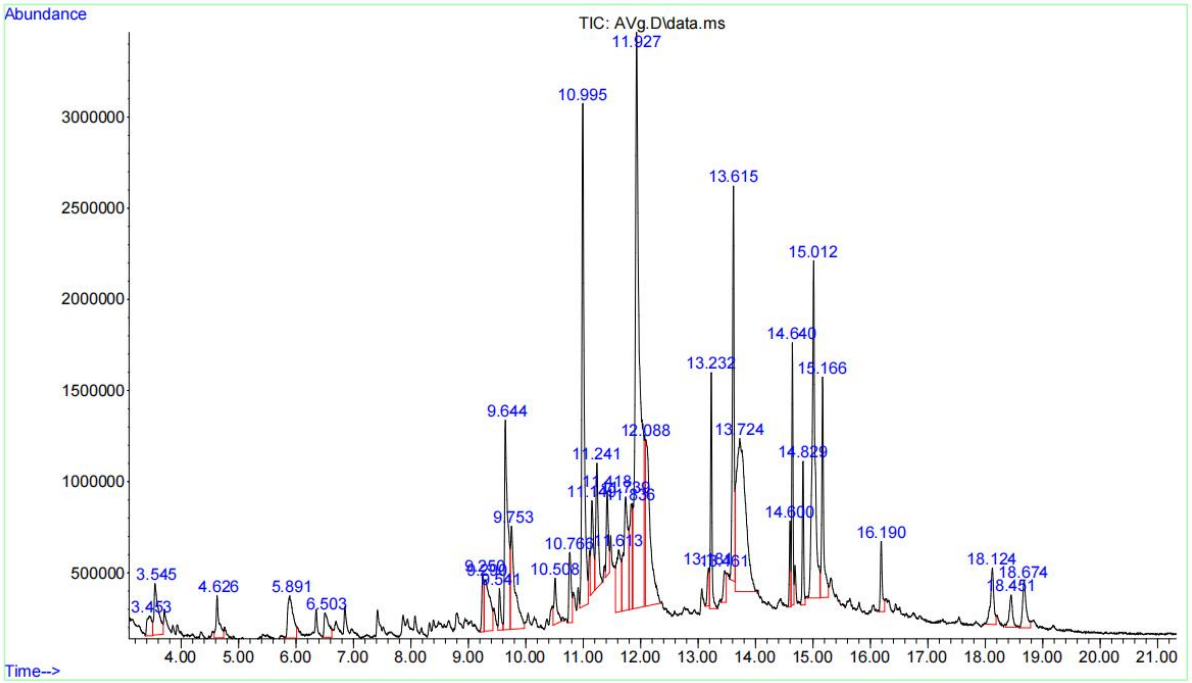


Figure 3 GCMS chromatogram of *Anthocleista vogelii* extract.

Table 3 illustrates the result of the GC-MS profiling of *Anthocleista vogelii* extract, the most abundant compound detected was 2,8-decadiyne (17.99 %), followed by pentyl octanoate (9.72%) and 1,4-cyclohexadiene-1,2-dicarboxylic anhydride (8.23%). Other major contributors included elaidic acid (7.13%), 2-methyl-tetrahydrothiophene (5.36%), and palmitic acid (4.81%), with several additional fatty acids and esters (stearic, oleic, linoleic, lauric) also represented.

In addition to lipid derivatives, the chromatogram showed the presence of aromatic acids (benzoic acid, methyl benzoate), heterocycles (benzothiopyranone, nitroimidazoles, thiophene derivatives), and sugar-related compounds such as levoglucosan. Hydrocarbons of both cyclic and polyunsaturated types were also detected, including cyclododecatriene and tricyclo[4.1.1.0(7,8)]oct-3-ene.

The GC–MS data indicate a chemically diverse mixture dominated by lipid-based constituents, supported by heteroaromatic and carbohydrate derivatives that may contribute to the extract's possible roles in membrane disruption, antimicrobial action and bioactive or pharmacologically relevant properties.

Table 3 GC-MS analysis of *Anthocleista vogelii* extract

S/N	**Rt (Min)**	**Compound Name**	**Area (%)**	**Molecular Formula**	**Molecular Weight (g/mL)**
1.	3.453	3,5-Dithiahexanol-5,5-dioxide	0.68	$C_4H_{10}O_3S_2$	170.250
2.	3.545	Cyclohexanol, 4-methyl-	1.59	$C_7H_{14}O$	114.19
3.	4.626	Benzoic acid, methyl ester	0.89	$C_8H_8O_2$	136.15
4.	5.891	Benzoic acid	1.60	$C_7H_6O_2$	122.12
5.	6.503	Bicyclo[3.3.1]non-2-en-	0.78	$C_9H_{14}O$	138.20

		9-ol, syn-			
6.	9.250	1H-2-Benzothiopyran-4(3H)-one	0.83	C_9H_6OS	150.20
7.	9.290	Octadecanoic acid	1.99	$C_{18}H_{36}O_2$	284.48
8.	9.541	Dodecanoic acid, methyl ester	0.59	$C_{13}H_{26}O_2$	214.34
9.	9.644	Tricyclo[4.1.1.0(7,8)]oct-3-ene	4.88	C_8H_{10}	106.1650
10.	9.753	.beta.-D-Glucopyranose, 1,6-anhydro-	3.10	$C_6H_{10}O_5$	162.1406
11.	10.508	1H-Imidazole, 2-methyl-4-nitro-	0.89	$C_4H_5N_3O_2$	127.10
12.	10.766	Cyclopropanecarboxylic acid, 3-ethenyl-2,2-dimethyl-	1.18	$C_8H_{12}O_2$	140.18
13.	10.995	1,4-Cyclohexadiene-1,2-	8.23	$C_8H_6O_3$	150.1314

		dicarboxylic anhydride			
14.	11.149	1-Cyclopenteneacetic acid, 5-oxo-	1.66	$C_7H_8O_3$	140–156
15.	11.241	3,6-Dimethyl-3,6-dihydro-pyran-2-one oxime	2.19	$C_7H_{11}NO_2$	141.17
16.	11.418	1,5,9-Cyclododecatriene, (Z,Z,Z)-	1.23	$C_{12}H_{18}$	162.27
17.	11.613	Trimethylsilyl 23-acetoxy-3,6,9,12,15,18,21-heptaoxatricosan-1-oate	2.02	$C_{29}H_{58}O_9Si$	574.87
18.	11.739	Methyl(methyl 4-O-methyl-.alpha.-d-mannopyranoside) uronate	3.54	$C_9H_{16}O_7$	236.09
19.	11.836	Dimethyl-	1.89	C_3H_6NP	87.06

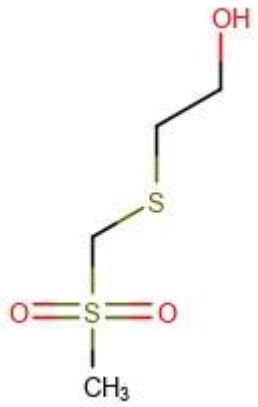
		cyano-phosphine			
20.	11.927	2,8-Decadiyne	17.99	$C_{10}H_{14}$	134.2182
21.	12.088	Thiophene, tetrahydro-2-methyl	5.36	$C_5H_{10}S$	102.20
22.	13.181	1H-Imidazole, 4-methyl-5-nitro	0.56	$C_4H_5N_3O_2$	127.10
23.	13..232	Hexadecanoic acid, methyl ester	2.46	$C_{17}H_{34}O_2$	270.45
24.	13.461	Hexadecyl nonyl ether	0.60	$C_{25}H_{52}O$	368.68
25.	13.615	n-Hexadecanoic acid	4.81	$C_{16}H_{32}O_2$	256.42
26.	13.724	Pentyl octanoate	9.72	$C_{13}H_{26}O_2$	214.34
27.	14.600	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	0.79	$C_{19}H_{34}O_2$	294.47
28.	14.640	9-Octadecenoic acid (Z)-,	2.30	$C_{19}H_{36}O_2$	296.49

		methyl ester			
29.	14.829	Methyl stearate	1.39	$C_{19}H_{38}O_2$	298.50
30.	15.012	9-Octadecenoic acid, (E)-	7.13	$C_{18}H_{34}O_2$	282.46
31.	15.166	Octadecanoic acid	2.92	$C_{18}H_{36}O_2$	284.48
32.	16.190	Glycidyl palmitate	0.95	$C_{19}H_{36}O_3$	312.50
33.	18.124	9-Oxabicyclo[6.1.0]nonane, cis-	1.31	$C_8H_{14}O$	126.20
34.	18.451	Glycidol stearate	0.74	$C_{21}H_{40}O_3$	340.55
35.	18.674	Octadecanoic acid, 2,3-dihydroxypropyl ester	1.20	$C_{21}H_{42}O_4$	358.56

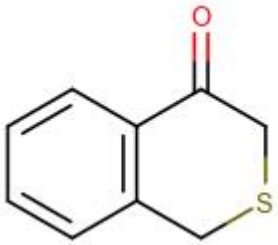
The chemical structures of the phytochemicals identified by GC-MS profiling are shown below;

1. Sulfur Heterocycles / Organosulfur Compounds

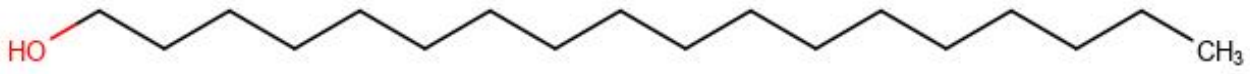
   a. 3,5-Dithiahexanol-5,5-dioxide



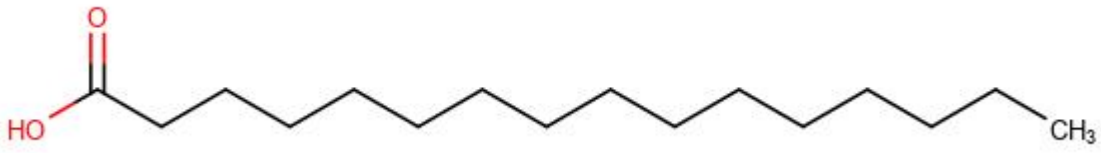
   b. 1H-2-Benzothiopyran-4(3H)-one



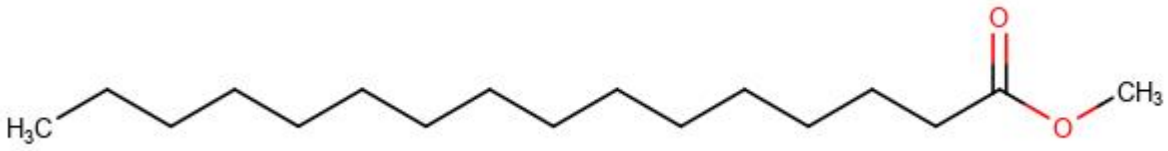
2. Fatty Acids (Free)
   a. Octadecanoic acid



