GCMS PROFILE OF ACETONE FRACTION OF *LONCHOCARPUS CYANESCENS*

BY

DAOMI DANIEL

BMS2101385

DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF MEDICAL SCIENCE

COLLEGE OF MEDICAL SCIENCE

UNIVERSITY OF BENIN

NOVEMBER, 2025

GCMS PROFILE OF ACETONE FRACTION OF *LONCHOCARPUS CYANESCENS*

BY

DAOMI DANIEL

BMS2101385

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY

NOVEMBER, 2025

CERTIFICATION

We the undersigned hereby certify that DAOMI DANIEL (BMS2101385) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

Signed

....

Dr. (Mrs.) N. Eluehike **(Date)**

(Project Supervisor)

....

Dr. N.B. Aguebor-Ogie **(Date)**

(Head of Department)

DEDICATION

This project is dedicated to Almighty God, the giver of life who has made it possible to complete my Bachelor of Science Degree (B.Sc) program in the Department of Medical Biochemistry and my entire family for their tender care and love for me.

ACKNOWLEDGEMENT

My gratitude goes for Almighty God for his grace in all my endeavors, unto him is all the glory. My sincere appreciation goes to my amiable supervisor Dr. (Mrs.) N. Eluehike, alongside the Head of Department, Dr. N. B. Aguebor-Ogie, as well as other lecturers in the department for their words of wisdom and encouragement.

ABSTRACT

Lonchocarpus cyanescens (Fabaceae), commonly known as Yoruba Indigo, is a medicinal plant widely utilized in West African ethnomedicine to treat skin infections, ulcers, and inflammatory conditions. This study aimed to characterize the phytochemical constituents of the acetone fraction of L. cyanescens leaves using Gas Chromatography-Mass Spectrometry (GC-MS) to provide a scientific basis for its traditional uses. Dried and powdered leaves were subjected to ethanolic maceration and sequential solvent partitioning to isolate the acetone fraction. Constituents were then identified by comparing their mass spectra against the NIST14 library. The GC-MS analysis led to the tentative identification of 40 distinct compounds, with many key components showing high spectral match quality scores (>80). The chemical profile was predominantly composed of aromatic hydrocarbons, with Benzene, 1,2,4-trimethyl- (19.05%) being the most abundant constituent. Other major components included various fatty acid methyl esters (FAMEs), such as Dodecanoic acid, methyl ester (5.18%) and 9-Octadecenoic acid (Z)-, methyl ester (4.18%). Biologically relevant minor compounds, including the monoterpene o-Cymene and the anti-inflammatory sesquiterpene Azulene, were also detected. These findings provide a chemical basis for the plant's traditional therapeutic uses and establish a valuable phytochemical fingerprint for future quality control and pharmacological research.

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Natural products remain a major source of pharmaceutical agents due to their rich chemical diversity and biological activity (Elshafie *et al.,* 2023). Many modern drugs originate from plant-derived phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, which possess antioxidant, anti-inflammatory, antimicrobial, and antitumor properties (Singh *et al.,* 2024; Shrivastava & Mishra, 2019). However, the synergistic nature of plant extracts often complicates the identification of specific active molecules, highlighting the need for detailed profiling of lesser-studied medicinal plants (Vaou *et al.,* 2022).

Lonchocarpus cyanescens, commonly known as *Yoruba Indigo* or *Iyarin*, is a perennial shrub of the Fabaceae family widely distributed in West Africa. Traditionally, its leaves are used to produce the culturally significant *adire* indigo dye (Amit & Colón, 2023), while various plant parts serve in ethnomedicine for treating skin infections, ulcers, leprosy, dysentery, and inflammatory disorders (Oladeji *et al.,* 2020; Peter *et al.,* 2024). Recent studies have reported its anti-inflammatory and aldose reductase-inhibitory effects, suggesting potential in diabetic and arthritic therapies. With increasing global demand for natural dyes and herbal remedies, *L. cyanescens* presents both economic and pharmacological relevance (Wouyou *et al.,* 2025).

To harness this potential, phytochemical characterization is essential for standardizing herbal formulations, validating traditional claims, and identifying novel therapeutic compounds (Iyoha *et al.,* 2025; Ogbuagu *et al.,* 2022). Among available analytical tools, Gas Chromatography–Mass

Spectrometry (GC-MS) remains one of the most powerful techniques for detecting volatile and semi-volatile bioactives such as terpenes and phenolics (Chiaia *et al.,* 2025). GC-MS has previously revealed secondary metabolites in *L. cyanescens* linked to antimicrobial and anti-inflammatory activity. Beyond compound identification, GC-MS supports quality control by detecting adulterants and ensuring consistency in herbal formulations (Salahshour *et al.,* 2020; Zhang *et al.,* 2023). Despite its ethnomedicinal prominence, comprehensive GC-MS profiling of specific solvent fractions of *L. cyanescens* remains limited, particularly for the acetone fraction.

1.2 Aim of the Study

The aim of this study is to determine the phytochemical constituents of the acetone fraction of *Lonchocarpus cyanescens* leaves using Gas Chromatography-Mass Spectrometry (GC-MS).

CHAPTER TWO

LITERATURE REVIEW

2.1 *Lonchocarpus cyanescens*

2.1.1 Botanical Classification and Distribution

Lonchocarpus cyanescens (Schumach. & Thonn.) Benth is a key medicinal plant belonging to the Fabaceae family, specifically classified under the Dalbergiae tribe of the Leguminosae order (Iyoha *et al.,* 2025). It is also known by the synonym Philenoptera cyanescens (Schumach. & Thonn.) Roberty (Moronkola and Oladosu, 2013).

2.1.1.1 Scientific Classification of *Lonchocarpus cyanescens*

Table 2.1 Scientific Classification of *L. cyanescens*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	Lonchocarpus
Species	cyanescens

Synonyms: Lonchocarpus cyaneus (Schumach. & Thonn.) Benth., Macrolobium cyanescens Schumach. & Thonn., Philenoptera cyanescens (Schumach. & Thonn.) Roberty (NMPPDB, 2025)

2.1.2 Morphological and Anatomical Details

The plant is generally described as a deciduous scandent shrub. Macroscopic examination reveals that the leaf shape is lanceolate, the margin is entire, the surface is glabrous, and the midrib is prominent with a papery texture (Moronkola and Oladosu, 2013; Adeku *et al.,* 2022).

Figure 2.1: Image of *Lonchocarpus cyanescens* plant leaves (NMPPDB*,* 2025)

Pharmacobotanical studies focusing on quality control are essential to prevent misidentification in traditional medicine (Adeku *et al.,* 2022). Microscopic features that aid in the identification of *L. cyanescens* include:

- **Stomata Presence**: Stomata are present on both the adaxial and abaxial surfaces (amphistomatic). Specifically, Paracytic stomata are observed on the adaxial surface, while Anisocytic stomata are found on the abaxial surface (Adeku *et al.,* 2022).
- **Epidermal Cells**: Epidermal cells on the adaxial surface possess wavy/undulating anticlinal walls. The epidermal cells on the abaxial surface are smooth to slightly wavy (Adeku *et al.,* 2022).
- **Trichomes**: The adaxial surface bears unicellular trichomes, while the abaxial surface possesses multicellular, uniseriate trichomes. Importantly, preliminary studies noted the relative absence of numerous trichomes in L. cyanescens compared to other related species (Adeku *et al.,* 2022).
- **Internal Structure**: The transverse section of the leaf appears concave on the abaxial surface and convex on the adaxial surface. The cuticle is moderately thick, and the vascular bundle is arc-shaped (Adeku *et al.,* 2022).
- **Chemical Markers:** Preliminary phytochemical screening revealed that the powdered leaves contain key secondary metabolites, including alkaloids, anthraquinones, cardiac glycosides, tannins, saponins, steroids, and flavonoids (Adeku *et al.,* 2022).

2.1.3 Distribution and Physicochemical Constants

L. cyanescens is native to West Africa and is cultivated extensively across countries such as Nigeria, Ghana, Cameroon, Ivory Coast, Togo, Sierra Leone, Benin, and Guinea (Karlina *et al.,* 2025; Amit and Colon, 2023). The plant thrives particularly in coastal and forested regions (Karlina *et al.,* 2025).

In standardization efforts, the powdered leaves of L. cyanescens showed measurable physicochemical constants, including a moisture content of 14.000 ± 2.800 and a total ash content of 14.500 ± 2.100 (Adeku *et al.,* 2022).

2.1.4 Ethnobotanical Uses

1. **Indigo Dye (Adire)**

The plant is widely recognized as the African Tarum plant or West African Indigo (Karlina *et al.,* 2025; Amit and Colon, 2023). Its historical use as a dye source dates back to the 11th century for textiles, such as the Yoruba adire cloth (NMPPDB, 2025). The young tender leaves and flowers are traditionally used to produce the dye (Baa-Poku and Enu-kwEsi, 2016).

2. **Traditional Dye Production and Chemical Identity Debate**

The dye extracted from *L. cyanescens* is conventionally referred to as indigo (Moronkola and Oladosu, 2013). Traditionally, the leaves are believed to contain indoxyl, which yields indigotin, the compound present in indigo dyestuff (Moronkola and Oladosu, 2013). The customary process involves pounding the leaves and flowers, soaking them in a pot with a white cloth, which imparts a blue colour after approximately three to five days (Baa-Poku and Enu-kwEsi, 2016).

However, the identity of the dye component has been subject to modern scientific scrutiny. One study using UHPLC-HRMS analysis surprisingly suggested that the probable dye component responsible for the blue coloration was gentian violet (a triphenylmethane synthetic dye, also known as crystal violet) (Amit and Colon, 2023). This conclusion was based on the dye extract's UV-Vis spectrum showing an absorbance maximum at 590 nm, which is comparable to that of crystal violet, and a high-confidence match with library data (Amit and Colon, 2023). The authors noted that this finding suggests potential contamination or adulteration of the specific extract sample used in that analysis, as gentian violet is unlikely to be natural to the plant (Amit and Colon, 2023).

3. Alternative Dye Application (Histology)

Beyond textile dyeing, L. cyanescens has been investigated as a source for natural dyes in histology, potentially serving as an alternative to hematoxylin (Karlina *et al.,* 2025). The dye derived from L. cyanescens is predominantly blue (Karlina *et al.,* 2025). It exhibits a strong binding affinity for cells and tissues, particularly for testicular tissue. Research attributes the blue staining observed in testicular tissue primarily to the presence of tannins and flavonoids. Optimal staining results on testicular tissue were achieved with a staining time of 5 minutes (Karlina *et al.,* 2025).

4. Non-Dye Uses

In addition to its role as a dye, the plant’s hard, durable timber is utilized for carpentry and construction in rural communities. The species also contributes to agroforestry by enhancing soil fertility through nitrogen fixation (Moronkola and Oladosu, 2013; NMPPDB, 2025).

2.1.5 Ethnomedicinal Applications

L. cyanescens is utilized in African ethnomedicine to address various disease conditions, a practice substantiated by its rich phytochemical profile (Adeku *et al.,* 2022).

1. **Phytochemical Basis**

Phytochemical screening confirms the presence of saponins, tannins, steroids, terpenoids, cardiac glycosides, phlobatannins, and flavonoids in the root, stem, and leaf methanol and hexane fractions (Sonibare *et al.,* 2014; Moronkola and Oladosu, 2013). These constituents are believed to be responsible for the plant's acclaimed biological activities, including anti-inflammatory, anti-arthritic, anti-microbial, and anti-ulcer effects. Furthermore, chemical analyses of the leaf extract have specifically yielded two triterpenoids (Moronkola and Oladosu, 2013).

