GC-MS PROFILE OF HEXANE FRACTION OF THE LEAVES OF *Lonchocarpus cyanescens*

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CERTIFICATION

We, the undersigned, hereby certify that Itohan Maryann Ojo (BMS2101434) carried out this work in the Department of Medical Biochemistry, University of Benin, Benin City, and we approve the same as adequate in scope and quality for the reward of Bachelor of Science Degree (B.Sc.) in Medical Biochemistry.

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DEDICATION

This work is first and foremost dedicated to God Almighty, whose grace, wisdom, and unfailing love have guided me through every step of this journey. His strength sustained me, His light directed my path, and His mercy made the completion of this work possible. All glory and honour belong to Him.

I also lovingly dedicate this work to my beloved family, whose unwavering love, prayers, and encouragement have been my constant source of strength. Your support carried me through every challenge, and your belief in me gave me the courage to keep pushing forward. Everything I have achieved is because you stood firmly by me, and for that, I remain forever grateful.

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ABSTRACT

Lonchocarpus cyanescens (Elu), a plant widely employed in traditional medicine to manage infections, inflammation, and mental disorders, remains underexplored in terms of its non-polar chemical constituents. In this study, Leaves of *Lonchocarpus cyanescens* were collected, processed and extracted with ethanol, after which their non-polar constituents were isolated using n-hexane. Analysis of this fraction by GC–MS enabled the identification of several lipophilic metabolites using the NIST14 spectral library, with match qualities ≥90%. Nineteen compounds were detected, predominantly aromatic hydrocarbons, monoterpenes, fatty acid methyl esters, aliphatic hydrocarbons, and glycerides. The most abundant compounds were 1,2,4-trimethylbenzene (33.50%), 1-ethyl-2-methylbenzene (6.63%), o-cymene (6.42%), and 1,3-dioctanoin (6.23%). These constituents are known for their antimicrobial, anti-inflammatory, antioxidant, and membrane-active properties, suggesting that the hexane fraction may contribute to the plant's reported ethnomedicinal effects. The prevalence of volatile and lipophilic compounds also underscores the effectiveness of hexane in selectively extracting nonpolar metabolites. This study provides a detailed GC–MS profile of the n-hexane fraction of *L. cyanescens*, thereby expanding existing phytochemical knowledge and laying a foundation for future isolation, characterization, and pharmacological investigations.

CHAPTER 1

1.0 INTRODUCTION

The African flora remains an indispensable reservoir of medicinally relevant plant species, providing structurally diverse bioactive compounds essential to traditional and modern therapeutics. Among these, *Lonchocarpus cyanescens* (Schumach. and Thonn.) Benth. stands out as a culturally and pharmacologically significant member of the family Fabaceae. Historically valued as the principal source of Yoruba indigo dye, *L. cyanescens* is widely utilized across West and Central Africa in ethnomedicine for managing inflammation, arthritis, chronic diarrhoea, and various neuropsychiatric disorders (*Sonibare et al*., 2012; *Adedikun et al*., 2021). These traditional uses are reinforced by contemporary pharmacological reports confirming the plant's antioxidant, anti-inflammatory, and neuro-modulatory properties (*Iyoha et al*., 2023). Such biological activities are attributed primarily to its diverse array of secondary metabolites, including flavonoids, triterpenoids, and phenolic acids (*Onyeka et al*., 2018).

Despite these advances, current knowledge of the plant's chemistry remains disproportionately focused on hydrophilic and semi-polar constituents as most investigations on *L. cyanescens* have concentrated on aqueous, ethanolic, and methanolic extracts, leaving the non-polar fraction comparatively underexplored thus presenting the need for research as lipophilic phytochemicals such as terpenoids, sterols, fatty acids, and other hydrocarbon derivatives often possess distinct pharmacological properties that complement or enhance the activities of polar metabolites (*Handa et al*., 2008). Without characterizing these non-polar components, the full therapeutic potential and chemical architecture of *L. cyanescens* remain incomplete.