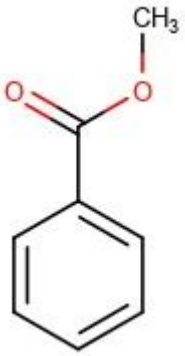
   b. n-Hexadecanoic acid



3. Fatty Acid Esters / Methyl Esters
   a. Hexadecanoic acid, methyl ester (Methyl palmitate)



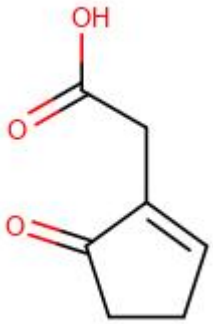
b. Benzoic acid, methyl ester



4. Terpenoids / Terpene-like Hydrocarbons & Alcohols

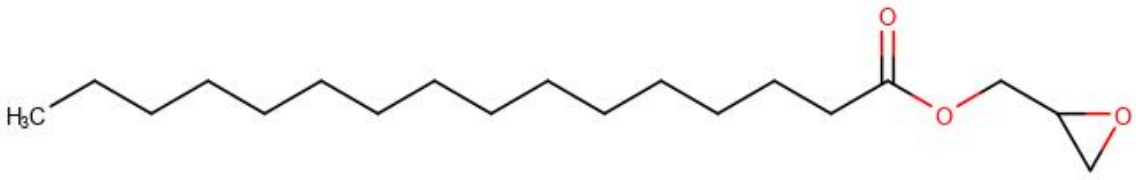
a. Cyclohexanol, 4-methyl-

b. 1-Cyclopenteneacetic acid, 5-oxo-

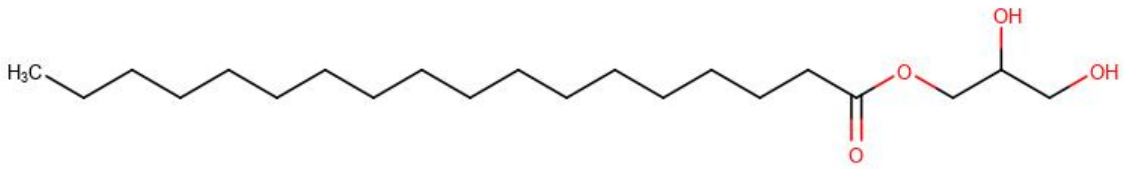


5. Glycerides / Glycidyl Esters (Epoxy Esters)

a. Glycidyl palmitate

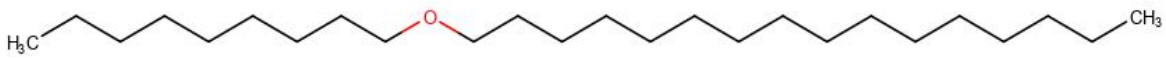


b. Octadecanoic acid, 2,3-dihydroxypropyl ester



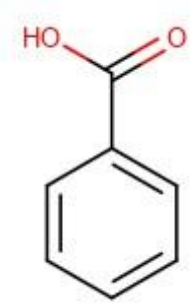
6. Fatty Ethers / Long-Chain Ethers

a. Hexadecyl nonyl ether



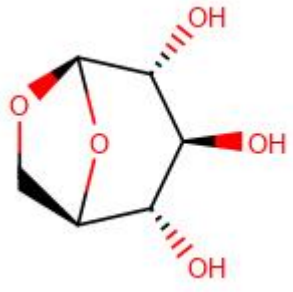
7. Aromatic Acids & Derivatives

a. Benzoic acid

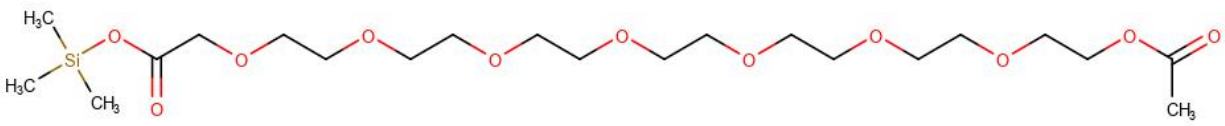


8. Carbohydrates / Glycosides / Sugar Derivatives

a. β-D-Glucopyranose, 1,6-anhydro- (Levoglucosan)

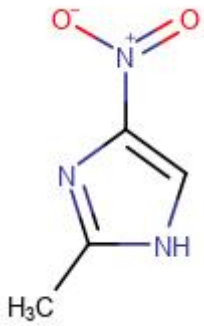


b. Trimethylsilyl 23-acetoxy-3,6,9,12,15,18,21-heptaoxatricosan-1-oate



9. Nitrogen Heterocycles (Imidazole Derivatives / Alkaloid-like)

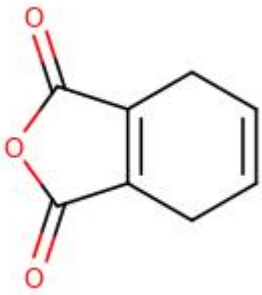
a. 1H-Imidazole, 2-methyl-4-nitro-



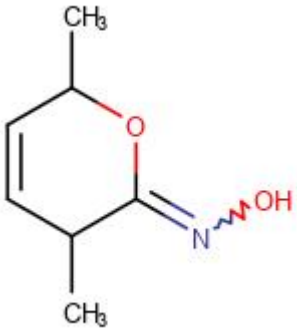
b. 1H-Imidazole, 4-methyl-5-nitro-

10. Lactones / Anhydrides / Pyranones (Oxygen Heterocycles)

a. 1,4-Cyclohexadiene-1,2-dicarboxylic anhydride

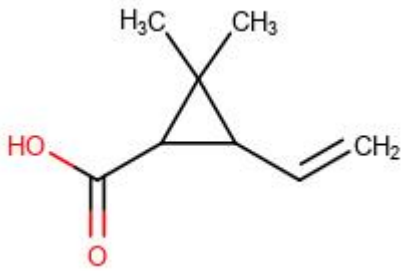


b. 3,6-Dimethyl-3,6-dihydro-pyran-2-one oxime

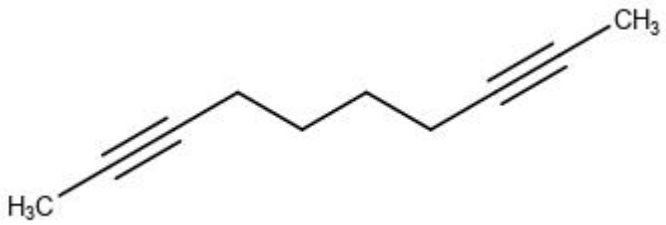


11. Aliphatic & Cycloalkane Derivatives / Small Hydrocarbons

a. Cyclopropanecarboxylic acid, 3-ethenyl-2,2-dimethyl-

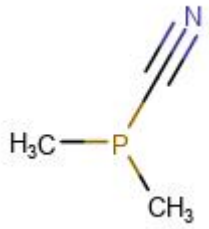


b. 2,8-Decadiyne



12. Organophosphorus Compounds

a. Dimethyl-cyano-phosphine



3.3 IN VITRO ANTIOXIDANT ACTIVITY

3.3.1 DPPH Radical Scavenging Activity

The antioxidant capacity of the *A. vogelii* root extract was assessed using the DPPH assay at varying concentrations ranging from 200 to 1000 µg/mL. Figure 4 represents the result of the DPPH radical scavenging activity. The results showed a gradual increase in absorbance with rising concentration, while the percentage inhibition values decreased correspondingly from 91.92% at 200 µg/mL to 81.28% at 1000 µg/mL.

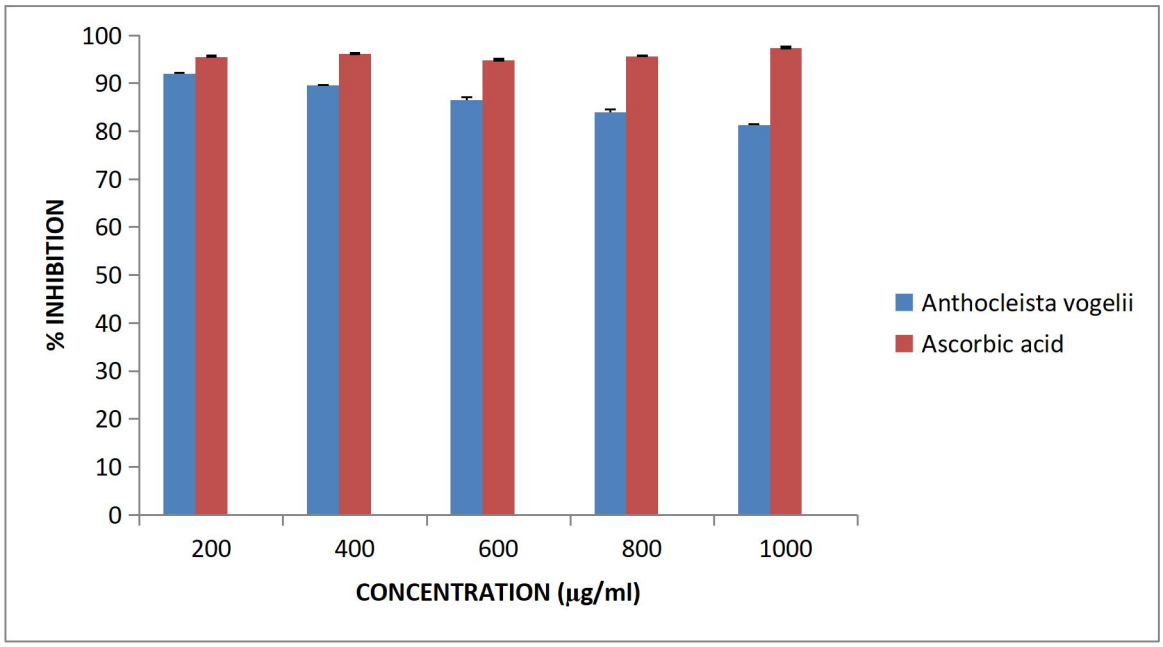


Figure 4 Comparative DPPH % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

3.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing activity of the *A. vogelii* root extract was evaluated using the FRAP method across concentrations ranging from 200 to 1000 µg/mL. The mean absorbance values showed a general upward trend with increasing concentration, while the corresponding percentage inhibition values decreased from 40.44% to 28.23%. It is represented by figure 5.