2. **Anti-inflammatory, Anti-Arthritic, and Anti-Ulcer Effects**

The roots of L. cyanescens are traditionally used to treat arthritis (Amit and Colon, 2023). Scientific literature reports that oleanane derivatives and glycyrrhetinic acid are the components responsible for the observed anti-inflammatory properties and the relief of peptic ulcers. Triterpenes found in the plant are noted for acting against arthritis. Moreover, an aqueous root extract was investigated and showed proven antiulcer and analgesic effects in rats, providing scientific support for this traditional use (Amit and Colon, 2023).

3. **Antioxidant Activity and Related Ailments**

The extract exhibits significant antioxidant and free radical scavenging activities. Studies using the DPPH and Ferric Reducing Antioxidant Power (FRAP) assays demonstrated that the antioxidant capacity is concentration-dependent (Samuel and Adaramoye, 2014).

The observed activity correlates strongly with the total phenolic and total flavonoid content (with correlation coefficients exceeding 0.99 using the DPPH method) (Samuel and Adaramoye, 2014).

The most active antioxidant fraction identified through bioassay-guided fractionation (Fraction F5) indicated the presence of flavonoids based on spectroscopic analysis (IR and ^{1}H NMR) (Samuel and Adaramoye, 2014).

This confirmed antioxidant property provides a scientific explanation for the plant's local use in treating pathophysiological conditions associated with oxidative stress, such as ulcer, arthritis, and neurodegenerative disorders.

4. Central Nervous System (CNS) Applications

L. cyanescens is a crucial component in traditional Nigerian formulations used to treat psychosis. Ethnobotanical surveys list it among plants utilized for mental illnesses in Nigeria (Isayaka *et al.,* 2025). Studies conducted in rodents have validated the antipsychotic property of both aqueous and ethanolic extracts (Amit and Colon, 2023).

Furthermore, an untargeted screening approach suggested the possible presence of bioactive molecules structurally similar to known drugs, potentially accounting for the traditional psychoactive or analgesic uses (Amit and Colon, 2023). These proposed candidates include:

- Palmitoylethanolamide (PEA): Known for analgesic, anti-inflammatory, and psychoactive effects.
- Lysergol: Recognized for hypotensive, psycho-tropic analgesic, and uterus- and intestine-stimulating properties.
- Cathinone: Associated with psychoactive properties.

Other Medicinal Uses

- **Antidiabetic Potential**: Leaf extracts have demonstrated hypoglycaemic potential by inhibiting diabetes-related enzymes, such as α-amylase and α-glucosidase (Desta *et al.,* 2022).
- **Insects and Pests**: The root powder possesses insecticidal properties used to control *Sitophilus zeamais* in maize and wheat storage. It has also been identified as an anti-malaria herb in Southwestern Nigerian ethno-medicine (Amit and Colon, 2023).
- **Reproductive and Internal Health**: A decoction of the root and stem is traditionally administered to women during or after childbirth, and is used to treat hernia. The plant is also listed for treating venereal diseases and possesses anti-tussive effects (Moronkola and Oladosu, 2013).

2.2 Phytochemistry of *L. cyanescens*

2.2.1 Known Classes of Compounds (Flavonoids, Terpenoids, Alkaloids)

The genus *Lonchocarpus* (Fabaceae, Papilionoideae) is scientifically recognized as a rich source of polyphenolic compounds (Isayaka *et al.,* 2025). These compounds are crucial secondary metabolites, which are involved in structural roles, defense mechanisms, and UV protection in plants (Isayaka *et al.,* 2025). Phytochemical investigations specifically focusing on *L. cyanescens* have consistently identified three key classes of compounds contributing to its traditional ethnomedicinal activities, the compounds are: Flavonoids, Terpenoids (including steroids), and Alkaloids.

A. Flavonoids

Flavonoids are polyphenolic compounds confirmed to be present in various parts of *L. cyanescens*. Flavonoids were detected in the powdered leaves of *L. cyanescens* (Sonibare *et al.,* 2014), the ethanol extract of the root, the leaf residue fraction, and in both aqueous and methanolic leaf extracts (Ajani *et al.,* 2017; Moronkola and Oladosu, 2013). The antioxidant activity of the leaf extract is highly associated with its flavonoid content (Samuel *et al.,* 2014). Strong correlations were observed between the total flavonoid content (TFC) and antioxidant activities measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (r^2 = 0.9926) and the Ferric Reducing Antioxidant Power (FRAP) method (r^2 = 0.8840) (Samuel *et al.,* 2014). The DPPH and FRAP assays showed that the crude *L. cyanescens* extract possessed antioxidant activity comparable to the standard antioxidant, catechin (Samuel *et al.,* 2014). Bioactivity-guided fractionation of the acetone leaf extract identified fraction F5 as the most potent fraction for antioxidant activity. Preliminary analysis using ^{1}H and Infra-red (IR) spectroscopy indicated the presence of flavonoid compounds within this highly active fraction (Samuel *et al.,* 2014). The broader *Lonchocarpus* genus is known to produce a diversity of flavonoids, including chalcones, flavans, aurones, pterocarpans, flavanols, flavones, isoflavones, and flavonols (Isayaka *et al.,* 2025). These flavonoids frequently contain distinctive substitutions, such as the prenyl (3-methyl-2-butenyl) group or dimethylpyrano group linkages (Isayaka *et al.,* 2025).

B. Terpenoids

Terpenoids, specifically triterpenoids and related steroid compounds, are highlighted for their pharmacological significance in *L. cyanescens*. Terpenoids were detected through phytochemical screening of cold methanol extracts of the leaf, stem, and root (Moronkola and Oladosu, 2013). Steroids were also identified in the powdered leaves (Sonibare *et al.,*2014). Gas Chromatography

(GC) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the volatile oils provided specific data on terpenoid components: The leaf essential oil, obtained at a yield of 0.03%, was dominated by the alcohol Phytol (62.5%), along with hexadecanoic acid (12.4%) (Moronkola and Oladosun, 2013). Alcohols made up 64.1% of the leaf oil composition. Two white crystalline triterpenoids were isolated from the ethyl acetate fraction obtained after partitioning the methanol leaf extract. Their structures were characterized using multiple spectroscopic techniques, including Infra-Red spectra, Electrospray Ionization (ESI) mass spectrometry, ^{1}H-NMR, and ^{13}C-NMR analyses (Moronkola and Oladosun, 2013). Known triterpenoids linked to the activity of *L. cyanescens* include:

- Lupeol (a triterpene) has been reported in *L. cyanescens*.
- Oleanane derivatives and glycyrrhetinic acid (a pentacyclic triterpenoid) are responsible for the claimed anti-inflammatory properties and the relief of peptic ulcers associated with the plant.

Molecular docking simulations confirmed that *L. cyanescens* triterpenoids act as effective ligands for ulcer treatment. The OH derivative ligand showed a high binding affinity (E_i = -7.2 kcal/mol) and inhibition constant (K_i = 5.21μM) against the 1AFC ulcer target receptor (Adejoro *et al.,* 2017).

C. **Alkaloids**

Alkaloids are a class of nitrogen-containing compounds detected primarily in the more polar extracts of the plant. Alkaloids were identified in the powdered leaves, the ethanol extract of the root, and the aqueous bark extract (where they were slightly present) (Oribhabor and Akanse, 2020). In studies comparing leaf extracts, alkaloids were detected in the aqueous extract but were notably absent in the methanolic extract, suggesting highly polar solvents are required for their

extraction (Ajani *et al.,* 2017). The presence of alkaloids, alongside saponins and oxalates, in the bark aqueous extract contributes to the extract's piscicidal effects (fish-poisoning activity) (Oribhabor and Akanse, 2020). Untargeted screening of the leaf extract using Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS) proposed the probable presence of alkaloids such as Lysergol and Cathinone. Lysergol is known for hypotensive, analgesic, and psychotropic effects, while Cathinone possesses psychoactive properties (Amit and Colon, 2023).

2.3 Phytochemical Studies on *L. cyanescens*

Phytochemical studies on *L. cyanescens* have employed standard chemical screening methods and advanced analytical techniques to correlate traditional uses with molecular composition.

2.3.1Qualitative Phytochemical Screening Results

Multiple studies across different plant parts confirm a broad array of metabolites:

Root Extracts: The ethanol extract of the roots confirmed the presence of saponin, alkaloid, flavonoids, and tannins. These constituents are considered responsible for the root's anti-inflammatory and analgesic effects observed in mice (Umoh and Nwafor, 2013).

Methanol Extracts (Stem, Leaf, Root): Comprehensive cold methanol extraction revealed seven major classes of secondary metabolites: saponin, tannin, steroid, terpenoid, cardiac glycoside, phlobatannins, and flavonoids.

Leaf Extracts (Polarity Comparison): A detailed study highlighted the solvent-dependent nature of metabolite extraction:

- Phenol, flavonoid, tannins, and phlobatannins were detected in both aqueous and methanolic leaf extracts (Ajani *et al.,* 2017).
- Saponins, alkaloids, cardiac glycosides, and quinones were exclusively found in the aqueous (more polar) extract (Ajani *et al.,* 2017).

Bark Extracts: The aqueous bark extract, used traditionally as a fish poison, contains oxalates (copiously present), saponins (moderately present), and slight amounts of alkaloids, flavonoids, glycosides, and phytates (Oribhabor and Akanse, 2020).

2.3.2 Quantitative and Structural Characterization Studies

Triterpenoid Isolation and Characterization: Two triterpenoids were isolated as white crystalline solids from the leaf ethyl acetate fraction. Their structures (identified as 1, R=H and 2, R=CH3) were characterized by spectroscopic methods including IR, ESI-MS, ^{1}H-NMR, and ^{13}C-NMR (Moronkola and Oladosun, 2013).

Volatile Oil Analysis: GC-MS determined the volatile oil composition of the leaf and stem. The leaf oil yield was 0.03%, dominated by Phytol (62.5%). The stem oil yield was 0.17%, predominantly composed of octadecenoic acid (24.1%) and hexadecanoic acid (17.2%). The leaf oil composition contained 64.1% alcohols, while the stem oil contained 49.6% acids (Moronkola and Oladosun, 2013).

Bioassay-Guided Fractionation (Antioxidant): Acetone leaf extracts showed antioxidant activity comparable to catechin. This activity was strongly correlated with Total Phenolic Content (TPC, $r^2 = 0.9906$) and Total Flavonoid Content (TFC, $r^2 = 0.9926$). Fraction 5 (F5) displayed the highest antioxidant effect in the DPPH method and contained flavonoids based on spectroscopic profile (Samuel *et al.,* 2014; Samuel *et al.,* 2014).

Enzyme Inhibition (Aldose Reductase): Fractions of *L. cyanescens* leaf extract were investigated for their potential to inhibit Aldose Reductase (AR), an enzyme linked to diabetic complications like cataracts (Ajani *et al.,* 2017). All tested fractions showed significant AR inhibition. The aqueous fraction exhibited the strongest activity (IC_{50}, 0.06 ± 0.02 mM^{-1}). Kinetic analysis showed that the ethyl acetate, hexane, and methanol fractions demonstrated competitive inhibition, while the aqueous and chloroform fractions showed mixed inhibition. The authors suggested this anticataract effect is due to the plant's phenolic constituents (Ajani *et al.,* 2017).

Untargeted LC-HRMS Screening: A high-resolution mass spectrometry study identified several potential bioactive secondary metabolites in the leaf extract, including Citral, Palmitoylethanolamide (PEA), Azaperol, Lysergol, Cathinone, and Methyl-4-Boc-piperazine-2-acetate. This advanced technique also proposed Gentian violet ($C_{25}H_{29}N_3$) as the most probable dye component based on accurate mass and fragmentation patterns, though the presence of this synthetic dye may suggest potential contamination in the sample, requiring further corroboration (Amit and Colon, 2023).