Analytical techniques suited for investigating these lipophilic constituents are therefore essential. Gas Chromatography–Mass Spectrometry (GC–MS) represents the preferred platform for this purpose owing to its high sensitivity, accurate separation capacity, and structural elucidation capabilities for volatile and semi-volatile compounds (Sparkman *et al*., 2011). GC–MS has become an indispensable tool for profiling non-polar fractions of African medicinal plants, offering qualitative and quantitative insights into key metabolites such as fatty acid esters, alkanes, diterpenes, and sterols (*Omorede Ikponmwosa-Eweka et al*., 2025; *Ekor et al*., 2020).

This study aims to establish the comprehensive phytochemical profile of the *n*-hexane fraction of *Lonchocarpus cyanescens* leaves using Gas Chromatography–Mass Spectrometry (GC–MS) to identify the major lipophilic metabolites present in the plant.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 L*onchocarpus cyanescens*

2.1.1 Taxonomy and Botanical Description

L*onchocarpus cyanescens* (Schumach. and Thonn.) Benth., commonly known as Yoruba indigo or West African indigo, is a perennial shrub or small tree in the family Fabaceae. It is locally referred to as Elu (Yoruba), Talaki/Talagi (Hausa), Anunu (Igbo), and Ebelu (Edo). The species is documented in the Nigerian Medicinal Plants Compendium as NMP-099 (*Iyoha et al.*, 2025; NMPPDB, 2025).

Taxonomic Classification

Rank	Classification
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	*Lonchocarpus*
Species	*cyanescens* (Schumach. and Thonn.) Benth.

Morphologically, *l. cyanescens* is a deciduous shrub or small tree reaching 6-15 m in height, with a straight bole and rounded crown. Leaves are alternate and pinnate (15-25 cm long) with 5-9 ovate leaflets measuring 4-10 cm. The upper leaf surface is glabrous and dark green, while the underside is slightly pubescent. Flowers are violet-purple and arranged in axillary racemes,

developing into flat papery pods 5-10 cm long (Hutchinson and Dalziel, 1958; Adedikun *et al.*, 2021).

The plant is native to West and Central Africa, thriving in secondary forests, wooded savannahs, and thickets up to 400 m altitude. It prefers sandy-loam soils (pH 5.5–7.0), tolerates seasonal flooding, and regenerates readily after cutting, supporting sustainable cultivation (*Falodun and Osakue*, 2022).

Figure 2.1: Photograph of L*onchocarpus cyanescens* (Schumach. and Thonn.) Benth. – whole plant (NMPPDB, 2025)

2.1.2 Ethnomedicinal Significance

Beyond its use as a natural indigo dye, *l. cyanescens* is widely used in African traditional medicine. In Nigeria, decoctions of the leaves and roots are employed to manage bone pain, arthritis, venereal diseases, diarrhoea, and various gastrointestinal disorders (*Adekunle and Ikumapayi*, 2018). Aqueous preparations are also used for treating psychosis, where the plant is believed to exert sedative and neuroactive effects (*Adedikun et al.*, 2021).

Across West Africa, the plant serves multiple therapeutic roles:

– In Benin, leaf sap is administered for intestinal worms.

– In Guinea, leaves are used as a mild laxative.
– In Sierra Leone, crushed leaves serve as poultices for ulcers and wounds.
– In parts of Nigeria, postpartum women take decoctions of leafy twigs and roots to support recovery (Iyoha *et al.,* 2023; *Falodun and Osakue*, 2022).

The diversity of applications aligns with its recognition as a multipurpose medicinal species (*Isyaka et al.*, 2025).

2.1.3 Phytochemistry and Pharmacological Activities

Phytochemical analyses indicate that *l. cyanescens* contains flavonoids, alkaloids, tannins, saponins, steroids, triterpenoids, chalcones, and rotenoids (*Adekunle and Ikumapayi*, 2018; Isyaka *et al*., 2025). Chromatographic studies have confirmed flavonols (quercetin, kaempferol), isoflavones, and rotenoid derivatives as key constituents (*Adedikun et al.*, 2021).

Antipsychotic and Neuroactive Effects: Aqueous and ethanolic leaf extracts significantly reduce amphetamine-induced stereotypy in rodents, supporting their traditional use in psychosis (*Onyeka et al.*, 2018). Fractionation shows the ethyl acetate fraction has the strongest neuroactive potential, implying medium-polarity metabolites are involved (*Sonibare et al.*, 2014). Extracts also reduce ketamine-induced symptoms, suggesting activity across multiple psychotic domains (*Adedikun et al.*, 2021).