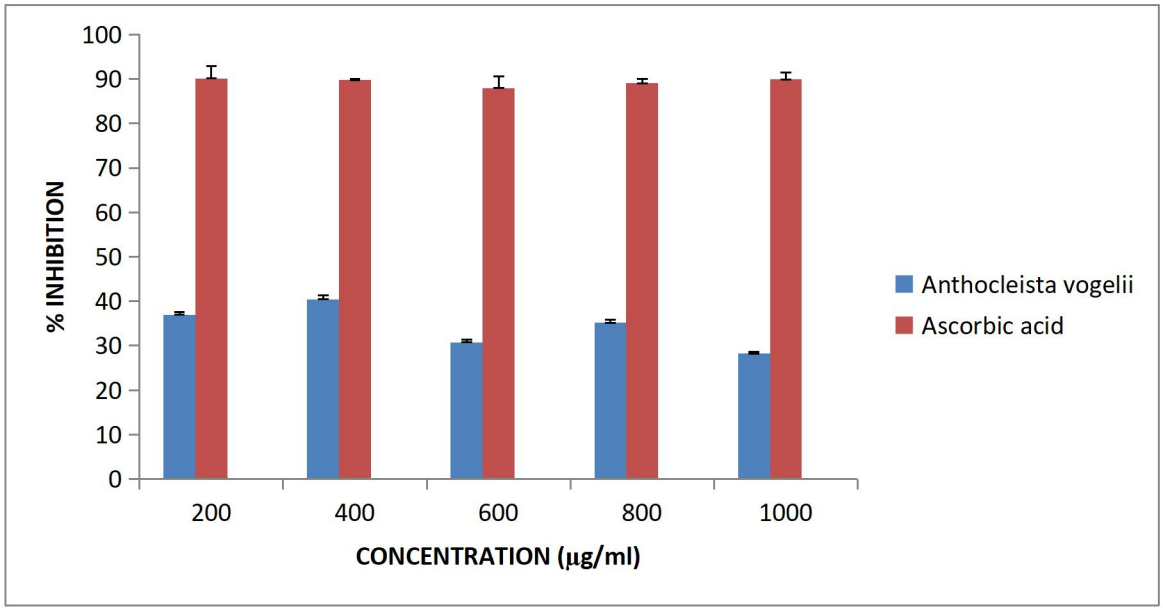


Figure 5 Comparative FRAP % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

3.3.3 ABTS Radical Scavenging Activity

The ABTS assay was conducted to further assess the antioxidant efficiency of the *A. vogelii* root extract across concentrations ranging from 200 to 1000 µg/mL. The extract exhibited high radical scavenging activity, with percentage inhibition values decreasing gradually from 93.24% at 200 mg/mL to 71.02% at 1000 µg/mL. Correspondingly, absorbance readings increased with concentration. These results indicate that the extract is highly effective in neutralizing $ABTS^{+}$ radicals, particularly at lower concentrations.

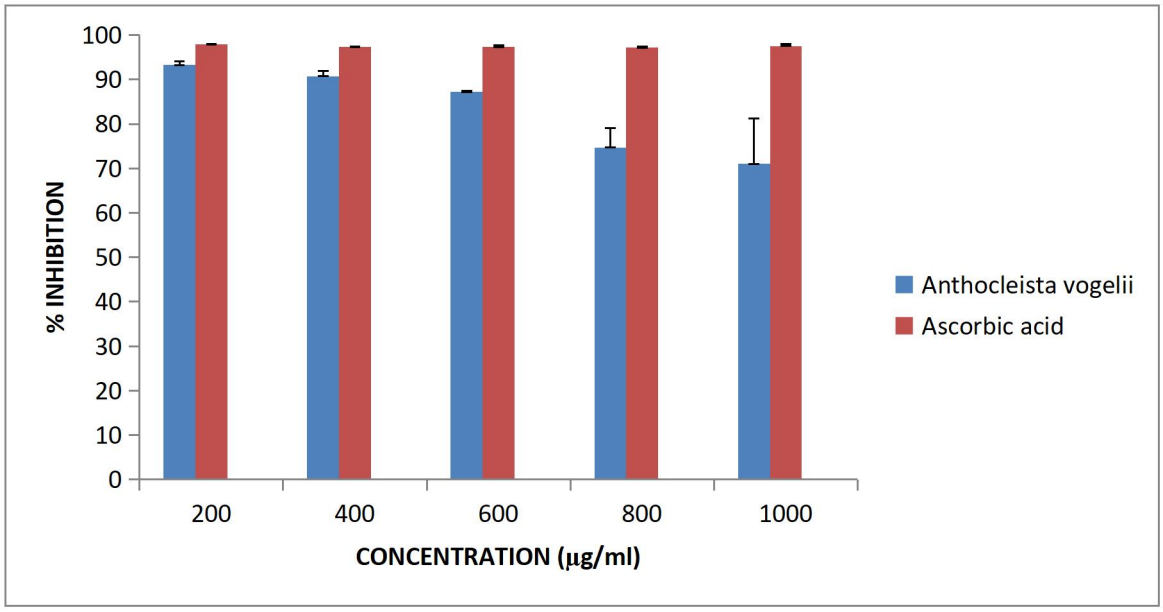


Figure 6 Comparative ABTS % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

3.3.4 Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity of the *A. vogelii* root extract was determined at concentrations ranging from 200 to 1000 µg/mL. Figure 7 represents the result of the TAC assay. The mean absorbance values increased progressively with concentration, while the percentage inhibition showed a steady increase from 76.17% at 200 µg/mL to 89.15% at 1000 µg/mL. This trend reflects a strong overall antioxidant capacity.

.

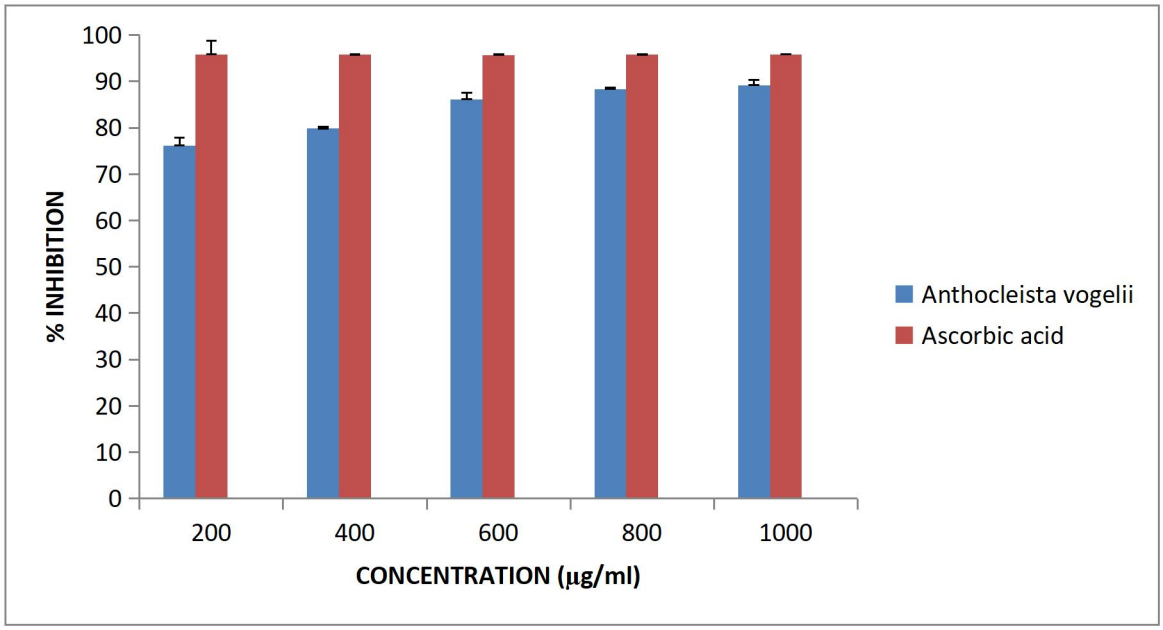


Figure 7 Comparative TAC % Inhibition at 200–1000 µg/mL of Plant Extract and Ascorbic acid

3.4 ANTI-MICROBIAL ASSAY

3.4.1 Determination of Zone of Inhibition

Table 4 shows the result of the susceptibility assay of *Anthocleistaq vogelii* against selected clinical isolates (measured in mm). The root bark extract of *A.vogelii* exhibited moderate antibacterial activity against all tested microorganisms, with inhibition zones ranging from 13 mm (*Klebsiella pneumonia)* to 18 mm (*Enterobacter cloacae*). Ciprofloxacin (analytical grade) displayed significantly higher activity, producing inhibition zones between 20 mm and 40 mm.

Table 4 Zones of inhibition caused by *Anthocleista vogelii* root extract on selected bacteria

S/N	Bacteria	Zone of inhibition (mm) (***Anthocleista vogelii)***	Zone of inhibition Ciprofloxacin (mm)
1	*Klebsiella pneumoniae*	13	20
2	*Escherichia coli*	14	20
3	*Pseudomonas aeruginosa*	14	20
4	*Bacillus subtilis*	14	34
5	*Staphylococcus aureus*	16	40
6	*Enterobacter cloacae*	18	36

The antibacterial activity of 200 mg/ml hydro-ethanolic fraction of *Anthocleista vogelii* was compared to 0.5 mg/ml ciprofloxacin using an independent samples t-test (assuming equal variances)**.** The analysis yielded an absolute t-value (3.477) > t-critical (2.228) and the p-value (0.0059) < 0.05, the null hypothesis (H_0) was rejected. This indicates that there was a statistically significant difference between the antibacterial activities of the plant extract and the standard antibiotic indicating a statistically significant difference ($p < 0.001$) in antibacterial efficacy between the two treatments. This suggests that the ethanolic fraction demonstrates strong antibacterial activity, although its effect does not exceed that of ciprofloxacin.

3.4.2 Minimum Inhibitory Concentration (MIC)

The MIC assay is represented by Table 4 below. The assay was conducted to determine the lowest concentration of *Anthocleista vogelii* root extract capable of inhibiting visible growth of selected clinical bacterial isolates. Five concentrations (200, 100, 50, 25, and 12.5 mg/mL) were tested. Complete inhibition of all bacterial isolates was observed at 200 mg/mL and 100 mg/mL. At 50 mg/mL, growth inhibition was recorded for *Escherichia coli* and *Staphylococcus aureus*, while the other isolates showed resistance. At 25 mg/mL, only *E. coli* was inhibited, and at the lowest concentration tested (12.5 mg/mL), no inhibitory activity was detected against any organism.