2.4 Pharmacological Properties of *L. cyanescens*

Lonchocarpus cyanescens (West Africa indigo) is a deciduous scandent shrub whose traditional use in Africa spans several ailments, including skin diseases, ulcers, leprosy, intestinal issues, diarrhea, venereal diseases, and arthritis (Samuel *et al.,* 2014; Ajani *et al.,* 2017). The reported bioactivity of *L. cyanescens* includes anti-inflammatory, anti-arthritic, and ulcer-relieving effects (Isayaka *et al.,* 2025). Phytochemical screening of the leaves and stem bark reveals the presence of several bioactive constituents, such as phenols, flavonoids, tannins, saponins, and alkaloids.

These chemical components are believed to underpin the plant's diverse pharmacological applications (Ajani *et al.,* 2017).

2.4.1 Anti-inflammatory and Antioxidant Activity

L. cyanescens has been empirically studied and validated for its anti-inflammatory and antioxidant properties, supporting its long-standing use in traditional medicine for conditions like arthritis and ulcers (Adejoro *et al.,* 2017).

Anti-inflammatory Activity

The root of *L. cyanescens* is a common remedy in Nigeria for treating pain and inflammatory disorders (Umoh and Nwafor, 2013). Studies using ethanol extracts and fractions of the root in mice models confirmed marked anti-inflammatory and analgesic effects (Umoh and Nwafor, 2013).

In studies investigating acute inflammation:

The extract dose-dependently demonstrated significant anti-inflammatory effects against carrageenan-induced oedema in mice, reaching maximum suppression after four hours of pretreatment. This effect was comparable to that of acetyl salicylic acid (ASA, 100mg/kg), a known cyclo-oxygenase inhibitor, suggesting a systemic anti-inflammatory effect (Umoh and Nwafor, 2013).

The extract suppressed acute oedema induced by topical administration of xylene, indicating an anti-phlogistic effect, which supports topical use where anti-inflammatory action is needed.

Among the partitioned fractions of the root extract, the butanol fraction exhibited the highest anti-inflammatory activity in inhibiting paw oedema caused by carrageenan (Umoh and Nwafor, 2013).

The presence of bioactive constituents such as flavonoids and tannins in *L. cyanescens* root extracts is implicated in the observed anti-inflammatory effects. Furthermore, molecular docking studies involving triterpenoids of *L. cyanescens* suggest an inhibitory activity against ulcer-related proteins (1AFC, 1AXM, and 2AXM), which aligns with the plant's traditional use for relieving ulcers (Adejoro *et al.,* 2017).

Antioxidant Activity

Antioxidants are crucial for preventing oxidative damage caused by free radicals, which are implicated in degenerative diseases like cancer, ulcer, arthritis, and inflammatory conditions. *L. cyanescens* extracts possess significant antioxidant capacity, which is linked to its phenolic and flavonoid content (Samuel *et al.,* 2014).

Key findings regarding antioxidant activity in leaf extracts include:

The antioxidant activity, measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging and FRAP (Ferric Reducing Antioxidant Power) methods, was concentration-dependent and compared favorably to the standard antioxidant catechin (Samuel *et al.,* 2014).

A strong correlation was observed between the antioxidant activity (DPPH method) and the total phenol and flavonoid contents, with correlation coefficients of 0.9906 and 0.9926, respectively (Adejoro *et al.,* 2017).

The aqueous fraction showed particularly potent antioxidant activity, exhibiting the strongest DPPH scavenging activity ($IC_{50,}$ 0.050 ±0.009mg/ml), highest metal chelating activity (88.6%), greatest reducing power ($IC_{50,}$ 0.211 ± 0.003mg/ml), and lowest hydroxyl radical scavenging IC_{50} (0.664 ± 0.039mg/ml) (Ajani *et al.,* 2017).

In one study, the ethyl acetate fraction of *L. cyanescens* (LC) exhibited the highest overall antioxidant activity across several assays, including metal chelating ability *(IC_{50}*, 0.673 ± 0.061 g/ml*)* and DPPH scavenging (IC_{50} 0.245 ± 0.007 g/ml) (Falade *et al.,* 2025).

Preliminary spectroscopic investigations confirmed that the most active fraction (F5) contained essentially phyto-phenolic compounds (flavonoids) (Samuel *et al.,* 2014)**.**

In safety studies on normal Wistar albino rats, chronic administration of aqueous and methanol extracts of *L. cyanescens* leaves (200 mg/kg body weight for 12 weeks) did not significantly alter activities of endogenous antioxidant enzymes (catalase, SOD, GPx) or concentrations of reduced glutathione (GSH) and malondialdehyde (MDA), suggesting the extracts maintain the oxidative status of normal rats without inducing oxidative stress (Iyoha *et al.,* 2023).

2.4.2 Antimicrobial Properties

L. cyanescens has been reported in literature to possess antiviral, antifungal, anti-protozoa, and antibacterial pharmacological properties.

Antibacterial and Antifungal Activity: The stem bark extracts (aqueous, methanolic, and chloroform) were screened for antimicrobial sensitivity against several human pathogens. The crude extracts demonstrated sensitivity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*, with activity observed at a concentration as low as 1.0×10^{-4}mg/ml (Nwokonkwo *et al.,* 2017).

The chloroform extract (SCE) was noted to be more potent against *E. coli* and *P. aeruginosa* compared to the positive control (Amoxicillin) at the corresponding concentrations used.

The aqueous extract (SAE) showed potential as an antibacterial agent against *S. typhi*.

The extracts did not inhibit the growth of *Klebsiella pneumonia* and *Streptococcus pneumonia* (Nwokonkwo *et al.,* 2017).

Antiprotozoal Activity (related species): Studies on flavonoids isolated from other *Lonchocarpus* species, specifically *L. xuul* and *L. yucatanensis*, demonstrated promising antiprotozoal and cytotoxic activities (Larque *et al.,* 2024). Flavone 6 and chalcone 7 were highly active against *Leishmania* parasites and against Leukemia P388DI and adenocarcinoma prostate PC-3 cell cultures (Horta *et al.,* 2023). Flavan 3 exhibited significant antiplasmodial activity against *Plasmodium falciparum* (Sabri *et al.,* 2025).

The antiprotozoal activity observed in these related metabolites often correlated with a significant cytotoxic effect, suggesting a general antiproliferative mechanism.

2.4.3 Other Reported Bioactivities

Aldose Reductase Inhibition (ARI)

ARI studies focus on discovering effective agents against cataracts, a common secondary complication of diabetes, mediated by the polyol pathway and the aldose reductase enzyme (Li *et al.,* 2025). *L. cyanescens* leaves were investigated for their *in vitro* ARI activity using partially purified aldose reductase (AR) from goat lens (Ajani *et al.,* 2017).

All tested fractions demonstrated significant inhibition of AR activity.

The aqueous fraction (IC_{50} 0.06 ± 0.02 mg/ml) and the methanolic fraction *(IC_{50}* 0.09 ± 0.01mg/ml) showed the strongest ARI capacity. Kinetic studies revealed that the ethyl acetate, hexane, and methanol fractions demonstrated competitive inhibition (Ajani *et al.,* 2017)**.**

The aqueous and chloroform fractions displayed mixed inhibition**,** characterized by significantly different V_{max} and K_m values compared to the substrate (DL-glyceraldehyde). The presence of phenols and flavonoids is suggested to be responsible for the ARI activity. These results confirm the potential of *L. cyanescens* for developing an anticataract agent (Ajani *et al.,* 2017).

Antidiabetic and Hypoglycemic Activity

The ethanol leaf extract of *L. cyanescens* has been reported to possess a hypoglycaemic effect, including activity in starch-loaded rats. One proposed mechanism is the inhibition of diabetes-related enzymes**, sp**ecifically pancreatic α-amylase and intestinal α-glucosidase (Desta *et al.,* 2022).

Analgesic Activity

The ethanol extract and fractions derived from the *L. cyanescens* root exhibited dose- and time-dependent analgesic properties in mice. The extract reduced pain induced chemically (acetic acid-induced writhing and formalin-induced hind paw licking) and thermally (hotplate-induced pain). The analgesic effects, comparable to Aspirin, were most prominent in the butanol fraction (Umoh and Nwafor, 2013)**.**

Antipsychotic Activity

L. cyanescens is traditionally used in Nigerian medicine to cure mental illnesses (psychosis). (Isyaka *et al.,* 2025). Aqueous and ethanolic extracts of *L. cyanescens* have demonstrated antipsychotic properties in rodents (Sonibare *et al.,* 2024). Furthermore, solvent-partitioned fractions of the leaf extract have shown antipsychotic activity in mice (Sonibare et al., 2024).

2.5 Principles of Gas Chromatography-Mass Spectrometry (GC-MS)

The analysis of natural products, particularly those derived from medicinal plants, necessitates powerful and reliable analytical techniques to delineate their complex chemical composition. Gas Chromatography-Mass Spectrometry (GC-MS) stands out as a fundamental tool in this field, integrating high separation capacity with robust structural identification capabilities (Ranjan *et al.,*2023).

2.5.1 Overview of the GC-MS Technique

Gas Chromatography-Mass Spectrometry (GC-MS) is an immensely potent and adaptable instrumental technique widely employed across Applied Sciences and Technology. This method achieves separation, identification, and quantification by synergizing two distinct analytical methodologies: gas chromatography (GC) and mass spectrometry (MS) (Ranjan *et al.,* 2023).

The Gas Chromatography (GC) component focuses on achieving the analytical separation of complex chemical mixtures into their individual components (Ranjan *et al.,* 2023). The separation process commences when the sample is introduced into the GC inlet port and instantaneously vaporized, typically without decomposition. The volatilized constituents are then carried through a high-efficiency column, often a capillary column, by an inert carrier gas (such as helium), where segregation occurs based on the chemical properties and volatilities of the components. GC methods are particularly effective for analyzing compounds whose boiling points are typically below 200ºC. Modern GC systems often feature temperature programming and controlled gas purging, which are crucial for resolving complex mixtures.

Once separated, the individual components exit the GC column and are introduced into the Mass Spectrometry (MS) component, which serves the function of identifying and quantifying the constituents. In the MS ion source, the sample molecules are ionized, often via electron ionization (EI). The mass spectrometer then produces a mass spectrum by measuring the abundance of ions according to their mass-to-charge ratio (*m/z*). GC instruments coupled with MS often employ a single quadrupole mass spectrometer, frequently denoted as GC-MS.

A critical aspect of the MS detection process involves comparing the generated mass spectra of unknown compounds against extensive library databases stored in the accompanying computer system. These reference libraries contain known mass spectra for a multitude of compounds. Identification is achieved by matching the fragmentation patterns and molecular weight data with these databases, complemented by retention time analysis. For instance, one GC-MS method utilizes a scan mass range from *m/z* 30 to 600, while another uses *m/z* 40 to 700 for scanning. Quantification in capillary GC analysis is determined by graphing peak areas, which reflects peak concentration.

GC-MS techniques are often regarded as the "gold standard" for analyzing volatile flavor and fragrance compounds. Recent methodological advances often involve combining GC-MS with other high-performance systems to enhance its capabilities. For example, methods such as Headspace Solid-Phase Microextraction (HS-SPME) are often paired with GC-MS for isolating volatile compounds prior to analysis, particularly in food volatilomics studies. Furthermore, two-dimensional gas chromatography-mass spectrometry (GC × GC-MS) offers advantages for the analysis of complex chemical mixtures.

2.5.2 Application of GC-MS in Medicinal Plant Profiling

GC-MS plays an essential role in natural product research, providing foundational data necessary for the scientific validation of traditional medicinal plants and accelerating the discovery of novel therapeutic compounds (Asmaey, 2024).