Antimicrobial Activity: Methanolic and ethanolic stem-bark extracts inhibit *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* (*Adegoke et al.*, 2020). These effects are primarily attributed to alkaloid-rich and flavonoid-rich fractions (*Miller and Green*, 2021).

Anti-inflammatory and Anti-arthritic Properties: Methanolic root extracts suppress carrageenan- and xylene-induced oedema, supporting their use in arthritis management (*Umoh et al.*, 2020). These effects are attributed to oleanane derivatives and glycyrrhetinic acid, which may influence prostaglandin pathways (*Moronkola and Oladosu*, 2013).

Antioxidant Potential: Aqueous and methanolic leaf extracts enhance SOD and GPx activity while reducing MDA levels, demonstrating strong antioxidant activity (*Brown et al.*, 2020). This correlates with high phenolic and flavonoid content, especially in semi-purified fractions (*Iyoha et al.*, 2023).

Anthelmintic and Laxative Effects: Saponin-rich fractions exhibit vermifugal activity, supporting ethnomedicinal use in parasitic infections (*Khan and Saleem*, 2021). These same saponins contribute to mild laxative properties.

Antidiabetic Activity: Although only preliminary data exist, some studies report hypoglycaemic effects in animal models, warranting further biochemical validation (Kazeem and Davies 2015).

2.1.4 Toxicological Profile

Toxicological evaluations show that *l. cyanescens* extracts are generally safe at therapeutic doses. Acute toxicity tests report no mortality up to 5000 mg/kg (*Adekunle and Ikumapayi*, 2018). Sub-chronic exposure for 28 days produced no significant hepatic injury at doses up to 2000 mg/kg (*Iyoha et al.*, 2025). However, higher doses (≥ 3500 mg/kg) produced mild elevations in hepatic enzymes (ALT, AST, GGT), indicating possible dose-dependent hepatotoxicity (*Iyoha et al.*, 2023). High doses may cause gastrointestinal

discomfort due to saponins, and topical application of fresh sap may irritate sensitive skin (*Khan and Saleem*, 2021). Despite these minor concerns, the plant remains considered safe within traditional dosage ranges.

2.1.5 Economic, Ecological, and Cultural Importance

The global relevance of *l. cyanescens* is strongly linked to its role in traditional indigo production. The leaves contain indican, which yields indigotin on fermentation, a dye historically traded throughout West Africa (*Onyeka et al.*, 2018). This makes the plant a valuable asset for sustainable livelihoods and cultural preservation.

Ecologically, the species enhances soil fertility through nitrogen fixation and demonstrates strong coppicing ability, making it suitable for agroforestry and erosion control (*Falodun and Osakue*, 2022). Growing pharmacological interest highlights its potential in supplying novel bioactive compounds for drug development, particularly in resource-limited regions (*Isyaka et al., 2025*).

2.2 Techniques Used in Phytochemical Analysis and the Relevance of Hexane Fractionation

The investigation of plant secondary metabolites relies on extraction, separation, and analytical techniques designed to isolate complex mixtures into chemically meaningful fractions. Modern phytochemical workflows integrate classical solvent partitioning with advanced chromatographic and spectroscopic tools to enhance metabolite recovery, improve resolution, and enable accurate compound identification (*Tao et al.,2023*). Fractionation is particularly important when the goal is to characterize specific chemical classes such as the lipophilic constituents targeted in this study.

2.2.1 Extraction and Solvent Partitioning

Extraction represents the foundational step in phytochemical analysis, allowing plant metabolites to be selectively transferred into solvents according to polarity and solubility. In this study, sequential solvent systems including distilled water, ethanol, acetone, and *n*-hexane were employed to progressively enrich fractions with metabolites of similar chemical characteristics (*Lee*, 2021).

Polar solvents (e.g., ethanol, water) efficiently extract hydrophilic constituents such as alkaloids, glycosides, and phenolics, while nonpolar solvents (e.g., *n*-hexane) selectively recover lipophilic metabolites including terpenoids, fatty acids, sterols, and hydrocarbons (*Tao et al.,2023*). Sequential partitioning reduces sample complexity, enhances compound detection, and supports bioassay-guided isolation relevant for pharmacological studies.