Table 5 Minimum Inhibitory Concentration of *Anthocleista vogelii* extract

S/N	**Bacteria**	**200 mg/mL**	**100 mg/mL**	**50 mg/mL**	**25 mg/mL**	**12.5 mg/mL**
1	*Klebsiella pneumoniae*	NG	NG	G	G	G
2	*Escherichia coli*	NG	NG	NG	NG	G
3	*Pseudomonas aeruginosa*	NG	NG	G	G	G
4	*Bacillus subtilis*	NG	NG	G	G	G
5	*Staphylococcus aureus*	NG	NG	NG	G	G
6	*Enterobacter cloacae*	NG	NG	G	G	G

3.4.3 Minimum Bactericidal Concentration (MBC)

The MBC assay was performed to determine the lowest concentration of *Anthocleista vogelii* extract capable of killing the selected bacterial isolates. Three concentrations (200, 100, and 50 mg/mL) were tested. At 200 mg/mL, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterobacter cloacae* showed no visible growth, confirming a bactericidal effect at this concentration. At 100 mg/mL, complete inhibition of *Staphylococcus aureus* was observed, while other organisms exhibited growth. At 50 mg/mL, visible growth occurred for all isolates, indicating no bactericidal activity at this concentration. Table 6 shows the result gotten from the MBC assay of *Anthocleista vogelii.*

Table 6 Minimum Bactericidal Concentration of *Anthocleista vogelii* extract

S/N	Bacteria	200 mg/mL	100 mg/mL	50 mg/mL
1	*Klebsiella pneumoniae*	G	G	G
2	*Escherichia coli*	NG	G	G
3	*Pseudomonas aeruginosa*	NG	G	G
4	*Bacillus subtilis*	G	G	G
5	*Staphylococcus aureus*	NG	NG	G
6	*Enterobacter cloacae*	NG	G	G

3.4.4 Mode of Antibacterial Activity

The table below presents the minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and corresponding MBC/MIC ratios for clinical isolates exposed to the ethanolic aerial extract of *Anthocleista vogelii*. The MIC represents the lowest extract concentration (mg/mL) that suppresses visible microbial growth, while the MBC indicates the lowest concentration that eliminates the organism, shown by the absence of growth after subculturing. The MBC/MIC ratio is then used to determine the nature of antimicrobial activity, where values ≤ 4 suggest a bactericidal effect and values > 4 indicate a bacteriostatic effect. These parameters provide insight into whether the extract primarily inhibits bacterial growth or actively kills the organisms under in-vitro conditions.

Table 7 Mode of antibacterial activity of *Anthocleista vogelii*

Bacteria	MIC	MBC	MBC/MIC	Classification
Staphylococcus aureus	50	100	100/50 = 2	Bactericidal
Enterobacter cloacae	100	200	200/100 = 2	Bactericidal
Pseudomonas aeruginosa	100	200	200/100 = 2	Bactericidal
Escherichia coli	25	200	200/25 = 8	Bactericidal

CHAPTER FOUR

4.0 DISCUSSION

The discussion provides a comprehensive interpretation of the findings obtained from the phytochemical, antioxidant, and antimicrobial analyses of *Anthocleista vogelii* Planch (Gentianaceae) root bark.

The chromatographic and biological assays employed includes; High Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC–MS), DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP, TAC, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and microbial susceptibility testing which are widely recognised methods for validating ethnomedicinal claims and identifying bioactive components responsible for antioxidant and antibacterial properties.

In this study, HPLC and GC–MS analyses were utilized to elucidate the chemical constituents of *A. vogelii*, while antioxidant assays such as DPPH, FRAP ABTS, and TAC, along with antimicrobial screening, were employed to assess its functional bioactivities.

4.1 PHYTOCHEMICAL CONSTITUENTS (HPLC & GC–MS FINDINGS)

The chromatographic analyses of the *Anthocleista vogelii* root bark extract revealed a diverse array of secondary metabolites, which are likely to underpin the antioxidant and antibacterial activities observed in this study. Both high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) data provide insightful evidence of the chemical complexity present.

4.1.1 High Performance Liquid Chromatography (HPLC Analysis)

The HPLC profiling (Table 2) identified 19 compounds across various chemical classes.

Flavonoids (Kaempferol, Naringenin, Flavones, and Flavonones)

The flavonoid class, represented in the extract by kaempferol (3.3343 µg/mL), naringenin (7.4327 µg/mL), flavones (5.7253 µg/mL), and flavonones (8.7424 µg/mL), forms a crucial antioxidant backbone in *Anthocleista vogelii*. These compounds are polyphenolic structures capable of donating hydrogen atoms or electrons to neutralize reactive oxygen species (ROS), thereby inhibiting lipid peroxidation and protecting cellular biomolecules (Veiko *et al*., 2023). The strong antioxidant profile aligns with the DPPH and FRAP results previously reported for *A. vogelii* root extract (Aiwonegbe *et al*., 2025), highlighting the contribution of these flavonoids to the plant's redox-balancing properties.

Kaempferol and naringenin are particularly important due to their dual antioxidant and antimicrobial effects. Kaempferol has been shown to destabilize bacterial membranes and inhibit virulence factors in *Staphylococcus aureus* and *Escherichia coli* (Periferakis *et al*., 2022), while naringenin modifies bacterial membrane integrity and inhibits hemolytic toxins (Veiko *et al*., 2023). Their presence thus supports the ethnomedicinal use of *A. vogelii* in treating infectious and inflammatory conditions such as wounds and gastrointestinal disorders (Enwemiwe, 2021). Chemically, their hydroxyl groups at C-3, C-5, and C-4′ contribute to radical scavenging, while conjugated double bonds enhance resonance stability, explaining their potent antioxidant responses.

Phenolic Compounds (Proanthocyanidins, Tannins, and Anthocyanins)

The detection of proanthocyanidins (10.4914 µg/mL), tannins (12.9619 µg/mL), and anthocyanins (2.0696 µg/mL) indicates a high concentration of phenolic antioxidants in the

hydro-ethanolic extract. These compounds function as chain-breaking antioxidants that scavenge superoxide radicals and chelate metal ions, preventing oxidative stress and cellular damage (Aiwonegbe *et al*., 2025). In *A. vogelii*, the abundance of tannins may explain its traditional application for wound healing and gastrointestinal protection, as tannins form complexes with microbial proteins, leading to cell wall disruption and enzyme inactivation (Anyanwu *et al*., 2015).

Proanthocyanidins, which are polymeric flavan-3-ols, contribute to both antioxidant defense and antimicrobial protection. Their polymeric hydroxyl groups confer strong reducing power, while their ability to bind bacterial adhesins and toxins explains the extract's inhibitory action against *Staphylococcus aureus* and *Pseudomonas aeruginosa* observed in previous studies (Enwemiwe, 2021). Anthocyanins, although less concentrated, add to the overall antioxidant synergy, as their chromophoric structure allows electron delocalization, enhancing free radical neutralization.

Steroids and Triterpenoids (Steroids and Sapogenin)

Steroids (8.2903 µg/mL) and sapogenin (10.9960 µg/mL) represent the terpenoid-based class of compounds in the extract. These molecules are known for their membrane-active antimicrobial properties and for modulating oxidative stress via lipid peroxidation inhibition (Oladimeji *et al*., 2015). Steroids in *A. vogelii* likely reinforce the plant's broad antimicrobial activity through disruption of bacterial lipid bilayers, leading to leakage of cellular contents and death (Anyanwua *et al*., 2023).

Sapogenins, which are aglycone derivatives of saponins, exhibit surface-active properties that contribute to cell membrane permeabilization in pathogens. Beyond their antimicrobial role, sapogenins have been shown to regulate antioxidant enzyme activities, including catalase and superoxide dismutase, in oxidative stress models (Aiwonegbe *et al*., 2025). This biochemical

versatility may underlie *A. vogelii'*s traditional use as an antipyretic and in the management of liver-related disorders, where oxidative imbalance is a common feature.

Alkaloids (Ammodendrine, Sparteine, Ephedrine, and Aphyllidine)

The alkaloid fraction, prominently represented by ammodendrine (22.0694 µg/mL) and sparteine (15.9760 µg/mL), contributes significantly to *A. vogelii's* antimicrobial efficacy. Alkaloids are nitrogen-containing compounds known for their ability to intercalate with microbial DNA and inhibit nucleic acid synthesis (Anyanwu *et al*., 2015). The strong presence of ammodendrine may explain the plant's pronounced activity against Gram-positive organisms such as *Staphylococcus aureus* (Enwemiwe, 2021).

Sparteine and ephedrine may exhibit synergistic effects, acting through neurochemical and membrane-modifying mechanisms that hinder bacterial respiration and energy metabolism. Their basic nitrogen centers facilitate binding to acidic phospholipids in bacterial membranes, destabilizing structural integrity. Additionally, ephedrine's phenethylamine backbone provides redox potential that indirectly enhances antioxidant response by reducing reactive oxygen species (ROS) accumulation in tissues. These dual actions highlight the complex defense role of alkaloids in the plant's pharmacological profile.

Glycosides (Cardiac and Cyanogenic Glycosides)

The extract showed notable concentrations of cyanogenic glycosides (20.1936 µg/mL) and cardiac glycosides (4.0095 µg/mL). Cyanogenic glycosides, while potentially toxic in excess, can generate low levels of hydrogen cyanide that exert antimicrobial effects by inhibiting microbial respiratory enzymes (Anyanwu *et al*., 2015). This property supports *A. vogelii's* ethnomedicinal use in infection control and its mild antimicrobial potency observed in vitro (Enwemiwe, 2021).

Cardiac glycosides, on the other hand, are known to interact with Na^+/K^+-ATPase in both eukaryotic and microbial membranes, leading to ion imbalance and cell death. Their presence also aligns with the diuretic and cardiovascular modulatory properties of *A. vogelii* reported in animal studies (Anyanwua *et al*., 2023). From an antioxidant perspective, these glycosides stabilize membrane lipids and modulate intracellular calcium homeostasis, reducing oxidative stress in cardiac and hepatic tissues.