GC-MS profiling is highly advantageous for characterizing the volatile components of plant extracts, making it particularly useful for the analysis of essential oils. However, it is also effective for identifying various semi-volatile secondary metabolites present in extracts (de Melo Sacramento *et al.,* 2025). These metabolites often include large compound classes such as flavonoids, sesquiterpenes, alkaloids, and saponins, whose presence is frequently correlated with desired bioactivities such as anti-diabetic, antioxidant, and anti-inflammatory properties (Amakiri *et al.,* 2024). The technique is also used to facilitate the separation of specific fatty acids in biological samples, often requiring specialized high-polarity columns (Ranjan *et al.,* 2023).

A significant application of GC-MS in phytochemical analysis is dereplication, which refers to the rapid, early identification of known compounds present in a mixture, thereby saving time and resources that would otherwise be spent on traditional isolation and characterization (Odjobo *et al.,* 2020).

However, the suitability of GC-MS for natural products is constrained by the volatility and thermal stability of the molecules (Gaudencio *et al.,* 2023). Many natural products are not volatile enough for GC analysis, and the high operating temperatures (>300ºC in the inlet, column, or ion source) can cause thermally non-stable compounds to degrade or undergo structural rearrangement, potentially resulting in multi-peak phenomena (Odjobo *et al.,* 2020; Gaudencio *et al.,* 2023). When

analyzing non-volatile, highly polar extracts (such as methanol or aqueous extracts), derivatization is typically necessary to make the compounds amenable to GC conditions (Odjobo *et al.,* 2020).

Specific Examples of GC-MS Profiling:

- In anti-diabetic research, GC-MS has been used to characterize fractions of *Loranthus micranthus* methanol extract, correlating identified compounds (like 1,2,3-Propanetriol diacetate, Hexadecanoic acid methyl ester, and Squalene) with subsequent *in vivo* antidiabetic and antihyperlipidemic activities in rats (Channabasava *et al.,* 2015).
- The analysis of the aqueous leaf extract of *Vernonia amygdalina* utilized GC-MS to identify major bioactive constituents, including flavonoids and sesquiterpenes, which support the plant's traditional use in diabetes management due to their known antioxidant and anti-inflammatory roles (Amakiri *et al.,* 2024). Specific compounds identified included 3-Amino-6-phenyl-1H-pyrazolo[3,4-b]pyridine-4-carbonitrile, noted for its anti-inflammatory effects (Amakiri *et al.,* 2024).
- For seed oil extracts of *Citrullus colocynthis*, GC-MS identified about fifty-five bioactive compounds. Major compounds identified included Rhodoxanthin (a carotene) and Ceanothine C (an alkaloid) (Thamer and Thamer, 2023).
- GC-MS profiling of *Diplopterys pubipetala* leaves detected 25 volatile compounds, noting that the compound Ethyl hexadecanoate was found in all partitions and the crude extract, known for its antimicrobial, antioxidant, and flavor-enhancing functions (de Melo Sacramento *et al.,* 2025). The fresh leaves primarily contained 3-Hexen-1-ol (47.98% area).(de Melo Sacramento *et al.,* 2025)
- In the broader analytical field of volatilomics, GC-MS combined with chemometrics is widely used to ascertain the authentication, origin, and detection of adulteration in products

like meat, honey, coffee, olive oil, and spices, by identifying volatile organic compounds (VOCs) that serve as fingerprints (Kaldeli *et al.,* 2024). For example, SPME/GC-MS profiling is crucial for distinguishing honey based on its floral, entomological, and geographical origin (Kaldeli *et al.,* 2024).

2.6 Role of Solvent Fractionation in Phytochemical Analysis

The initial extraction step is essential for recovering and isolating bioactive phytochemicals from raw plant material (Gomathi *et al.,* 2015). Since plant extracts are intrinsically complex mixtures and often contain active ingredients at low concentrations, solvent fractionation is a crucial methodological step preceding detailed instrumental analysis like GC-MS (Zhang *et al.,* 2023).

Solvent Fractionation and Polarity:

Fractionation, often implemented through solvent-solvent partition or column chromatography, involves the systematic use of a series of solvents, typically arranged in increasing order of polarity, to separate the crude extract (Rumidatul *et al.,* 2021). This controlled separation process isolates and concentrates specific classes of compounds, making subsequent GC-MS analysis more feasible and targeted (Odjobo *et al.,* 2020).

The selection of the solvent directly dictates the quantity and chemical character of the isolated metabolites (Khan *et al.,* 2022; Ouerfelli *et al.,* 2022). Polar solvents, such as water and methanol, efficiently extract highly polar compounds like phenolic acids and flavonoids (Darwin *et al.,* 2025). Conversely, less polar solvents, like hexane, dichloromethane, or ethyl acetate, preferentially extract non-polar or moderately polar constituents, such as many terpenoids, essential oils, and lipids (de Melo Sacramento *et al.,* 2025; Al-Nuri *et al.,* 2022).

Fractionation as a Pre-Treatment for GC-MS:

Crude extracts, especially those obtained using highly polar solvents (e.g., methanol or aqueous extracts), frequently contain polymeric substances, polysaccharides, and highly polar components that are non-volatile under the typical GC run temperatures (which can range from 80ºC to 300ºC) (Odjobo *et al.,* 2020). Non-volatile substances can degrade the sample or interfere with GC-MS results, potentially causing column damage or poor baseline quality (Gaudencio *et al.,* 2023).

Therefore, fractionation serves several key roles:

Purification and Clean-Up: Fractionation using techniques like small column cleaning with silica gel or alumina helps clean the extract, removing non-volatile polymeric or highly polar substances, thus creating a "free-flowing extract" that improves GC-MS performance (Odjobo *et al.,* 2020).

Concentration: It concentrates less polar, volatile compounds into specific fractions (e.g., hexane fraction or dichloromethane fraction), making their detection and profiling by GC-MS easier (de Melo Sacramento *et al.,* 2025).

Targeted Bioactivity Study: Fractionation allows researchers to correlate the activity of a specific fraction with its refined phytochemical profile identified by GC-MS.

2.7 Role of GC-MS in Phytochemical Profiling

GC-MS analysis is essential for phytochemical characterization. It is a reliable, rapid, and sensitive technique, capable of both qualitatively and quantitatively identifying a wide array of organic compounds, requiring only a small volume of plant extract (Ishak *et al.,* 2024). GC-MS is particularly valued for the detection and identification of volatile and semi-volatile bioactives, including terpenes, phenolic compounds, fatty acids, and sterols (Ishak *et al.,* 2024). Furthermore,

GC-MS aids in establishing quality control standards by helping to detect adulterants and ensure consistency across herbal formulations.

2.8 Profiling the Acetone Fraction

Fractionation of crude extracts using solvents of varying polarity is a standard procedure in natural product research, starting typically from the least polar solvent and progressing towards the most polar (e.g., n-hexane, chloroform, acetone, methanol, water). Acetone is characterized as an intermediate polar solvent and holds high utility in the extraction process (Abubakar *et al.,* 2020).

Acetone extraction is known to readily dissolve non-polar components over highly polar substances (Lee *et al.,* 2024). It is specifically advantageous for extracting lipophilic compounds, such as vegetable oils, carotenoids, and phenolic compounds, which are key agents often associated with the antibacterial properties of herbal products (Barde *et al.,* 2025). Previous phytochemical analyses of acetone extracts from other plant species have successfully yielded high concentrations of significant compounds, including sesquiterpenoids/terpenoids like epiglobulol, and high percentages of triterpenoids such as alpha-amyrin (16.6644%) and beta-amyrin (4.6159%) (Barde *et al.,* 2025; Ishak *et al.,* 2024). Acetone extracts have demonstrated efficiency in isolating components exhibiting strong antioxidant and anti-inflammatory activities (Channabasava *et al.,* 2015). For example, the acetone fraction of *Guiera senegalensis* extract showed high concentrations of eupafolin, pyrogallol, hydroquinone, and catechol, correlating with high antioxidant and antibacterial activity (Satti *et al.,* 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and Apparatus

All equipment was calibrated and maintained according to manufacturer guidelines and standard laboratory protocols to ensure reproducibility (Harvey *et al.,* 2015). The following equipment and apparatus were utilized:

- Mechanical grinder (Model: IKA MF 10 basic, IKA Works, Germany).
- Rotary evaporator (Model: Heidolph Laborota 4000, Heidolph Instruments, Germany).
- Weighing balance (Model: Sartorius BP 211D, Sartorius AG, Germany).
- Soxhlet extractor.
- Vacuum filtration system (Whatman filter paper No. 1, GE Healthcare, USA).
- Gas Chromatograph-Mass Spectrometer (GC-MS; Model: Agilent 7890A GC coupled with 5975C MSD, Agilent Technologies, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness).
- Autosampler (Agilent 7683B) for sample injection.
- Ultrasonic bath.
- Desiccator.
- Voucher specimen storage at herbarium facilities.

3.1.2 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and procured from reputable suppliers. The following were employed:

- Ethanol.
- Acetone (99.5% purity, Merck, Germany) for fractionation.
- Hexane, chloroform, and ethyl acetate.
- Anhydrous sodium sulfate.
- Methanol.
- Helium gas (ultra-high purity, 99.999%, Air Liquide, Nigeria) as carrier gas for GC-MS.

3.2 Methods

3.2.1 Plant Material Collection and Authentication

Fresh leaves of *Lonchocarpus cyanescens* were collected from a local herb dealer in Lagos State, Nigeria, in August 2025. The plant material was authenticated by a taxonomist at the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria, and assigned voucher number UBHFO29. A specimen was deposited in the herbarium for reference. Collection adhered to ethical guidelines for sustainable harvesting, ensuring minimal environmental impact.

3.2.2 Preparation of Plant Material

The collected leaves were sorted to remove debris and washed with distilled water to eliminate surface contaminants. They were air-dried under shade at room temperature (25–30°C) for three

weeks to prevent degradation of thermolabile compounds, following standard drying protocols to achieve a constant weight (moisture content <10%) (Ajani *et al.,* 2016). The dried leaves (yield: approximately 500 g from 2 kg fresh) were then pulverized into a fine powder using a clean mechanical grinder. The powder was sieved (mesh size 40) for uniformity and stored in airtight polyethylene bags at room temperature until extraction to avoid moisture absorption and microbial contamination (Adejoro *et al.,* 2017).

3.2.3 Extraction and Fractionation

The powdered leaves (200 g) were subjected to maceration extraction using 95% ethanol (1:10 w/v ratio) at room temperature for 72 hours with occasional stirring to facilitate solvent penetration. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator to yield the crude ethanol extract (yield: 15–20% w/w). This method was chosen for its efficiency in extracting a broad spectrum of polar and semi-polar phytochemicals (Zhang *et al.,* 2023).

The crude extract (10 g) was then fractionated using a solvent partitioning method with increasing polarity to isolate the acetone fraction. The extract was suspended in distilled water (100 mL) and sequentially partitioned with hexane (3 × 100 mL), chloroform (3 × 100 mL), ethyl acetate (3 × 100 mL), and acetone (3 × 100 mL). Each fraction was dried over anhydrous sodium sulfate, filtered, and concentrated via rotary evaporation. The acetone fraction (yield: 2–3 g) was selected for analysis due to its mid-polarity, which targets semi-volatile bioactives like terpenoids and phenolics compatible with GC-MS (Amit & Colón, 2023; from bioassay-guided studies on *L. cyanescens*). Fractions were stored at -20°C in amber vials to prevent photodegradation until analysis.