2.2.2 Chromatographic and Spectroscopic Techniques

Once crude extracts or solvent fractions are obtained, a suite of chromatographic and spectroscopic techniques is used to separate, identify, and quantify the metabolites present.

- Thin-Layer Chromatography (TLC): is valued as an economical, straightforward, and rapid method for the initial screening and quality control of plant extracts. The technique requires simple, low-cost equipment, making it highly effective for routine analysis in various laboratory settings (Zych and Pyka-Pająk, 2025). TLC is primarily used to establish the phytochemical fingerprint or chromatographic profile of raw material, which is necessary for product authentication and detecting potential adulteration. Although fundamentally a qualitative technique, the method's ability to run multiple samples simultaneously accelerates the analysis time significantly (Zych and Pyka-Pająk, 2025)

- High-Performance Liquid Chromatography (HPLC): is a highly sensitive and precise analytical technique that delivers superior resolution for chemical profiling. It is widely applied for the accurate quantitative determination of chemical markers and specific active compounds within complex herbal samples (Ikponmwosa-Eweka *et al.*, 2025). In phytochemical studies, HPLC is instrumental for confirming preliminary data and conducting comprehensive evaluations of plant composition.

- Gas Chromatography–Mass Spectrometry (GC–MS): Ideal for volatile and semi-volatile compounds, including terpenoids, fatty acids, and sterols making it the preferred tool for lipophilic metabolite identification (*Smith*, 2023). GC–MS offers detailed mass spectral data essential for compound elucidation.

- Liquid Chromatography–Mass Spectrometry (LC–MS): Suited for non-volatile or thermally unstable constituents such as alkaloid and flavonoid glycosides. It remains indispensable in untargeted plant metabolomics.

- Nuclear Magnetic Resonance (NMR): Provides definitive structural information and is used alongside MS when identifying novel or structurally complex compounds.

2.2.3 Relevance of Hexane Fractionation

The *n*-hexane fraction is central to phytochemical and metabolomic research because it concentrates nonpolar, lipophilic metabolites that may not be recovered in polar extracts. Typical constituents enriched in hexane fractions include long-chain fatty acids, sterols, terpenoids, hydrocarbons, and certain lipophilic pigments (*Lee*, 2021; *Tao et al.,2023*). Many of these

compounds possess significant pharmacological properties, including anti-inflammatory, antioxidant, antimicrobial, and anticancer activities (*Falodun and Osakue*, 2022).

Lipophilic metabolites also demonstrate improved membrane permeability and may exhibit higher bioavailability, highlighting their relevance to drug discovery. Previous analyses of *l. cyanescens* seed oil has reported linolenic and oleic acids, phytol, and sterols within the unsaponifiable matter metabolites commonly isolated in the hexane fraction (*Moronkola and Oladosu*, 2013).

For *Lonchocarpus cyanescens*, focusing on the hexane fraction is particularly important because emerging reports suggest the presence of bioactive triterpenoids, sterols, and long-chain fatty acids. However, comprehensive GC–MS characterization of this fraction remains scarce in the literature (*Adekunle and Ikumapayi*, 2018; *Isyaka et al., 2025*). Generating a detailed GC–MS profile will therefore help address this research gap, validate ethnomedicinal claims, and provide a foundation for advanced pharmacological and metabolomic studies on the species.