Phenolic Acid Derivatives and Chelators (Phytate and Oxalate)

The presence of phytate (8.8109 µg/mL) and oxalate (1.3133 µg/mL) highlights the extract's secondary antioxidant defense mechanism via metal chelation. Phytates sequester transition metals such as Fe^{2+} and Cu^{2+}, preventing Fenton-type reactions that generate hydroxyl radicals (Aiwonegbe *et al*., 2025). This contributes to the sustained antioxidant stability of the extract, complementing the radical scavenging activities of flavonoids and tannins.

Oxalates, although traditionally regarded as anti-nutritional, may also contribute to redox balance through calcium complexation, indirectly reducing pro-oxidant metal availability. These compounds thus support the extract's antioxidant persistence and may aid in detoxification processes within tissues. Their occurrence aligns with *A. vogelii's* traditional reputation as a cleansing and restorative herbal remedy (Anyanwu *et al*., 2015).

4.1.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS data (Table 3) identified 35 compounds, predominated by long-chain and unsaturated fatty acids and their esters (e.g., 2,8-decadiyne at 17.99 % area, pentyl octanoate at 9.72 %, palmitic acid at 4.81 %). These non-polar constituents expand the functional chemical scope of the extract. Long-chain fatty acids (LCFAs) and their derivatives are increasingly recognised for antimicrobial and anti-virulence effects for example, they may

inhibit biofilm formation, modulate quorum sensing, and destabilise bacterial membranes (Borreby *et al*., 2023).

Specifically, palmitic and octanoate derivatives may contribute to membrane fluidity changes and induction of membrane-stress responses in bacteria, thus enhancing susceptibility. Additional aromatic acid derivatives and heterocycles (e.g., benzoic acid, thiophene derivatives) may act synergistically, offering acidification of micro-environment or oxidative stress induction within microbial cells. The co-occurrence of these fatty acids with phenolics and alkaloids suggests that the extract targets both membrane and intracellular systems of microbes.

When viewed together with the HPLC profile, the findings suggest that the root bark extract of *A. vogelii* may exert a dual mode of action: its polar constituents likely function as radical scavengers or enzyme-modulating agents, while the more lipophilic compounds may interact directly with microbial membranes or influence virulence processes. The interaction between hydrophilic and lipophilic metabolites is increasingly recognised as a key contributor to the synergistic bioactivity often observed in complex plant extracts (Okafor *et al.,* 2024).

4.1.3 Implications for Biological Activities

The compound profile thus provides a mechanistic basis for the antioxidant and antibacterial results presented earlier. For antioxidant activity, flavonoids and phenolics identified support hydrogen-donating and metal-reducing actions in assays like DPPH and FRAP. For antimicrobial activity, the combination of alkaloids, glycosides and fatty acids suggests multi-modal actions including membrane disruption, metabolic inhibition, and efflux interference. Literature emphasises that plant flavonoids (e.g., kaempferol) display anti-MRSA activity and may reverse antibiotic resistance (Yan *et al*., 2024). Similarly, reviews of

fatty acids demonstrate their capacity to act as signalling molecules against pathogens (Borreby *et al.*, 2023).

In summary, the chemical fingerprint of *A. vogelii* root bark supports its ethnomedicinal use for infections and oxidative stress conditions in West Africa and justifies further fractionation and isolation studies to validate specific lead compounds for therapeutic development.

4.2 *IN VITRO* ANTIOXIDANT ACTIVITY

The antioxidant potential of the 70% ethanolic extract of Anthocleista vogelii root bark was evaluated using four complementary assays DPPH, FRAP, TAC, and ABTS. These assays measure distinct but interrelated mechanisms of antioxidant action, including free radical scavenging, ferric ion reduction, and total antioxidant capacity. The extract exhibited strong and concentration-dependent antioxidant activities, which are closely associated with the presence of phenolic and flavonoid compounds detected by HPLC (kaempferol, naringenin, proanthocyanidins, tannins, flavanones, sapogenin) and fatty acid derivatives identified by GC-MS (benzoic acid methyl ester, octadecanoic acid, and hexadecanoic acid methyl ester).

4.2.1 DPPH Radical Scavenging Activity

The *Anthocleista vogelii* root extract demonstrated significant free radical scavenging activity in the DPPH assay, with percentage inhibition values ranging from 81.28% to 91.92% as the concentration increased from 1000 to 200 µg/mL. The strong inhibition observed at lower concentrations suggests a high antioxidant capacity, likely due to the presence of flavonoids such as kaempferol, and fatty acid esters, both of which were identified in the GC-MS and HPLC profiles.

Kaempferol is well-documented for its potent antioxidant mechanisms, primarily through hydrogen atom donation and radical stabilization (Periferakis *et al*., 2022; Bangar, 2023). These compounds scavenge DPPH radicals by donating electrons, converting reactive species

into more stable molecular forms. The observed high inhibition rate at low concentrations may also be attributed to synergistic effects among polyphenolic compounds and terpenoids, which enhance overall free radical quenching efficiency. Additionally, fatty acid methyl esters (FAMEs) identified in the GC–MS spectrum has been reported to possess antioxidant properties due to their ability to stabilize lipid radicals and inhibit peroxidation reactions (Araújo *et al*., 2024).

4.2.2 Ferric Reducing Antioxidant Power (FRAP) Assay

In the FRAP analysis, *A. vogelii* exhibited moderate to strong reducing activity, with inhibition values ranging from 28.23% to 40.44%. The reducing capacity reflects the extract's ability to donate electrons to Fe^{3+} ions, converting them to Fe^{2+}. This redox-based mechanism suggests the involvement of phenolic hydroxyl groups and conjugated systems, which facilitate electron transfer (Bangar, 2023).

The moderate activity observed in this assay compared to DPPH and ABTS may be due to the relative proportion of phenolic compounds present or potential structural differences in the antioxidant constituents. The kaempferol detected through HPLC possesses multiple hydroxyl groups that contribute to ferric reduction (Periferakis *et al*., 2022), while FAMEs and n-9 fatty acids enhance the lipid-phase antioxidant interactions, improving redox stabilization (Araújo *et al*., 2024; Huang *et al*., 2010). Together, these components suggest that *A. vogelii*'s antioxidant mechanism involves both hydrophilic and lipophilic systems, contributing to its traditional use in oxidative stress-related conditions.

4.2.3 ABTS Radical Scavenging Activity

The ABTS assay results also confirmed substantial antioxidant potential, with inhibition values ranging from 71.02% to 93.24% across the tested concentrations. The high radical

scavenging effect, particularly at 200 µg/mL (93.24%), aligns with the DPPH results, reinforcing the extract's ability to neutralize both hydrophilic and lipophilic radicals.

Flavonoids are known to chelate transition metal ions, reduce radical formation, and stabilize $ABTS^{\bullet+}$ radicals through electron transfer mechanisms (Bangar, 2023; Li *et al*., 2024). The consistency between DPPH and ABTS inhibition supports the presence of both hydrogen-donating and electron-transfer antioxidants within the *A. vogelii* extract. The participation of unsaturated fatty acid esters (identified via GC–MS) further strengthens this observation, as these compounds can scavenge peroxyl radicals and terminate chain oxidation processes (Huang *et al*., 2010).

4.2.4 Total Antioxidant Capacity (TAC) Assay

The TAC assay revealed consistently high antioxidant potential, with inhibition values ranging between 76.24% and 89.24%. These results indicate that *A. vogelii* root extract possesses a broad-spectrum antioxidant effect, likely due to the combined action of polyphenols, flavonoids, fatty acid esters, and terpenoids.

The high TAC values suggest the extract's cumulative capacity to scavenge a wide range of reactive oxygen species (ROS). This aligns with earlier chromatographic findings that revealed kaempferol, linoleic acid derivatives, and fatty acid methyl esters as major bioactive components (Araújo *et al*., 2024; Periferakis *et al*., 2022). These compounds are known to act through both primary antioxidant mechanisms (radical scavenging and metal chelation) and secondary mechanisms (membrane protection and enzyme modulation). Li *et al*. (2024) also demonstrated that kaempferol modulates membrane interactions and restores cellular redox balance, providing a mechanistic link to the extract's observed antioxidant performance.

4.3 ANTIMICROBIAL ASSAY

The 70% ethanolic root bark extract of *Anthocleista vogelii* exhibited broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, consistent with its ethnomedicinal use in treating infectious diseases. The zones of inhibition recorded ranged between 13 mm and 18 mm, with *Staphylococcus aureus* showing the highest susceptibility (16 mm), followed closely by *Enterobacter cloacae* (18 mm), while *Klebsiella pneumoniae* demonstrated the lowest inhibition (13 mm). In comparison, the standard antibiotic ciprofloxacin produced larger zones (20-40 mm), reflecting its potent synthetic nature. Nonetheless, the appreciable inhibitory diameters from the plant extract highlight a significant antimicrobial potential, particularly considering its crude, unrefined composition.

The observed inhibitory effects align closely with the phytochemical constituents identified from the HPLC and GC-MS profiles of the extract. Notably, kaempferol, proanthocyanidins, fatty acid methyl esters, alkaloids, and phenolic compounds were predominant. Kaempferol, in particular, has been extensively reported to exhibit antimicrobial activity through multiple mechanisms, including disruption of bacterial cell membranes, inhibition of nucleic acid synthesis, and attenuation of quorum sensing pathways (Periferakis *et al*., 2022). Its ability to interfere with bacterial communication and biofilm formation enhances susceptibility to both natural and synthetic antimicrobials (Li *et al*., 2024). This property may explain the extract's activity against *E. coli* and *P. aeruginosa*, organisms typically known for robust biofilm formation and multidrug resistance.

The results from the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests further reinforce these findings. Complete growth inhibition was observed at 100–200 mg/mL for most test organisms, while bactericidal effects were evident at concentrations up to 200 mg/mL. This moderate potency suggests a bacteriostatic-to-bactericidal transition at higher concentrations, likely influenced by the synergistic effects of

multiple bioactive compounds. The presence of fatty acid derivatives, such as n-6, n-7, and n-9 fatty acids and their esters identified via GC–MS, could contribute to this effect. According to Huang et al. (2010), these lipid compounds integrate into bacterial membranes, disrupting their structural integrity and causing leakage of cellular contents. Similarly, Araújo *et al*. (2024) reported that fatty acid methyl esters derived from plant oils exhibit significant antibacterial and antifungal properties, primarily through interference with membrane permeability and metabolic enzyme systems.