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

The acetone fraction (1 mg) was dissolved in 1 mL HPLC-grade methanol, vortexed for 5 minutes, and filtered through a 0.45 μm PTFE syringe filter to prepare the sample. Analysis was performed using an Agilent 7890A GC system coupled with a 5975C MSD, following standard protocols for plant extract profiling (Harvey *et al.,* 2015; Zhang *et al.,* 2023).

Instrument parameters:

- Column: HP-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W).
- Injection: 1 μL splitless mode at 250°C injector temperature.
- Carrier Gas: Helium at 1.0 mL/min constant flow.
- Oven Temperature Program: Initial 50°C (hold 2 min), ramp 10°C/min to 280°C (hold 10 min); total run time ~35 min.
- Ion Source: Electron ionization (EI) at 70 eV, 230°C.
- Quadrupole: 150°C.
- Scan Mode: Full scan m/z 50–550.
- Data Acquisition: Agilent MassHunter software (version B.07.00).

Compounds were separated based on volatility and column interactions, vaporized in the hot inlet, carried by inert gas (helium), and detected in the MS. Retention times (RT) were recorded, and mass spectra were generated via fragmentation (mass-to-charge ratio, m/z, and relative abundance). Identification involved comparing spectra and RT with the NIST14 mass spectral library (minimum quality match: 70), supplemented by literature data for confirmation (Ajani *et*

al., 2016). Relative quantification was based on peak area percentages normalized to the total ion chromatogram (TIC). The analysis was conducted in triplicate for reproducibility.

3.3 Statistical Analysis

Data from GC-MS were processed using Agilent MassHunter Qualitative Analysis software for peak integration and deconvolution. Relative abundances (area%) were calculated as the mean ± standard deviation (SD) from triplicate runs. Descriptive statistics were applied to summarize compound distributions, including means for RT and area%, and grouping by chemical class. All calculations were performed using Microsoft Excel 2021 and GraphPad Prism version 9.0.

CHAPTER FOUR

RESULTS

4.1 Total Ion Chromatogram (TIC)

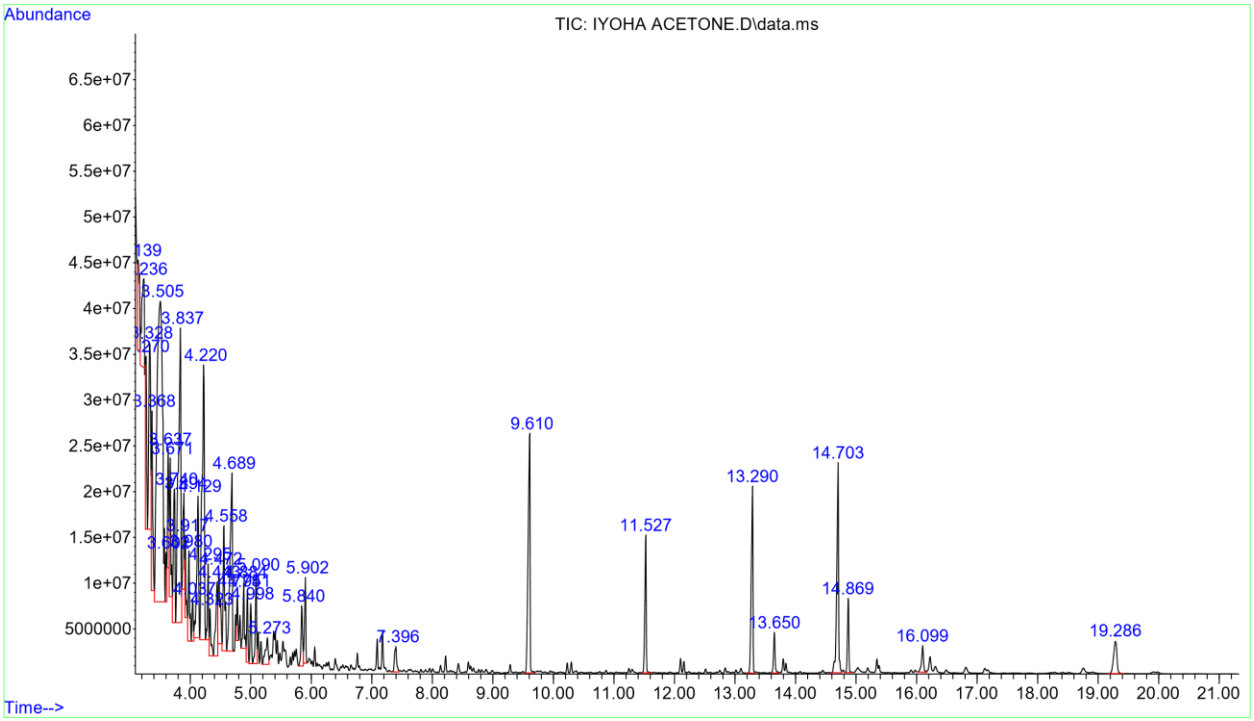

Figure 4.1: Total Ion Chromatogram (TIC) of Acetone Fraction from *Lonchocarpus cyanescens* Leaves

The GC-MS chromatogram of the acetone fraction is presented in Figure 4.1. The profile shows a complex mixture of volatile and semi-volatile compounds eluted between 3.14 and 19.29 minutes. The most abundant peaks were observed in the early region of the chromatogram, specifically at retention times (RT) of 3.505 min (19.05%), 3.837 min (8.40%), and 4.220 min (7.38%). Later-eluting peaks, such as those at 9.610 min, 13.290 min, and 14.703 min, were also prominent.

4.2 Identified Phytochemical Compounds

The compounds identified in the acetone fraction are listed in Table 4.1. The table includes the peak number, retention time (RT), relative peak area (Area %), the identified compound, its chemical class, and the NIST library match quality (Qual).

Table 4.1: Phytochemical compounds identified in the acetone fraction of *Lonchocarpus cyanescens* leaves by GC-MS.

Pk#	RT (min)	Area%	Top 3 Proposed Compounds (Ref#, CAS#, Qual)	Chemical Class
1	3.139	1.77	1. Benzene, 1-ethyl-3-methyl- (9609, 000620-14-4, **81**) 2. Benzene, 1-ethyl-4-methyl- (9610, 000622-96-8, **76**) 3. 1,3-Cyclopentadiene, 5-(1-methylpropylidene)- (9641, 003141-02-4, **72**)	Aromatic hydrocarbon
2	3.236	2.66	1. Benzene, 1,2,3-trimethyl- (9592, 000526-73-8, 91) 2. Benzene, 1,2,4-trimethyl- (9590, 000095-63-6, 91) 3. 2,3-Heptadien-5-yne, 2,4-dimethyl- (9627, 041898-89-9, 87)	Aromatic hydrocarbon
3	3.27	1.93	1. 1H-Indene, octahydro-5-methyl- (17327, 019744-64-0, 49) 2. Carbonic acid, 2,2,2-trichloroethyl cyclohexylmethyl ester (147333, 1000357-89-8, 47) 3. 4,5-Nonadiene, 2-methyl- (17302, 055956-32-6, 46)	Hydrocarbon (indene derivative)
4	3.328	4.04	1. Benzene, 1,2,4-trimethyl- (9590, 000095-63-6, 64) 2. Benzene, 1-ethyl-2-methyl- (9608, 000611-14-3, 64) 3. Benzene, 1-ethyl-3-methyl- (9609, 000620-14-4, 64)	Aromatic hydrocarbon
5	3.368	2.21	1. Cyclohexane, 1,4-dimethyl- (6863, 000589-90-2, **43**) 2. Cyclohexane, 1,4-dimethyl-, cis- (6927, 000624-29-3, **43**)	Cycloalkane

			3. Cyclohexane, 1,1-dimethyl- (6872, 000590-66-9, 38)	
6	3.505	19.05	1. Benzene, 1,2,4-trimethyl- (9590, 000095-63-6, 83) 2. Benzene, 1,2,3-trimethyl- (9592, 000526-73-8, 83) 3. Benzene, 1-ethyl-3-methyl- (9609, 000620-14-4, 70)	Aromatic hydrocarbon
7	3.602	0.65	1. Bicyclo[3.1.0]hexan-2-one, 5-(1-methylethyl)- (18076, 000513-20-2, 58) 2. Cyclohexene, 3-methyl- (2915, 000591-48-0, 49) 3. 3-Octyne, 2,2-dimethyl- (17295, 019482-57-6, 47)	Bicyclic ketone
8	3.637	1.63	1. Benzene, (2-methylpropyl)- (15186, 000538-93-2, 35) 2. Cyclohexanone, 2-(2-propenyl)- (17992, 000094-66-6, 35) 3. Benzene, n-butyl- (15158, 000104-51-8, 35)	Aromatic hydrocarbon
9	3.671	1.78	1. Benzene, (1-methylpropyl)- (15187, 000135-98-8, 46) 2. Benzene, 1-methyl-4-propyl- (15193, 001074-55-1, 46) 3. Benzeneacetaldehyde, .alpha.-methyl- (15709, 000093-53-8, 46)	Aromatic hydrocarbon
10	3.74	2.44	1. Decane, 4-methyl- (29359, 002847-72-5, 90) 2. Decane, 4-methyl- (29367, 002847-72-5, 72) 3. Decane, 4-methyl- (29366, 002847-72-5, 64)	Alkane
11	3.837	8.4	1. Benzene, 1,2-diethyl- (15178, 000135-01-3, 76) 2. Benzene, 1,2-diethyl- (15173, 000135-01-3, 64) 3. Benzene, 1-ethyl-3-methyl- (9609, 000620-14-4, 64)	Aromatic hydrocarbon
12	3.894	1.56	1. Cyclohexane, butyl- (18420, 001678-93-9, 90) 2. Cyclohexane, butyl- (18421, 001678-93-9, 87) 3. Cyclohexane, butyl- (18418, 001678-93-9, 87)	Cycloalkane
13	3.917	0.69	1. 3-Methyl-2-(2-oxopropyl)furan (17780, 087773-62-4, 43) 2. Glutaric acid, 3-methylbut-2-en-1-yl dec-4-enyl ester (195426, 1000405-00-1, 35) 3. Decane, 3-methyl- (29362, 013151-34-3, 25)	Furan derivative
14	3.98	1.32	1. Indane (9120, 000496-11-7, 50) 2. Indane (9121, 000496-11-7, 50)	Hydrocarbon (indane)

			3. Benzene, 2-propenyl- (9125, 000300-57-2, 50)	
15	4.037	0.6	1. 2,3,4-Trimethyl-hex-3-enal (19190, 1000193-72-9, 52) 2. Cyclohexane, 1,2,4-trimethyl- (11829, 002234-75-5, 52) 3. Cyclohexane, 1,2,4-trimethyl- (11831, 002234-75-5, 50)	Aldehyde/cycloalkane
16	4.129	2.98	1. Benzene, 1-methyl-3-propyl- (15192, 001074-43-7, 95) 2. Benzene, 1-methyl-3-propyl- (15194, 001074-43-7, 87) 3. Benzene, (1-methylpropyl)- (15187, 000135-98-8, 80)	Aromatic hydrocarbon
17	4.22	7.38	1. Benzene, 1-ethyl-3,5-dimethyl- (15221, 000934-74-7, 90) 2. Benzene, 4-ethyl-1,2-dimethyl- (15224, 000934-80-5, 90) 3. Benzene, 2-ethyl-1,4-dimethyl- (15226, 001758-88-9, 90)	Aromatic hydrocarbon
18	4.295	0.94	1. Decane, 3-methyl- (29362, 013151-34-3, 81) 2. Undecane, 3,4-dimethyl- (51417, 017312-78-6, 64) 3. Decane, 3-methyl- (29369, 013151-34-3, 59)	Alkane
19	4.323	0.71	1. Benzene, 1-methyl-4-propyl- (15193, 001074-55-1, 81) 2. Benzeneacetaldehyde, .alpha.-methyl- (15710, 000093-53-8, 81) 3. Benzene, 1-methyl-2-propyl- (15191, 001074-17-5, 81)	Aromatic hydrocarbon
20	4.443	1.63	1. Benzene, 1-ethyl-2,3-dimethyl- (15216, 000933-98-2, 95) 2. Benzene, 4-ethyl-1,2-dimethyl- (15224, 000934-80-5, 95) 3. Benzene, 2-ethyl-1,4-dimethyl- (15226, 001758-88-9, 95)	Aromatic hydrocarbon
21	4.472	1.61	1. o-Cymene (15146, 000527-84-4, 94) 2. o-Cymene (15145, 000527-84-4, 94) 3. Benzene, 1-ethyl-2,4-dimethyl- (15217, 000874-41-9, 93)	Monoterpene
22	4.558	2.36	1. Benzene, 2-ethyl-1,4-dimethyl- (15226, 001758-88-9, 94) 2. Benzene, 1-ethyl-2,3-dimethyl- (15216, 000933-98-2, 94)	Aromatic hydrocarbon