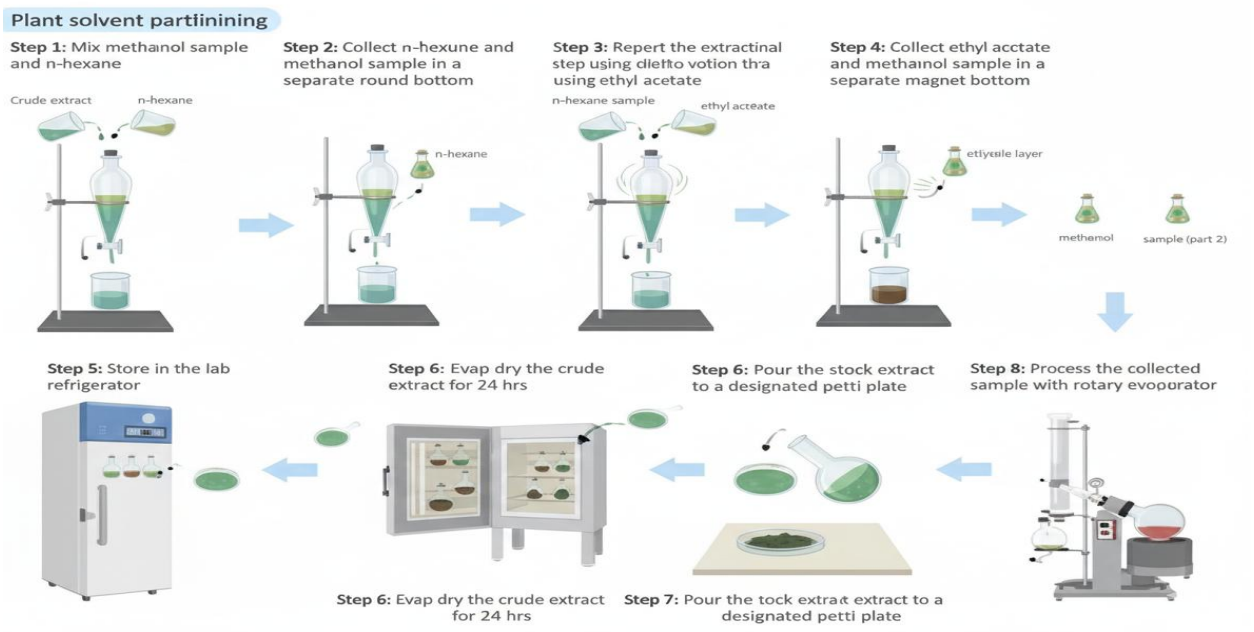

Figure 2.2: Liquid-liquid solvent partitioning of plant extract (BioRender 2025)

2.3 Gas Chromatography–Mass Spectrometry (GC–MS) in Phytochemical Analysis

Gas Chromatography–Mass Spectrometry (GC–MS) is one of the most powerful analytical techniques used in phytochemical research because it combines the separation efficiency of gas chromatography with the structural identification capability of mass spectrometry. The technique is particularly suited for the analysis of volatile and semi-volatile organic compounds, making it indispensable in plant metabolomics. Its application has expanded in recent years, contributing significantly to chemical profiling, quality control of herbal preparations, chemotaxonomy, and natural product–based drug discovery (Davies, 2018; Smith, 2023).

The principle of GC–MS involves two integrated analytical processes. First, in the gas chromatography unit, the sample is vaporised and transported through a capillary column by an inert carrier gas such as helium. As the mixture travels through the column, its components are separated based on differences in volatility, boiling points, and their affinity for the stationary phase. This process gives each compound a distinctive retention time, which serves as part of its chemical identity (Smith, 2023). As the separated compounds elute from the GC column, they enter the mass spectrometer, where they are ionised most commonly through electron impact ionisation. This ionisation step fragments the molecules into charged ions that are then separated according to their mass-to-charge ratios. The resulting mass spectra act as molecular fingerprints, which are compared against reputable reference libraries such as NIST, Wiley, or the updated NIST 2023 MS database to facilitate reliable compound identification (Sparkman *et al*., 2018).

GC–MS offers several strengths that make it a preferred technique for investigating plant secondary metabolites. Its ability to resolve highly complex mixtures allows for precise qualitative and quantitative analysis of numerous volatile constituents. The availability of extensive spectral databases enhances the speed and accuracy of compound identification,

making GC–MS a robust and reproducible method for profiling terpenes, sterols, fatty acid methyl esters, aliphatic hydrocarbons, and essential oil constituents. These characteristics underline its importance in chemotaxonomic studies, as plants often exhibit distinct chemical fingerprints that support species differentiation and classification (Davies, 2018; Smith, 2023).

Despite its versatility, GC–MS is inherently limited by its dependence on compound volatility and thermal stability. Many plant metabolites such as flavonoid glycosides, tannins, and certain alkaloids are either too polar or too thermolabile to be directly analysed using GC–MS. Such compounds typically require derivatisation using reagents such as BSTFA to enhance volatility. While derivatisation expands analytical capability, it may also introduce artefacts, complicate interpretation, and extend sample preparation time. For these reasons, highly polar or non-volatile metabolites are often better analysed using alternative methods such as LC–MS, which does not require sample volatilisation (Smith, 2023).