The enhanced inhibitory response observed against *S. aureus* and *E. cloacae* may also be attributed to the combined presence of kaempferol and proanthocyanidins. Flavonoids and tannin derivatives are known to chelate metal ions and form complexes with bacterial cell wall proteins, leading to structural deformation and lysis (Bangar, 2023). This mechanism aligns with the observed reduction in growth of Gram-positive species such as *B. subtilis* and *S. aureus*, whose thick peptidoglycan layers are susceptible to polyphenol binding. Furthermore, these phytochemicals have been linked to inhibition of virulence factors, potentially suppressing pathogenicity in infections such as staphylococcal wound infections, enterobacterial gastroenteritis, and *Klebsiella*-associated urinary tract infections, all conditions traditionally managed with *A. vogelii* extracts in African ethnomedicine.

The comparatively lower activity against *K. pneumoniae* (13 mm inhibition) may be due to its inherent resistance mechanisms, such as extended-spectrum β-lactamase (ESBL) production and robust capsule formation, which limit the penetration of phytochemicals. However, kaempferol's recently reported ability to restore antibiotic susceptibility in ESBL-producing E. coli (Li *et al*., 2024) suggests potential synergistic therapeutic value if used in combination with standard antibiotics. This finding supports the concept that bioactive compounds in *A. vogelii* could act as resistance modulators, potentiating conventional antimicrobials against resistant pathogens.

The hydro-ethanolic aerial fraction of *Anthocleista vogelii* was assessed for its antimicrobial mode of action against four bacterial strains. The calculated MBC/MIC ratios were as follows: *Staphylococcus aureus*: 2, *Pseudomonas aeruginosa*: 2, *Enterobacter cloacae*: 2, *Escherichia coli*: 8

These results indicate that the extract exerted a bactericidal effect on *S. aureus*, *P. aeruginosa*, and *E. cloacae*, as their MBC/MIC ratios were below or equal to 4. In contrast, the ratio for *E. coli* was 8, suggesting a bacteriostatic mechanism where the extract inhibits growth without necessarily killing the organism.

This distinction is clinically relevant, as bactericidal agents are often preferred in treating infections in immuno-compromised patients or in cases where bacterial eradication is critical. The differential activity observed may be attributed to variations in the cell wall structure, efflux mechanisms, or metabolic pathways among the tested bacteria, which influence their susceptibility to phytochemicals present in the extract (Emery Pharma, 2023).

Overall, the antimicrobial performance of the *A. vogelii* root bark extract can be attributed to the combined effects of multiple phytochemical classes acting through different mechanisms: membrane disruption (fatty acids and alkaloids), enzyme inhibition (flavonoids), oxidative stress induction (phenolics), and biofilm inhibition (kaempferol and proanthocyanidins). The integration of these actions produces a synergistic antimicrobial response capable of inhibiting both Gram-positive and Gram-negative bacteria. This pharmacological profile not only substantiates the traditional use of *A. vogelii* in managing infections such as diarrhea, wounds, and urinary tract infections but also underscores its potential as a source of novel antimicrobial agents in the face of rising antibiotic resistance.

CONCLUSION

This investigation delivers an in-depth chemical and biological assessment of *Anthocleista vogelii* root bark, using HPLC and GC-MS for profiling, antioxidant and antimicrobial assays for activity. HPLC cataloged polar elements like alkaloids, flavonoids, and tannins; GC-MS added volatiles like fatty acids and heterocycles.

Antibacterial tests showed moderate, wide-ranging inhibition, with bactericidal effects at elevated levels. Though less potent than standards, the metabolite diversity suggests synergistic benefits, validating cultural applications and marking the plant for drug exploration.

Ethnopharmacologically, results endorse *A. vogelii* in local care.

The delimitations of the study include the exclusion of other plant parts such as leaves, stem bark, and fruits, which may also possess bioactive compounds but are beyond the present scope. Additionally, the study does not cover toxicity testing, in-vivo pharmacological evaluation, or isolation of pure compounds, as these would require extended research and advanced analytical facilities and is recommended for further studies.

Ultimately, *A. vogelii* holds promise as a bioactive source, merging heritage with potential modern therapies.

REFERENCES

Abdallah, E.M., Alhatlani, B.Y., de Paula Menezes, R. & Martins, C.H.G. (2023). *Back to Nature: Medicinal Plants as Promising Sources for Antibacterial Drugs in the Post-Antibiotic Era*. Plants, 12(17), 3077. https://doi.org/10.3390/plants12173077

Adepoju, O.T., Adebiyi, A.O. and Adetunji, O.A. (2021) 'Antioxidant and antimicrobial potentials of selected medicinal plant extracts used in Nigeria' *Journal of Applied Pharmaceutical Science* 11(5) pp. 57–64.

Afolayan, A.J. & Meyer, J.J.M., 1997. The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens*. *Journal of Ethnopharmacology*, 57(3), pp.177-181. doi:10.1016/S0378-8741(97)00065-2.

Aiwonegbe, A., Ojeniyi, O., Akhidenor, F.I. and Odion, E. (2025) 'Phytochemical composition and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of *Anthocleista vogelii* (Gentianaceae) root extract', *Nigerian Journal of Pharmaceutical and Applied Science Research*, 14(3), pp. 21–28.

Ajayi, A.M., *et al*. (2023) '*Anthocleista vogelii* (Planch) stem bark ethanol extract prevents inflammatory and pain phenotypes in experimental rodent models by modulating oxido-inflammatory mediators', *Journal of Complementary and Integrative Medicine*.

Akah, P.A., Osonwa, E.U. and Obasi, O.E. (2021) 'Evaluation of hypoglycaemic and antioxidant effects of *Anthocleista vogelii* stem bark extract in alloxan-induced diabetic rats', *African Journal of Pharmaceutical Research and Development*, 13(2), pp. 89–96.

Akaahan, D.I., Ekere, A.T. and Mtomga, I., 2025. Quercetin: A flavonoid with antioxidant, anti-inflammatory, and anti-apoptotic properties – A review. *Benue Journal of Clinical Research*, 12(1), pp.17–33. Available at: https://ideas.repec.org/a/bjc/journl/v12y2025i1p17-33.html

Al-Rimawi, F, Sbeih, M., Amayreh, M., Qabaha, K., Al-Khatib, S. and Al-Natsheh, M. (2024) 'Evaluation of the antibacterial and antifungal properties of *Olea europaea* leaf extract and *Thymus vulgaris* oil', *BMC Complementary Medicine and Therapies*, 24(1), 297.

Angelini, P. (2024) 'Plant-Derived Antimicrobials and Their Crucial Role in Combating Antimicrobial Resistance', *Antibiotics*, 13(8), 746. doi: 10.3390/antibiotics13080746. mdpi.com+1

Alharbi, H.O.A., Alshebremi, M., Babiker, A.Y. and Rahmani, A.H., 2025. The role of quercetin in the management of pathogenesis through regulation of oxidative stress and inflammation. *Biomolecules*, 15(1), p.151. Available at: https://www.mdpi.com/2218-273X/15/1/151 Phenolic Compounds – Gallic Acid

Araújo, A.M., Rodrigues, E.A., Rodrigues, A.C., Filho, J.P., Maróstica Jr., M.R. & Santana, L.S. (2024). Antifungal and antioxidant activity of fatty acid methyl esters (FAMEs)

obtained from vegetable oils. Anais da Academia Brasileira de Ciências, 96(1), e20240120. https://doi.org/10.1590/0001-3765202420240120

Aryal, B., Raut, B.K., Bhattarai, S., Bhandari, S., Tandan, P., Gyawali, K., Sharma, K., Ranabhat, D., Thapa, R., Aryal, D., Ojha, A., Devkota, H.P. and Parajuli, N., 2022. Potential therapeutic applications of plant-derived alkaloids against inflammatory and neurodegenerative diseases. *Evidence-Based Complementary and Alternative Medicine*, 2022, https://doi.org/10.1155/2022/7299778.

Anyanwu, G.O., ur-Rehman, N., Onyeneke, C.E. & Rauf, K. (2015) 'Medicinal plants of the genus *Anthocleista* – A review of their ethnobotany, phytochemistry and pharmacology', *Journal of Ethnopharmacology*, 175, pp. 648–667. doi: 10.1016/j.jep.2015.09.032.

Anyanwua, G.O., Onyeneke, C.E., Ofoha, P.C., Rauf, K., Usunobun, U. & ur-Rehman, N. (2023) 'Diuretic, anti-diuretic and laxative activities of Anthocleista vogelii extracts in rats', African Journal of Traditional, Complementary and Alternative Medicines, 15, (1). doi: 10.21010/ajtcam.vi15.1.7.

Bader, C.D., Nichols, A.L., Yang, D. and Shen, B. (2023) 'Interplay of emerging and established technologies drives innovation in natural product antibiotic discovery', *Current Opinion in Microbiology*, 75, 102359.

Bangar, S.P. (2023). Kaempferol: A flavonoid with wider biological activities and mechanistic insight. Critical Reviews in Food Science and Nutrition, 63(2), 245–260. https://doi.org/10.1080/10408398.2022.2067121

Baranova, A.A., Alferova, V.A., Korshun, V.A. and Tyurin, A.P. (2023) 'Modern trends in natural antibiotic discovery', *Life*, 13(5), 1073.

Barba-Ostria, C., Carrera-Pacheco, S.E., Gonzalez-Pastor, R., Heredia-Moya, J., Mayorga-Ramos, A., Rodríguez-Pólit, C., Zúñiga-Miranda, J., Arias-Almeida, B. and Guamán, L.P. (2022) 'Evaluation of biological activity of natural compounds: Current trends and methods', *Molecules*, 27(14), 4490.

Bassey, R.A., Ndarake, E.I.-I., Ngele, B.A. and Iniobong, E.A. (2023) 'GC-MS analysis and phytochemical constituents in *Anthocleista vogelii* and *Leucaena leucocephala* obtained from Calabar, Cross River State, Southern Nigeria', *Research Journal of Science & Technology*, 3(2), pp. 68–81

Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996 Jul 15;239(1):70-6. doi: 10.1006/abio.1996.0292. PMID: 8660627.

Borreby, C. (2023). Anti-infective activities of long-chain fatty acids against foodborne pathogens. FEMS Microbiology Reviews, 47(4). https://doi.org/10.1093/femsre/fuad037

Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995) 'Use of a free radical method to evaluate antioxidant activity', *LWT – Food Science and Technology*, 28(1), pp. 25–30. doi: 10.1016/S0023-6438(95)80008-5.