			3. o-Cymene (15145, 000527-84-4, 93)	
23	4.689	4.53	1. Undecane (29354, 001120-21-4, 87) 2. Undecane (29355, 001120-21-4, 83) 3. Tetradecane (63624, 000629-59-4, 83)	Alkane
24	4.775	0.58	1. Benzene, 1-methyl-4-(1-methylpropyl)- (23448, 001595-16-0, 50) 2. Benzene, 1,3-diethyl-5-methyl- (23424, 002050-24-0, 49) 3. 2-Propenal, 3-(2-pyridinylamino)- (23814, 068970-82-1, 46)	Aromatic hydrocarbon
25	4.884	0.97	1. trans-Decalin, 2-methyl- (26210, 1000152-47-3, 97) 2. Naphthalene, decahydro-2-methyl- (26227, 002958-76-1, 97) 3. trans-4a-Methyl-decahydronaphthalene (26231, 002547-27-5, 94)	Decalin derivative
26	4.941	0.89	1. Benzene, 1-ethyl-2,3-dimethyl- (15216, 000933-98-2, 93) 2. Benzene, 1,2,3,4-tetramethyl- (15200, 000488-23-3, 76) 3. Benzene, 1,2,3,5-tetramethyl- (15203, 000527-53-7, 76)	Aromatic hydrocarbon
27	4.998	0.9	1. Benzene, 1,2,4,5-tetramethyl- (15208, 000095-93-2, 95) 2. Benzene, 1-ethyl-2,4-dimethyl- (15217, 000874-41-9, 91) 3. Benzene, 1,2,3,5-tetramethyl- (15203, 000527-53-7, 90)	Aromatic hydrocarbon
28	5.09	1.37	1. trans-4a-Methyl-decahydronaphthalene (26231, 002547-27-5, 93) 2. Naphthalene, decahydro-2-methyl- (26227, 002958-76-1, 90) 3. trans-Decalin, 2-methyl- (26210, 1000152-47-3, 90)	Naphthalene derivative
29	5.273	0.82	1. Benzene, (1,1-dimethylpropyl)- (23433, 002049-95-8, 52) 2. Benzene, (1,1-dimethylpropyl)- (23434, 002049-95-8, 50) 3. 3,4-Dihydro-2-quinoxalinol (23800, 1000332-36-8, 49)	Aromatic hydrocarbon
30	5.84	1.09	1. Azulene (12190, 000275-51-4, 94) 2. Naphthalene (12197, 000091-20-3, 94)	Sesquiterpene

			3. 1H-Indene, 1-methylene- (12199, 002471-84-3, 93)	
31	5.902	1.22	1. Dodecane (39972, 000112-40-3, 97) 2. Octadecane, 1-chloro- (148105, 003386-33-2, 83) 3. Tridecane (51408, 000629-50-5, 83)	Alkane
32	7.396	0.62	1. Decanoic acid, methyl ester (52763, 000110-42-9, 97) 2. Decanoic acid, methyl ester (52773, 000110-42-9, 93) 3. Decanoic acid, methyl ester (52771, 000110-42-9, 64)	Fatty acid methyl ester (FAME)
33	9.61	5.18	1. Dodecanoic acid, methyl ester (78067, 000111-82-0, 98) 2. Undecanoic acid, 10-methyl-, methyl ester (78102, 005129-56-6, 90) 3. Dodecanoic acid, methyl ester (78065, 000111-82-0, 87)	FAME
34	11.53	2.16	1. Methyl tetradecanoate (104286, 000124-10-7, 99) 2. Methyl tetradecanoate (104289, 000124-10-7, 96) 3. Methyl tetradecanoate (104287, 000124-10-7, 95)	FAME
35	13.29	3.47	1. Hexadecanoic acid, methyl ester (130813, 000112-39-0, 98) 2. Hexadecanoic acid, methyl ester (130821, 000112-39-0, 98) 3. Hexadecanoic acid, methyl ester (130819, 000112-39-0, 98)	FAME
36	13.65	0.7	1. Dibutyl phthalate (138058, 000084-74-2, 95) 2. 1,2-Benzenedicarboxylic acid, dihexyl ester (192079, 000084-75-3, 72) 3. Phthalic acid, hexadecyl propyl ester (251463, 1000309-06-5, 72)	Phthalate ester
37	14.7	4.18	1. 9-Octadecenoic acid (Z)-, methyl ester (155750, 000112-62-9, 99) 2. 6-Octadecenoic acid, methyl ester, (Z)- (155752, 002777-58-4, 99) 3. 9-Octadecenoic acid, methyl ester, (E)- (155754, 001937-62-8, 99)	FAME (unsaturated)
38	14.87	1.14	1. Methyl stearate (157883, 000112-61-8, 99) 2. Methyl stearate (157884, 000112-61-8, 99)	FAME

			3. Methyl stearate (157879, 000112-61-8, 99)	
39	16.1	0.59	1. Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis- (171279, 002566-91-8, 91) 2. Oxiraneoctanoic acid, 3-octyl-, methyl ester (171276, 002500-59-6, 87) 3. 2,2,4-Trimethyl-5-oxo-2,5-dihydro-3-furancarboxylic acid (40358, 094072-56-7, 38)	Epoxy fatty acid ester
40	19.29	1.21	1. Bis(2-ethylhexyl) phthalate (233373, 000117-81-7, 91) 2. Phthalic acid, di(oct-3-yl) ester (233383, 1000377-72-3, 86) 3. Diisooctyl phthalate (233361, 000131-20-4, 83)	Phthalate ester

This table presents the complete results from the GC-MS analysis of the acetone fraction, including all 40 detected peaks with retention times (RT), relative peak areas (Area%), and the top three proposed compound identifications from the NIST14 library (with reference numbers, CAS numbers, and quality match scores). Chemical classes are inferred based on compound structures.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Lonchocarpus cyanescens holds a significant place in West African traditional medicine, where it is widely employed for treating a range of ailments including skin infections, ulcers, leprosy, and inflammatory disorders (Oladeji *et al.,* 2020; Peter *et al.,* 2024). To investigate the chemical basis for these therapeutic claims, this study focused on the plant's acetone fraction. This fraction was specifically chosen due to acetone's intermediate polarity, which is effective for isolating a distinct profile of semi-volatile and moderately polar phytochemicals, such as terpenoids and certain phenolics. Consequently, Gas Chromatography-Mass Spectrometry (GC-MS) was the analytical technique of choice, as it is exceptionally well-suited for separating, identifying, and profiling such volatile compounds. The analysis successfully identified 40 distinct compounds, providing a detailed chemical fingerprint that helps to rationalize the plant's traditional ethnomedicinal applications.

The most significant finding was the predominance of aromatic hydrocarbons, which collectively represent a substantial portion of the fraction's composition. Notably, Benzene, 1,2,4-trimethyl- (Pk# 6, 19.05%) was the most abundant compound detected. This compound, along with other isomers and derivatives such as Benzene, 1,2-diethyl- (Pk# 11, 8.40%) and Benzene, 1-ethyl-3,5-dimethyl- (Pk# 17, 7.38%), forms the chemical backbone of this fraction. While simple aromatic hydrocarbons are common in plant volatiles, their high concentration suggests a significant role. Various studies has linked certain trimethylbenzene isomers to antimicrobial and anti-inflammatory activities. This chemical profile aligns with the traditional use of *L. cyanescens* in

treating skin infections, ulcers, and inflammatory disorders as documented by Oladeji et al. (2020) and Peter et al. (2024). The lipophilic nature of these compounds would facilitate absorption through the skin, supporting their potential efficacy in topical remedies for conditions like leprosy and ulcers.

The second major chemical class identified was fatty acid methyl esters (FAMEs). Key compounds included Dodecanoic acid, methyl ester (Pk# 33, 5.18%), 9-Octadecenoic acid (Z)-, methyl ester (Pk# 37, 4.18%), and Hexadecanoic acid, methyl ester (Pk# 35, 3.47%). These compounds are derivatives of lauric, oleic, and palmitic acids, respectively, which are known to possess significant biological properties. For instance, hexadecanoic acid is reported to have antioxidant, anti-inflammatory, and antimicrobial effects. The presence of these FAMEs provides a strong scientific basis for the plant's use in wound healing and managing skin infections (Ganesan *et al.,* 2024; Shawer *et al.,* 2022).

Beyond the most abundant classes, several minor compounds of high biological relevance were detected. The identification of o-Cymene (Pk# 21, 1.61%), a monoterpene, is noteworthy. Monoterpenes are characteristic constituents of essential oils and are widely recognized for their potent antimicrobial and antiseptic properties. Its presence supports the plant’s use against infections. Even more compelling is the detection of Azulene (Pk# 30, 1.09%). Azulene is a sesquiterpene renowned for its vibrant blue color and potent anti-inflammatory and soothing properties, famously found in chamomile (Yeşilyurt *et al.,* 2024). This finding is particularly significant as it creates a direct molecular link between the plant's traditional use as a source for *adire* indigo dye and its application as an anti-inflammatory agent.

It is crucial to address the limitations of this study and the GC-MS technique. While GC-MS is excellent for volatile and semi-volatile compounds, it is less effective for identifying non-volatile,

highly polar, or thermally labile molecules (Khoury *et al.,* 2023). The absence of these classes here is likely an artifact of the analytical method rather than their true absence in the acetone fraction. Acetone is capable of extracting some of these compounds, but they may not have volatilized under the GC conditions. This highlights the need for complementary analytical techniques, such as Liquid Chromatography-Mass Spectrometry (LC-MS), to obtain a complete phytochemical inventory.

Furthermore, the quality of library matches (Qual) warrants careful consideration. While many key compounds were identified with high confidence (Qual > 80), several peaks had lower scores (e.g., Pk# 3, 1H-Indene, octahydro-5-methyl-, Qual=49; Pk# 8, Benzene, (2-methylpropyl)-, Qual=35). Such identifications should be considered tentative and would require confirmation through analysis with authentic standards or structural elucidation via Nuclear Magnetic Resonance (NMR) spectroscopy. Lastly, the detection of Dibutyl phthalate (Pk# 36) and Bis(2-ethylhexyl) phthalate (Pk# 40) is consistent with common laboratory contaminants originating from plasticizers (Mansuri *et al.,* 2025). While their presence must be reported for transparency, they are not considered endogenous phytochemicals of *L. cyanescens* and are excluded from biological interpretation.