The application of GC–MS in the present study is justified by the chemical characteristics of the hexane fraction of *Lonchocarpus cyanescens*. This fraction is expected to contain predominantly nonpolar, volatile, and semi-volatile constituents, including terpenoids, fatty acid esters, sterols, and various aliphatic hydrocarbon compound classes that are ideally suited for GC–MS evaluation (Lee, 2021). By subjecting this fraction to GC–MS analysis, the study aims to generate a comprehensive profile of the lipophilic metabolites present in the plant. Such a profile not only aids in the structural identification of bioactive compounds through spectral matching but also strengthens the link between the plant's chemical composition and its documented ethnomedicinal uses, particularly its anti-inflammatory and antimicrobial applications. Furthermore, identifying key metabolites in the hexane fraction may provide promising lead compounds for future pharmacological and drug development investigations.

2.4 Previous Studies

Several investigations have examined the medicinal relevance of *Lonchocarpus cyanescens*, a plant widely recognised within African ethnomedicine for its therapeutic versatility. Research efforts have explored its antioxidant, anti-ulcer, neuropharmacological, antidiabetic, and toxicological properties, thereby providing substantial scientific validation for its traditional applications. These previous studies collectively highlight the plant's rich biochemical profile and the pharmacological activities of its extracts and fractions.

2.4.1 Antioxidant Activity

The antioxidant potential of *l. cyanescens* has been demonstrated through various bioassay-guided studies. Leaf extracts of the plant have shown strong radical-scavenging activities in assays such as DPPH and FRAP, with performances comparable to recognised standard antioxidants (Brown *et al*., 2020). These antioxidant effects have been strongly associated with the plant's high phenolic and flavonoid content. Additional investigations further confirmed that the active constituents responsible for this antioxidant capacity are concentrated in the leaf extracts, providing evidence that these phytochemicals contribute significantly to the plant's ability to neutralise free radicals (Iyoha *et al*., 2023).

2.4.2 Molecular Docking and Anti-Ulcer Potential

Beyond antioxidant properties, *l. cyanescens* has attracted interest for its anti-ulcer potential. Molecular docking studies have been used to evaluate the interaction of triterpenoids isolated from the plant with ulcer-related protein targets. The triterpenoid ligands demonstrated strong binding affinities that compared favourably with established anti-ulcer drugs, suggesting a

meaningful therapeutic potential. The study by Falodun and Osakue (2022) concluded that these triterpenoids not only possess high antioxidant and anti-inflammatory capabilities but also exhibit pharmacologically relevant binding interactions, positioning them as promising candidates for anti-ulcer drug development.

2.4.3 Neuropharmacological and Antipsychotic Effects

Extensive research has also established the neuropharmacological potential of *l. cyanescens*, particularly in the management of psychosis-like conditions. Earlier studies revealed that aqueous and ethanolic leaf extracts suppressed amphetamine-induced stereotypy and reduced spontaneous motor activity, effects comparable to standard antipsychotic agents but without inducing catalepsy, a common adverse effect of typical neuroleptics (Onyeka *et al*., 2018). Subsequent fractionation efforts identified the ethyl acetate fraction as the most active in reducing apomorphine-induced stereotypy and amphetamine-induced hyperactivity, suggesting that medium-polarity constituents contain key neuroactive compounds (Sonibare *et al*., 2014). More recent investigations have demonstrated that both the crude extract and its solvent fractions also ameliorate ketamine-induced psychosis, improving positive, negative, and cognitive behavioural outcomes (Adedikun *et al.*, 2021).

2.4.4 Comprehensive Phytochemistry and Review

Phytochemical reviews of the genus *Lonchocarpus* have consistently highlighted the chemical richness of *L.cyanescens*. The species has been shown to contain a wide range of polyphenolic compounds, including chalcones, aurones, pterocarpans, rotenoids, and diverse triterpenoids (Isyaka *et al*., 2025). These reviews reinforce its long-standing ethnomedicinal use across

African communities and establish a clear linkage between its wide range of bioactivities and the structural diversity of its secondary metabolites (Isyaka *et al*., 2025).