Chen, S., Wang, X., Cheng, Y., Gao, H. and Chen, X., 2023. A review of classification, biosynthesis, biological activities and potential applications of flavonoids. *Molecules*, 28(13), p.4982. https://doi.org/10.3390/molecules28134982

Edet, U., Ekanem, A., Essien, E. and Akpan, A., 2018. Comparative evaluation of phytochemicals and antibacterial activity of the roots and leaves of *Anthocleista vogelii* on some clinical isolates. *ResearchGate*. Available at: https://www.researchgate.net/publication/326462575

Egbuna, C., Dable-Tupas, G., Tijjani, H., Patrick-Iwuanyanwu, K.C. and Olayemi, O. (2022) 'Pharmacognostic and biological potential of the genus *Anthocleista* (Gentianaceae): A review of current evidence', *Journal of Ethnopharmacology*, 293, 115372.

Ekpo, D.E., Akpabio, U.D. and Inyang, E.E. (2021) 'Comparative phytochemical analysis and antioxidant activity of methanolic extracts of *Anthocleista djalonensis* and *Anthocleista vogelii*', *Journal of Applied Chemical Science*, 8(2), pp. 33–40.

Enwemiwe, O. (2021) 'Anti-bacterial and phytochemical properties of Anthocleista vogelii on bacteria causing wound and enteric infection', Undergraduate Project, Michael Okpara University of Agriculture, Umudike.

Ezeorah, M.C., Uchendu, I.K. and Akinlabi, O.O. (2023) 'Hepatoprotective potential of *Anthocleista vogelii* extract in carbon tetrachloride-induced liver injury in rats', *Heliyon*, 9(12), e22419.

Godara, R., *et al*. (2019) 'GC-MS of bioactive compounds from Jeevanti: antimicrobial, antioxidant and lipoxygenase inhibitory activities', Pharmacognosy Journal, 11(1), pp. 129-140.

Gonzalez-Pastor, R., Carrera-Pacheco, S.E., Zúñiga-Miranda, J., Rodríguez-Pólit, C., Mayorga-Ramos, A., Guamán, L.P. and Barba-Ostria, C. (2023) 'Current landscape of methods to evaluate antimicrobial activity of natural extracts', *Molecules*, 28(3),

Hadidi, M., Liñán-Atero, R., Tarahi, M., Christodoulou, M.C. and Aghababaei, F., 2025. The potential health benefits of gallic acid: therapeutic and food applications. *Antioxidants*, 13(8), p.1001. Available at: https://www.mdpi.com/2076-3921/13/8/1001

Huang, C.B., George, B. & Ebersole, J.L. (2010). Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. BMC Microbiology, 10, 233. https://doi.org/10.1186/1471-2180-10-233

Ikpe, E.E., Archibong, U.D., Michael, C.U. and Ukpong, E.G., 2020. Chemical composition of *Anthocleista vogelii* and anti-oxidant effect of its methanolic and acetone extracts.

Chemical Science International Journal, 29(8), pp.7–18. https://doi.org/10.9734/CSJI/2020/v29i830196

Ishak, A., Mazonakis, N., Spernovasilis, N., Akinosoglou, K., & Tsioutis, C. (2025). Bactericidal versus bacteriostatic antibacterials: clinical significance, differences and synergistic potential in clinical practice. *Journal of Antimicrobial Chemotherapy*, 80(1), 1–17. https://doi.org/10.1093/jac/dkae380

Janet Liu (2023). Understanding Antimicrobial Testing: A Guide to MIC, MBC, and More. Retrieved from https://emerypharma.com/blog/understanding-antimicrobial-testing-a-guide-to-mic-mbc-and-more/

Kaur, C. and Kapoor, H.C. (2021) 'Antioxidants in fruits and vegetables: The millennium's health', *International Journal of Food Science and Nutrition*, 72(4), pp. 493–507

Li, P.C., et al. (2024). Kaempferol restores the susceptibility of ESBLs E. coli by inhibiting biofilm formation and modulating membrane interactions. Frontiers in Microbiology, 15, Article 1474919. https://doi.org/10.3389/fmicb.2024.1474919

Kováč, J., Slobodníková, L., Trajčíková, E., Rendeková, K., Mučaji, P., Sychrová, A. and Bittner Fialová, S., 2022. Therapeutic potential of flavonoids and tannins in management of oral infectious diseases – A review. *Molecules*, 28(1), p.158. https://doi.org/10.3390/molecules28010158

Kumar, P., Gupta, A. and Singh, M. (2022) 'Comparative evaluation of antioxidant assays in medicinal plant extracts', *Plant Science Today*, 9(1), pp. 156–165.

Kumari, S., Yadav, S. and Sharma, A. (2022) 'Ethnopharmacological significance and phytochemical diversity of medicinal plants used in primary healthcare: A review', *Journal of Ethnopharmacology*, 288, 115078.

Kwon, J.Y., *et al*. (2017) 'An evidence of C16 fatty acid methyl esters extracted from microalga for effective antimicrobial and antioxidant property', Algal Research, 23, pp. 52-59. doi: 10.1016/j.algal.2017.01.006.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2022) 'Free radicals, antioxidants and functional foods: Impact on human health', *Pharmacognosy Reviews*, 16(32), pp. 118–126.

Ndirangu, J.W., Gichimu, B.M. and Mwangi, E.W. (2022) 'GC–MS analysis of bioactive compounds and in vitro antioxidant properties of selected Kenyan medicinal plants', *Heliyon*, 8(12), e12105.

Newman, D.J. and Cragg, G.M. (2020) 'Natural products as sources of new drugs over the nearly four decades from 1981 to 2019', *Journal of Natural Products*, 83(3), pp. 770–803.

Nguyen, T.T., Pham, V.H., Nguyen, Q.T. and Le, D.T. (2022) 'Advances in liquid chromatography–mass spectrometry for phytochemical analysis of herbal medicines', *Journal of Chromatography B*, 1209, 123473.

Nnamani, P.O., Agbo, M.O. and Afiukwa, C.A. (2022) 'Influence of collection period and drying method on the phytochemical content of selected medicinal plant roots in Southeastern Nigeria', *African Journal of Plant Science*, 16(9), pp. 241–250.

Nzor, J.N., Uwakwe, A.A. and Ogunka-Nnoka, C.U., 2024. Comparative analysis of *Anthocleista vogelii* leaf extracts: solvent influence on phytochemical composition, quantitative profile, and in-vitro antioxidant activities. *International Journal of Innovative Biochemistry & Microbiology Research*, 12(1), pp.1–7.

Odion, E.E., Edet, A.I., Ambe, D.A., Osigwe, C.C. and Odiete, E.C. (2024) 'Emilia praetermissa Milne-Redhead (Asteraceae): Phytochemical profiling and cytotoxic potential', African Journal of Traditional, Complementary and Alternative Medicines, 21(1), pp. 12–27. https://doi.org/10.21010/AJTCAMv21i1.2.

Ofor, P.O., Okorie, C.N. and Nwachukwu, E.O. (2023) 'Phytochemical constituents and antimicrobial efficacy of *Anthocleista* species from Southeastern Nigeria', *African Journal of Biotechnology*, 22(7), pp. 89–98.

Ogu, R.A., Eze, J.O. and Chukwuma, P.C. (2022) 'Systematic evaluation and ethnomedicinal diversity of *Anthocleista* species in West Africa', *South African Journal of Botany*, 148, pp. 67–75.

Ogunyemi, C.M., Onigbinde, A.O. and Ajibola, O.A. (2022) 'Phytochemical screening and pharmacological relevance of medicinal plants used in Nigeria: A review', *Heliyon*, 8(10), e10987.

Ojo, O.A., Ademiluyi, A.O., Onikanni, A.S. and Ogunlana, O.O. (2024) 'HPLC quantification of phenolic antioxidants in selected Nigerian medicinal plants and correlation with in vitro radical scavenging activity', *Heliyon*, 10(2), e23341.

Okafor, C.E., Ijoma, I.K., Igboamalu, C.A., Ezebalu, C.E., Eze, C.F., Osita-Chikeze, J.C., Uzor, C.E. & Ekwuekwe, A.L. (2024) 'Secondary metabolites, spectra characterization, and antioxidant correlation analysis of the polar and non-polar extracts of *Bryophyllum pinnatum* (Lam) Oken', *Biotechnologia*, 105(2), pp. 121–136. doi: 10.5114/bta.2024.139752.

Okafor, N.A., Nwankwo, E.J. and Chukwu, C.P. (2023) 'Ethnobotanical authentication and herbarium documentation of medicinal plants used in Eastern Nigeria', *Tropical Plant Research*, 10(1), pp. 34–42.

Okorie, C.N., Nwachukwu, E.O. and Ofor, P.O. (2023) 'Analytical techniques for phytochemical profiling of African ethnomedicinal plants: Trends and challenges', *African Journal of Pharmaceutical Research and Development*, 15(1), pp. 11–22.

Oladimeji, A., et al. (2015) 'Medicinal plants of the genus *Anthocleista* – A review of their ethnobotany, phytochemistry and pharmacology', *Journal of Ethnopharmacology*, 162, pp. 109–127. doi: 10.1016/j.jep.2014.12.009.

Owolabi, M.S., Adeleke, A.J. and Ogunleye, A.O. (2021) 'Ethnopharmacological relevance of medicinal plants in African traditional medicine and their phytochemical bases', *Journal of Herbal Medicine*, 28, 100434.

Perez, C., Pauli, M. and Bazerque, P. (1990) An Antibiotic Assay by Agar Well Diffusion Method. Acta Biologiae et Medicinae Experimentalis, 15, 113-115.

Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999 May 1;269(2):337-41. doi: 10.1006/abio.1999.4019. PMID: 10222007.

Proximate and phytochemical analysis of leaves of Anthocleista vogelii (Cabbage palm)*, 2024.* Kashmir Journal of Science*, 3(2). https://doi.org/10.63147/krjs.v3i2.52*

Periferakis, A., Periferakis, K., Badarau, I.A., Petran, E.M., Popa, D.C., Caruntu, A., Costache, R.S., Scheau, C., Caruntu, C. & Costache, D.O. (2022) 'Kaempferol: Antimicrobial properties, sources, clinical, and traditional applications', International Journal of Molecular Sciences, 23, 15054. doi: 10.3390/ijms232315054.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999 May;26(9-10):1231-7. doi: 10.1016/s0891-5849(98)00315-3. PMID: 10381194.

Silva, B.N., Bonilla-Luque, O.M., Possas, A., Ezzaky, Y., Elmoslih, A., Teixeira, J.A., Achemchem, F., Valero, A., Cadavez, V. and Gonzales-Barron, U. (2023) 'Meta-analysis of in vitro antimicrobial capacity of extracts and essential oils of *Syzygium aromaticum*, *Citrus L.* and *Origanum L.*: Contrasting the results of different antimicrobial susceptibility methods', *Foods*, 12(6), 1265.