5.2 Conclusion

This study successfully characterized the phytochemical composition of the acetone fraction of *Lonchocarpus cyanescens* leaves via GC-MS analysis, identifying 40 compounds. The chemical profile was dominated by aromatic hydrocarbons (notably Benzene, 1,2,4-trimethyl-) and fatty acid methyl esters (including esters of dodecanoic, hexadecanoic, and octadecenoic acids). Other biologically significant compounds, such as the monoterpene o-Cymene and the anti-inflammatory sesquiterpene Azulene, were also identified. The identified compounds provide substantial scientific evidence to support and rationalize the ethnomedicinal uses of *L. cyanescens*, particularly for treating inflammatory conditions, skin infections, and ulcers. The chemical profile serves as a valuable reference for quality control and standardization of herbal preparations derived from this plant. This research contributes to the broader understanding of the phytochemistry of *L. cyanescens* and highlights the therapeutic potential residing in its semi-volatile constituents.

REFERENCES

Abubakar, A. R. and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences*, ***12***(1): 1–10.

Adejoro, I. A., Waheed, S. O. and Adeboye, O. O. (2016). Molecular docking studies of Lonchocarpus cyanescens triterpenoids as inhibitors for malaria. *Journal of Physical Chemistry and Biophysics*, ***6***, 213.

Adejoro, I. A., Waheed, S. O., Adeboye, O. O. and Akhigbe, F. U. (2017). Molecular docking of the inhibitory activities of triterpenoids of Lonchocarpus cyanescens against ulcer. *Journal of Biophysical Chemistry*, ***8***(1): 1–11.

Adeku, E., Osundahunsi, O. F., Malomo, S. A., Asasile, I. I., Owolabi, O. M. and Oyewole, G. (2022). Phytochemical constituents and assessment of crude extracts from Boerhavia diffusa L. and Lonchocarpus sericeus (Poir.) Kunth ex DC. leaves for antioxidant and antibacterial activities. *Measurement: Food*, ***5***, 100018.

Ajani, E. O., Sabiu, S., Odufuwa, K. T., Ibrahim, T. B. and Salau, B. A. (2017). Evaluation of lens aldose reductase inhibitory and free radical scavenging potential of fractions of Lonchocarpus cyanescens: Potential for cataract remediation. *Pharmacognosy Journal*, ***9***(1): 62–69.

Al-Nuri, M., Abu-Reidah, I. M., Alhajeh, A. A., Omar, G., Adwan, G. and Warad, I. (2022). GC–MS-based metabolites profiling, in vitro antioxidant, anticancer, and antimicrobial properties of different solvent extracts from the botanical parts of Micromeria fruticosa (Lamiaceae). *Processes*, ***10***(5): 1016.

Amakiri, O. K., Ezekwe, A. S. and Wokocha, P. G. (2024). Phytochemical profile and bioactive compounds in aqueous leaf extract of Vernonia amygdalina (Asteraceae): A GC-MS analysis. *Asian Journal of Research in Biochemistry*, ***14***(6): 117–123.

Amit, T. A. and Colón, L. A. (2023). Exploring natural dye and bioactive secondary metabolites in Lonchocarpus cyanescens Benth (Fabaceae) plant using liquid chromatography-high-resolution mass spectrometry and Compound DiscovererTM software. *ChemRxiv Journal*, ***1***(1): 1–37.

Arowona, I. T., Sonibare, M. A. and Umukoro, S. (2014). Antipsychotic property of solvent-partitioned fractions of Lonchocarpus cyanescens leaf extract in mice. *Journal of Basic and Clinical Physiology and Pharmacology*, ***25***(2): 235–240.

Asmaey, M. A. (2024). Utilizing GC–MS and UPLC–MS for rapid metabolomic analysis in medicinal plants. In *Propagation to Pharmacopeia*, 361-378.

Baa-Poku, F. and Enu-Kwesi, L. (2016). Ethnobotanical study of the use of natural dye plant species in the southern forest-savanna transition zone of Ghana. *Ghana Journal of Science*, **56**: 25–37.

Barde, A., Oloyede, R. B., Haruna, A., Muhammad, A., Bashir, A. I. J. and Jimoh, A. A. (2025). Phytochemical analysis and antibacterial activity of acetone extract of Terminalia catappa Linn. leaves. *Tropical Journal of Drug Research*, ***2***(2): 48–56.

Borges-Argáez, R., Balnbury, L., Flowers, A., Giménez-Turba, A., Ruiz, G., Waterman, P. G., and Peña-Rodríguez, L. M. (2007). Cytotoxic and antiprotozoal activity of flavonoids from Lonchocarpus spp. *Phytomedicine*, **14**(7–8): 530–533.

Channabasava, G. M., Chandrappa, C. P. and Umashankar, T. (2015). GC-MS study of two column fractions from methanol extracts of Loranthus micranthus and their in vivo antidiabetic activity on alloxan induced diabetic rats. *Journal of Diabetes and Metabolism*, ***6***(5): 536.

Chiaia, V., Galletta, M., Micalizzi, G., Mondello, L. and Salerno, T. M. G. (2025). A review of the recent advances in extraction techniques for volatilome of aromatic and fragrant plants. *Analytical and Bioanalytical Chemistry*, 1-27.

Cunha, G. M. A., Fontenele, J. B., Nobre Júnior, H. V., de Sousa, F. C. M., Silveira, E. R., Nogueira, N. A. P., de Moraes, M. O., Viana, G. S. B. and Costa-Lotufo, L. V. (2003). Cytotoxic activity of chalcones isolated from Lonchocarpus sericeus (Pocr.) Kunth. *Phytotherapy Research*, ***17***(2): 155–159.

Darwin, R., Valmon, R., Chithanna, S., Galla, S. H., Syed, S. H., Mohathasim Billah, A. A., Kumar Reddy, K. T. and Naga Venkata Arjun, U. V. (2025). Sustainable extraction and purification of phytochemicals: A review of green solvents and techniques. *Chemical Methodologies*, ***9***(5): 356–385.

Desta, G. T., Ferede, Y. A., Zewdu, W. S., Adugna, B. Y., Arega, T. and Alemu, M. A. (2022). Validation of antidiabetic and antihyperlipidemic effects of 80% methanolic extract of the Lonchocarpus laxiflorus leaves in streptozotocin-induced diabetic Swiss albino mice. *Evidence-Based Complementary and Alternative Medicine*, ***2***(1): 8-51.

Elshafie, H. S., Camele, I. and Mohamed, A. A. (2023). A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. *International journal of molecular sciences*, ***24***(4): 3266.

Falade, O. S., Patricia, A. F., Aderogba, M. A., Adeyanju, A. A. and Ajayi, O. S. (2025). Stabilisation of groundnut oil with gallic acid and leaf extracts of Lonchocarpus sericeus and Lonchocarpus cyanescens. *International Journal of Food Science and Technology*, ***60***(1): 76.

Ganesan, T., Subban, M., Christopher Leslee, D. B., Kuppannan, S. B. and Seedevi, P. (2024). Structural characterization of n-hexadecanoic acid from the leaves of Ipomoea eriocarpa

and its antioxidant and antibacterial activities. *Biomass Conversion and Biorefinery*, ***14***(13): 14547-14558.

Gaudêncio, S. P., Bayram, E., Lukić Bilela, L., Cueto, M., Díaz-Marrero, A. R., Haznedaroglu, B. Z., Jimenez, C., Mandalakis, M., Pereira, F., Reyes, F. and Tasdemir, D. (2023). Advanced methods for natural products discovery: Bioactivity screening, dereplication, metabolomics profiling, genomic sequencing, databases and informatic tools and structure elucidation. *Marine Drugs*, ***21***(5): 308.

Gomathi, D., Kalaiselvi, M., Ravikumar, G., Devaki, K. and Uma, C. (2015). GC-MS analysis of bioactive components from ethanol extract of Evolvulus alsinoides (L.) L. *Journal of Food Science and Technology*, ***52***(2): 1212–1217.

Horta, B., Freitas-Silva, J., Silva, J., Dias, F., Teixeira, A. L., Medeiros, R., Cidade, H., Pinto, M. and Cerqueira, F. (2023). Antitumor effect of chalcone derivatives against human prostate (LNCaP and PC-3): cervix HPV-positive (HeLa) and lymphocyte (Jurkat) cell lines and their effect on macrophage functions. *Molecules*, ***28***(5): 2159.

Ishak, S. F., Rajab, N. F. and Basri, D. F. (2024). Analysis of GC-MS from acetone extract of Canarium odontophyllum Miq stem bark (Dabai). *Biomedical and Pharmacology Journal*, ***17***(2): 1009–1020.

Isyaka, S. M., Olayemi, O. A. and Abdullahi, M. A. (2025). Phytochemistry and medicinal uses of Lonchocarpus species: Systematic review of a promising medicinal plant. *South Asian Research Journal of Natural Products*, ***8***(1): 32–44.

Iyoha, A. I., Ogboye, P. O. and Onoagbe, I. O. (2025). Safety Evaluation of Methanolic Leaf Extract of Lonchocarpus cynanecens on the Liver of Wistar Rat. *Sahel Journal of Life Sciences FUDMA*, ***3***(2): 49-54.

Iyoha, A. I., Onoagbe, I. O. and Abu, O. D. (2023). Effects of aqueous and methanolic leaf extracts of Lonchocarpus cyanescens leaf on oxidative status in normal albino Wistar rats. *Nigerian Journal of Life Sciences*, ***13***(1 & 2): 7–10.

Kaldeli, A., Zakidou, P. and Paraskevopoulou, A. (2024). Volatilomics as a tool to ascertain food adulteration, authenticity and origin. *Comprehensive Reviews in Food Science and Food Safety*, ***23***(4): e13387.

Karlina, I., Pusparini, N. A. O., Maharesi, C. E., Saeed, F., Nuriliani, A., Retnoaji, B., Saragih, H., Septriani, N. I. and Rohmah, Z. (2025). Natural dye as an alternative to hematoxylin-eosin staining on histological preparations. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, ***10***(2): 139–147.

Kazeem, M. I. and Davies, T. C. (2016). Hypoglycaemic potential of leaf extracts of Lonchocarpus cyanescens (Schum. and Thonn.) Benth. *Transactions of the Royal Society of South Africa*, ***71***(1): 1–6.

Khan, M., Khan, M., Al-Wahaibi, L. H., Alkhathlan, H. Z. and Al-Taweel, A. M. (2022). Comprehensive phytochemical analysis of various solvent extracts of Artemisia judaica and their potential anticancer and antimicrobial activities. *Life*, ***12***(11): 1885.

Khoury, D., Millet, M., Jabali, Y. and Delhomme, O. (2023). Analytical procedure for the concomitant analysis of 242 polar and non-polar organic compounds of different functional groups in fog water. *Microchemical Journal*, ***185***: 108235.

Kiria, M. J. (2018). Antimicrobial activity and constituents of the root bark of Lonchocarpus eriocalyx [Master's thesis, University of Nairobi].

Larqué, H., Chávez Montes, A., Zamora-Chimal, J., Looh-Hernández, M., Luevano, J. H. and del Olmo, E. (2024). Bioguided assay of polyphenols isolated from medicinal Mayan species and its activity against Leishmania mexicana. *Pharmacognosy Journal*, ***16***(1): 174–181.

Li, Y., Pan, A. P. and Yu, A. Y. (2025). Recent progression of pathogenesis and treatment for diabetic cataracts. *Seminars in Ophthalmology*, ***40***(4): 275–282.

Lytovchenko, A., Beleggia, R., Schauer, N., Isaacson, T., Leuendorf, J. E., Hellmann, H., Rose, J. K. C. and Fernie, A. R. (2009). Application of GC-MS for the detection of lipophilic compounds in diverse plant tissues. *Plant Methods*, ***5***: 4.

Magalhães, A. F., Tozzi, A. M. G. A., Magalhães, E. G., Sannomiya, M., Soriano, M. D. P. C. and Perez, M. A. F. (2007). Flavonoids of Lonchocarpus montanus A.M.G. Azevedo and biological activity. *Anais da Academia Brasileira de Ciências*, ***79***(3): 351–367.

Maji, S. R., Roy, C. and Sinha, S. K. (2023). Gas chromatography–mass spectrometry (GC-MS): A comprehensive review of synergistic combinations and their applications in the past two decades. *Journal of Analytical Sciences and Applied Biotechnology*, ***5***(2): 72–85.

Mansuri, A., Trivedi, C., Chokshi, S., Jantrania, K. and Kumar, A. (2025). Phthalate Exposure: Prevalence, Health Effects, Regulatory Frameworks, and Remediation. *Chemical Research in Toxicology*, ***38***(8): 1291-1308.

Mathe, E., Sethoga, L., Mapfumari, S., Adeniran, O., Mokgotho, P., Shai, J. and Gololo, S. (2024). Phytochemical screening and characterization of volatile compounds from three medicinal plants with reported anticancer properties using GC-MS. *Life*, ***14***(11): 1375.

Mohammed, H. A., Qureshi, K. A., Ali, H. M., Al-Omar, M. S., Khan, O. and Mohammed, S. A. A. (2022). Volatile oil of Artemisia judaica L. cultivated in Saudi Arabia: GC–MS analysis, anti-inflammatory, antioxidant and antimicrobial activity. *Antioxidants*, ***11***(2): 332.

Moriasi, G. A., Ireri, A. M., Nelson, E. M. and Ngugi, M. P. (2021). In vivo anti-inflammatory, anti-nociceptive and in vitro antioxidant efficacy and acute oral toxicity effects of the aqueous and methanolic stem bark extracts of Lonchocarpus eriocalyx (Harms.). *Heliyon*, *7*(5): 7-145.

Moronkola, D. O. and Oladosu, I. A. (2013). Chemical compositions of Lonchocarpus cyanescens Benth., (Fabaceae)—Case study of its volatile oils and two triterpenoids. *American Journal of Plant Sciences*, ***4***(8): 1653–1659.

NMPPDB. (2025). Lonchocarpus cyanescens. NMPPDB (National Medicinal Plant Products Database).

Nwokonkwo, D. C., Okpani, A. N. and Elom, N. (2017). Extraction, phytochemical, antibacterial screening and spectroscopic analysis of the crude samples of stem bark extract of Lonchocarpus cyanescens. *Der Pharma Chemica*, ***9***(8): 1–5.

Odjobo, B. O., Ichoron, N., Igoli, N. P. and Igoli, J. O. (2020). Using GC-MS for natural product analysis in Nigeria: Problems and prospects. *Journal of the Chemical Society of Nigeria*, ***45***(5): 798–803.

Ogbuagu, O. O., Mbata, A. O., Balogun, O. D., Oladapo, O., Ojo, O. O. and Muonde, M. (2022). Novel phytochemicals in traditional medicine: Isolation and pharmacological profiling of bioactive compounds. *International Journal of Medical and All Body Health Research*, ***3***(1): 63-71.

Oladeji, O. S., Oluyori, A. P., Bankole, D. T. and Afolabi, T. Y. (2020). Natural products as sources of antimalarial drugs: ethnobotanical and ethnopharmacological studies. *Scientifica*, ***20***(1): 7076139.

Oliveira, M., De Souza, L. C. R. R., Silva, D. E. F., Val, C. M. S., Carvalho, C. E. S., Carvalheiro, T. S. C. and Andrade, A. G. (2025). Diplopterys pubipetala (Malpighiaceae): Insights into antioxidant, antibacterial and antifungal activities with chemical composition analysis via UHPLC-MS/MS and GC/MS. *Molecules*, ***30***(4): 946.

Oribhabor, B. J. and Akanse, N. N. (2020). Assessment of ichthyotoxicity of Lonchocarpus cyanescens on the African catfish, Clarias gariepinus fingerlings and anuran tadpoles. *Research Journal of Environmental Toxicology*, ***14***(1): 8–15.

Peter, O. B., Iyoha, E. and Augustine, O. O. (2024). The effect of aqueous extract of Lonchocarpus cyanencens leaf on the histology of the liver of Wistar rats. *Sokoto Journal of Medical Laboratory Science*, ***9***(3): 168-172.

Qi-yue, Y., Zhang, T., He, Y.-n., Huang, S.-j., Deng, X., Han, L. and Chun-guang, X. (2020). From natural dye to herbal medicine: A systematic review of chemical constituents, pharmacological effects and clinical applications of *Indigo naturalis*. *Chinese Medicine*, ***15***, (127).

Rumidatul, A., Aryantha, I. N. P. and Sulistyawati, E. (2021). Phytochemicals screening, GC/MS characterization and antioxidant activity of Falcataria moluccana Miq. Barneby and J. W. Grimes methanolic extract. *Pharmacognosy Journal*, ***13***(2): 450–456.

Sabri, A. A. M., Begum, T., Uddin, A. H., Khattak, M. M. A. K., Azmi, S. N. H., Iqubal, S. S., Zakaria, Z. A. and Ahmed, Q. U. (2025). The antiparasitic potential of flavonols: A systematic review. *International Journal of Allied Health Sciences*, ***9***(2).

Salahshour, B., Sadeghi, S., Nazari, H. and Soltaninejad, K. (2020). Determining undeclared synthetic pharmaceuticals as adulterants in weight loss herbal medicines. *International Journal of Medical Toxicology and Forensic Medicine*, ***10***(1): 26253.

Saleh, M. A., Shabaan, A. A., May, M. and Ali, Y. M. (2022). Topical application of indigo-plant leaves extract enhances healing of skin lesion in an excision wound model in rats. *Journal of Applied Biomedicine*, ***20***(4): 124–131.

Samuel, B., Adigun, O. and Adaramoye, O. (2014). Bioassay-guided investigation of *Lonchocarpus cyanescens* Benth leaves extracts for antioxidant activities. *African Journal of Biotechnology*, ***13***(22): 2240–2247.

Satti, A. A., Abdelgadir, A. A., Hago, S. A., Ahmed, E. M. and Elimam, Y. M. (2021). GC-MS analysis, antioxidant and antibacterial activity of acetone fractions obtained from Guiera Senegalensis leaves and Quercus Infectoria Nutgalls extracts. *Arabian Journal of Medicinal & Aromatic Plants*, ***7***(2): 284–294.

Schoch, C. L., Ciufo, S., Domrachev, M., Hotton, C. L., Kannan, S., Khovanskaya, R., Leipe, D., Mcveigh, R., O'Neill, K., Robbertse, B., Sharma, S., Soussov, V., Sullivan, J. P., Sun, L., Turner, S. and Karsch-Mizrachi, I. (2020). NCBI Taxonomy: A comprehensive update on curation, resources and tools. *Database (Oxford)*, ***2***, 62.

Shawer, E. E. S., Sabae, S. Z., El-Gamal, A. D. and Elsaied, H. E. (2022). Characterization of Bioactive Compounds with Antioxidant Activity and Antimicrobial Activity from Freshwater Cyanobacteria. *Egyptian Journal of Chemistry*, ***65***(9): 723-735.

Shrivastava, R. and Mishra, J. (2019). Extraction, phytochemical screening, isolation and identification of bioactive compounds from extract of the plant Euphorbia Thymifolia Linn. *J Drug Deliv Ther*, ***9***(3): 107-113.

Singh, D., Mittal, N., Verma, S., Singh, A. and Siddiqui, M. H. (2024). Applications of some advanced sequencing, analytical and computational approaches in medicinal plant research: a review. *Molecular Biology Reports*, ***51***(1): 23.

Sonibare, M. A., Oke, T. A. and Soladoye, M. O. (2014). A pharmacobotanical study of two medicinal species of Fabaceae. *Asian Pacific Journal of Tropical Biomedicine*, ***4***(2): 131–136.

Sonibare, M. A., Umukoro, S. and Shonibare, E. T. (2012). Antipsychotic property of aqueous and ethanolic extracts of Lonchocarpus cyanescens (Schumach and Thonn.) Benth. (Fabaceae) in rodents. *Journal of Natural Medicines*, ***66***(1): 127–132.

Sophia, A., Faiyazuddin, M., Alam, P., Hussain, M. T. and Shakeel, F. (2022). GC–MS characterization and evaluation of antimicrobial, anticancer and wound healing efficiency

of combined ethanolic extract of Tridax procumbens and Acalypha indica. *Journal of Molecular Structure*, ***1***(2), 131678.

Taïbi, K., Aït Abderrahim, L., Boussaid, M., Taïbi, F., Achir, M., Souana, K., Bacha, N. D., Derdour, M. and Benmouhoub, N. (2021). Flavonoids kaempferol-3-bioside, the 3-glucosides and 3-rutinosides of quercetin, kaempferol and isorhamnetin, 3-sophorosides of quercetin. *European Journal of Integrative Medicine*, ***44***: 101-339.

Tanih, N. F. and Ndip, R. N. (2012). Evaluation of the acetone and aqueous extracts of mature stem bark of Sclerocarya birrea for antioxidant and antimicrobial properties. *Evidence-Based Complementary and Alternative Medicine*, 834156.

Thamer, F. H. and Thamer, N. (2023). Gas chromatography–mass spectrometry (GC-MS) profiling reveals newly described bioactive compounds in Citrullus colocynthis (L.) seeds oil extracts. *Heliyon*, ***9***(6): e16861.

Umoh, U. F. and Nwafor, P. A. (2013). Anti-inflammatory and analgesic effects of Lonchocarpus cyanescens root in mice. *African Journal of Pharmacology and Therapeutics*, ***2***(3): 88–93.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsakris, Z., Rozos, G., Tsigalou, C. and Bezirtzoglou, E. (2022). Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. *Antibiotics*, ***11***(8): 1014.

Wouyou, G. H., Avocevou-Ayisso, C., Idohou, R., Assogbadjo, C. S., Boukari, N. S., Abba, M. S. I. and Assogbadjo, A. E. (2025). Cultural and socio-economic determinants of natural dye usage: A case of African dye plants in Benin. *Ethnobotany Research and Applications*, ***32***: 1-22.

Wu, C., Lee, S. L., Taylor, C., Li, J., Chan, Y. M., Agarwal, R., Temple, R., Throckmorton, D. and Tyner, K. (2020). Scientific and regulatory approach to botanical drug development: A U.S. FDA perspective. *Journal of Natural Products*, ***83***(2): 552–562.

Yeşilyurt, S., Gürgan, M. and Sertkahya, M. (2024). Biologically Active Compounds from Medicinal and Aromatic Plants for Industrial Applications. In *Medicinal and Aromatic Plants: Current Research Status, Value-Addition to Their Waste, and Agro-Industrial Potential (Vol I)* (pp. 1-11). Cham: Springer Nature Switzerland.

Yu, Q., Zhang, T., He, Y., Huang, S., Deng, X., Han, L. and Xie, C. (2020). From natural dye to herbal medicine: A systematic review of chemical constituents, pharmacological effects and clinical applications of indigo naturalis. *Chinese Medicine*, ***15***, 127.

Zhang, M., Zhao, J., Dai, X. and Li, X. (2023). Extraction and analysis of chemical compositions of natural products and plants. *Separations*, ***10***(12): 598.

Zhang, W., Zeng, Y., Jiao, M., Ye, C., Li, Y., Liu, C. and Wang, J. (2023). Integration of high-throughput omics technologies in medicinal plant research: The new era of natural drug discovery. *Frontiers in Plant Science*, ***14***: 1073848.