Singh, A., Dutta, P.K., Kumar, H., Kureel, A.K. and Rai, A.K., 2019. Improved antibacterial and antioxidant activities of gallic acid grafted chitin-glucan complex. *Journal of Polymer Research*, 26, p.234. Available at: https://link.springer.com/article/10.1007/s10965-019-1893-3

Singh, S., Rajput, V.S. and Kumar, P. (2020) 'Thin layer chromatographic analysis in standardization of herbal drugs: A review', *Journal of Pharmacognosy and Phytochemistry*, 9(5), pp. 1623–1631.

Singh, R.H., 2024. Reserpine: A survey of its pharmacology. In: *Handbook of Pharmacology*. Springer. Available at: https://link.springer.com/content/pdf/10.1007/978-1-4615-7175-9_13.pdf

Tankeshwar, A. (2023). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). *Microbe Online*. Retrieved from https://microbeonline.com/minimum-inhibitory-concentration-and-minimum-bactericidal-concentration-mbc/

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. & Byrne, D.H., 2023. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19(6–7), pp.669–675.

Tittikpina, N.K., Nana, F., Fontanay, S., Duval, R.E. & Kirkiacharian, S., 2020. Antibacterial and antioxidant activities of *Anthocleista vogelii* extracts from Togo. *South African Journal of Botany*, 133, pp.174–180.

Uchendu, I.K., Akinlabi, O.O. and Ezeorah, M.C. (2024) 'Comparative antibacterial activity of methanol extracts of *Anthocleista vogelii* and *Anthocleista djalonensis*', *Heliyon*, 10(1), e24519.

Veiko, A.G., Olchowik-Grabarek, E., Sekowski, S., Roszkowska, A., Lapshina, E.A., Dobrzynska, I., Zamaraeva, M. & Zavodnik, I.B. (2023) 'Antimicrobial activity of quercetin, naringenin and catechin: Flavonoids inhibit Staphylococcus aureus-induced hemolysis and modify membranes of bacteria and erythrocytes', Molecules, 28(3), 1252. doi: 10.3390/molecules28031252.

Wang, Y., Zhang, Y., Shi, Y.Q., Pan, X. & Lu, Y., 2022. Synergistic antibacterial effects of herbal extracts and antibiotics: A new strategy against resistant pathogens. *Frontiers in Pharmacology*, 13, p.839598.

Wiegand, I., Hilpert, K. & Hancock, R.E.W., 2021. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2), pp.163–175.

World Health Organization, 2023. *World health statistics 2023: Monitoring health for the SDGs, sustainable development goals*. Geneva: WHO. Available at: https://www.who.int/data/gho/publications/world-health-statistics [Accessed 30 October 2025].

Wu, C., Wang, X., Wang, H., Liu, Y. & Liu, X., 2020. Ammodendrine: A promising antimicrobial alkaloid from *Lupinus* species. *Natural Product Communications*, 15(7), p.1934578X20934256. https://doi.org/10.1177/1934578X20934256

Xie, Y., Yang, W., Tang, F., Chen, X. & Ren, L., 2022. Antibacterial activity of flavonoids and their structure–activity relationship. *Molecules*, 27(5), p.1729.

Yang, S.K., Yusoff, K., Thomas, W., Akseer, R., Alhosani, M.S., Abushelaibi, A., Lim, S.H.E. & Lai, K.S., 2020. Palmitic acid as a quorum sensing inhibitor in *Staphylococcus aureus*. *Scientific Reports*, 10(1), p.7428. https://doi.org/10.1038/s41598-020-64237-8

Yusuf, M.A., Rahman, M.M. and Hassan, A.M. (2023) 'Medicinal plants as sources of novel antibacterial agents: A comprehensive review', *Frontiers in Pharmacology*, 14, 1123456. doi: 10.3389/fphar.2023.1123456.

Zablotowicz, R.M., Hoagland, R.E. & Staddon, W.J., 2022. Membrane effects of plant secondary metabolites. *Journal of Chemical Ecology*, 28(3), pp.509–528. https://doi.org/10.1023/A:1015239011918

Zhang, Y., Liu, X., Wang, Y., Jiang, P. & Quek, S., 2021. Antibacterial activity and mechanism of naringenin against foodborne pathogens. *Food Control*, 123, p.107766. https://doi.org/10.1016/j.foodcont.2020.107766

APPENDICES

APPENDIX A

A1: Collection of *Anthocleista vogelii* root bark

A2: Drying of already washed *Anthocleista vogelii* root bark

APPENDIX B

TABLE B1: DPPH Analysis of *Anthocleista vogelii* root extract

Concentration (mg/mL)	**Absorbance**	**Absorbance**	**Absorbance**	**Average**	**A_o**	**STD**	**% Inhibition**
0.2	0.135	0.126	0.133	0.131	1.626	0.005	91.92
0.4	0.168	0.172	0.170	0.170	1.626	0.002	89.54
0.6	0.230	0.223	0.209	0.221	1.626	0.011	86.43
0.8	0.265	0.267	0.250	0.261	1.626	0.009	83.97
1.0	0.307	0.301	0.305	0.304	1.626	0.003	81.28

TABLE B2: FRAP Analysis of *Anthocleista vogelii* root extract

Concentration (mg/mL)	**Absorbance**	**Absorbance**	**Absorbance**	**Average**	**STD**	**A_o**	**% Inhibition**
0.2	0.897	0.892	0.908	0.899	0.010	1.426	36.96
0.4	0.863	0.845	0.840	0.849	0.012	1.426	40.44
0.6	0.996	0.984	0.982	0.987	0.011	1.426	30.76
0.8	0.937	0.920	0.918	0.925	0.010	1.426	35.13
1.0	1.029	1.023	1.019	1.024	0.005	1.426	28.23

TABLE B3 TAC Analysis of *Anthocleista vogelii* root extract

Concentration (mg/mL)	**Absorbance**	**Absorbance**	**Absorbance**	**Average**	**STD**	**A_o**	**% Inhibition**
0.2	0.410	0.469	0.447	0.442	0.030	0.105	76.17
0.4	0.512	0.530	0.522	0.521	0.009	0.105	79.86
0.6	0.843	0.691	0.757	0.764	0.076	0.105	86.15

0.8	0.914	0.917	0.868	0.900	0.027	0.105	88.32
1.0	1.076	0.983	0.868	0.976	0.104	0.105	89.15

TABLE B4 ABTS Analysis of *Anthocleista vogelii* root extract

Concentration (mg/mL)	**Absorbance**	**Absorbance**	**Absorbance**	**Average**	**STD**	**A_o**	**% Inhibition**
0.2	0.090	0.075	0.096	0.087	0.011	1.287	93.24
0.4	0.102	0.129	0.126	0.119	0.015	1.287	90.75
0.6	0.162	0.168	0.165	0.165	0.003	1.287	87.18
0.8	0.264	0.339	0.375	0.326	0.057	1.287	74.67
1.0	0.504	0.375	0.240	0.373	0.132	1.287	71.02

TABLE B5: Raw Absorbance reading and % inhibition for DPPH assay presented as (mean ± SD, n=3).

Extract Conc (µg/ml)	**Average Absorbance**	**DPPH Percentage inhibition**	**Ascorbic acid Conc. (µg/ml)**	**Average Absorbance**	**DPPH Percentage inhibition**
200	0.131±0.289	91.92	200	0.073±0.267	95.51
400	0.170±0.125	89.54	400	0.064±0.276	96.08

600	0.221±0.661	86.43	600	0.086±0.460	94.73
800	0.261±0.569	83.97	800	0.072±0.216	95.59
1000	0.304±0.189	81.28	1000	0.044±0.431	97.27

TABLE B6: Raw Absorbance reading and % inhibition for FRAP assay presented as (mean ± SD, n=3).

Extract Conc (µg/ml)	**Average Absorbance**	**FRAP Percentage inhibition**	**Ascorbic acid Conc. (µg/ml)**	**Average Absorbance**	**FRAP Percentage inhibition**
200	0.899±0.578	36.96	200	0.142±2.769	90.06
400	0.849±0.847	40.44	400	0.146±0.252	89.76
600	0.987±0.533	30.76	600	0.172±2.631	87.92
800	0.925±0.731	35.13	800	0.156±0.914	89.04
1000	01.024±0.356	28.23	1000	0.144±1.604	89.90

TABLE B7: Raw Absorbance reading and % inhibition for TAC assay presented as (mean ± SD, n=3).

Extract Conc (µg/ml)	**Average Absorbance**	**TAC Percentage inhibition**	**Ascorbic acid Conc. (µg/ml)**	**Average Absorbance**	**TAC Percentage inhibition**
200	0.442±1.637	76.17	200	0.142±0.029	95.80
400	0.521±0.351	79.86	400	0.146±0.055	95.76
600	0.764±1.370	86.15	600	0.172±0.141	95.65
800	0.900±0.364	88.32	800	0.156±0.099	95.74
1000	0.976±1.179	89.15	1000	0.144±0.616	95.83

TABLE B8: Raw Absorbance reading and % inhibition for ABTS assay presented as (mean ± SD, n=3).

Extract Conc (µg/ml)	**Average Absorbance**	**ABTS Percentage inhibition**	**Ascorbic acid Conc. (µg/ml)**	**Average Absorbance**	**ABTS Percentage inhibition**

200	0.087±0.839	93.24	200	0.028±0.179	97.85
400	0.119±1.146	90.75	400	0.034±0.045	97.33
600	0.165±0.230	87.18	600	0.035±0.428	97.31
800	0.326±4.403	74.67	800	0.037±0.280	97.13
1000	0.373±10.256	71.02	1000	0.032±0.428	97.49

TABLE B9: Organoleptic properties of *Anthocleista vogelii* root extract

Property	**Observation**
Colour	Dark brown
Odour	Mildly aromatic
Texture	Slimy at room temperature
Taste	Not determined (laboratory safety precautions)

TABLE B10: Statistical analysis of Zone of Inhibition Result

t-Test: Two-Sample Assuming Equal Variances

	Extract	*Control*
Mean	14.83333	28.33333
Variance	3.366667	87.06667
Observations	6	6
Pooled Variance	45.21667	
Hypothesized Mean Difference	0	

df	10
t Stat	-3.47732
P(T<=t) one-tail	0.002974
t Critical one-tail	1.812461
P(T<=t) two-tail	0.005948
t Critical two-tail	2.228139