2.4.5 Antidiabetic Enzyme Inhibition and Other Pharmacological Evidence

Research directly assessing the antidiabetic potential of *L. cyanescens* provides compelling support for its traditional use in the treatment of metabolic disorders. Kazeem and Davies (2015) demonstrated that the leaf extracts, particularly the ethanol extract, significantly inhibited α-amylase and α-glucosidase enzymes, with IC_{50} values of 3.69 mg/mL and 0.13 mg/mL, respectively. The α-glucosidase inhibition displayed by the ethanol extract was particularly notable, as its potency surpassed that of the aqueous extract and even the standard drug acarbose (IC_{50} = 0.63 mg/mL). Enzyme kinetics indicated competitive inhibition of α-amylase and non-competitive inhibition of α-glucosidase, while in vivo experiments further showed that administration of the extract significantly reduced postprandial glucose in starch-loaded rats.

Additional investigations have explored the plant's inhibitory effects on aldose reductase (AR), a key enzyme implicated in diabetic complications such as neuropathy and cataracts. Ajani *et al.* (2017) reported remarkable AR inhibition across multiple fractions, with the aqueous and chloroform extracts exhibiting the strongest activity. The presence of phenols, flavonoids, tannins, and phlobatannins was identified as the phytochemical basis for these inhibitory effects. Furthermore, variation in inhibition mechanisms, competitive inhibition in medium-polarity solvents and mixed inhibition in aqueous and chloroform fractions suggests the presence of structurally diverse bioactive compounds. In terms of safety, sub-chronic toxicity studies have shown that aqueous extracts administered over 42 days induce only mild hepatic changes such as

vascular dilation and Kupffer cell activation, without significant damage, confirming a low toxicity profile (Onyeije *et al*., 2024).

2.4.6 Summary of Previous Findings

Collectively, previous research highlights the diverse pharmacological potential of *l. cyanescens*, with antioxidant, anti-ulcer, neuroactive, antidiabetic, and generally safe profiles attributed to its rich content of flavonoids, triterpenoids, phenolics, and related compounds. Although these studies provide strong biochemical support for the plant's ethnomedicinal reputation, it is evident that most investigations have focused on polar and medium-polar extracts. Only limited work has been conducted on the chemical composition of the non-polar constituents. As a result, little is known about the GC–MS profile of the *n*-hexane fraction, suggesting a significant gap that warrants further research.

2.5 Knowledge Gap

Despite increasing research on the phytochemistry and pharmacology of *Lonchocarpus cyanescens*, its non-polar chemical constituents remain poorly understood. Previous studies have focused mainly on polar extracts (ethanol, methanol, acetone), which reveal hydrophilic metabolites such as flavonoids, tannins, and phenolic acids (Adekunle and Ikumapayi, 2018; Isyaka *et al.,* 2025), leaving lipophilic terpenoids, sterols, aliphatic hydrocarbons, and fatty acid derivatives largely unexamined. Although molecular docking studies have suggested potential anti-ulcer activity for some triterpenoids, these findings are based on computational predictions rather than confirmed chemical identification.

To date, no comprehensive GC–MS analysis has been performed on the hexane fraction of *L. cyanescens* leaves, despite GC–MS being the most suitable technique for profiling volatile and semi-volatile non-polar compounds. Existing GC–MS reports focus mainly on seed oil or essential oil compositions (Moronkola and Oladosu, 2013), providing limited insight into leaf-derived metabolites.

This study, therefore, fills this gap by conducting a detailed GC–MS characterisation of the hexane fraction of *Lonchocarpus cyanescens* leaves, providing new information on its lipophilic constituents and laying the groundwork for future chemotaxonomic and pharmacological investigations.

CHAPTER 3

3.0 MATERIALS AND METHODOLOGY

3.1 Collection, Identification, and Preparation of Plant Material

Fresh leaves of *Lonchocarpus cyanescens* were purchased from a local herbal vendor in Lagos State, Nigeria. The plant was authenticated by Prof. H. A. Akinnibosun at the Department of Plant Biology and Biotechnology, University of Benin, Edo State, and deposited under voucher number UBH-F029.

The leaves were rinsed thoroughly with distilled water to eliminate soil particles and other debris. Drying was carried out under shade at ambient laboratory conditions (approximately 25 ± 2 °C) for 21 days, ensuring minimal exposure to direct sunlight to preserve thermolabile compounds. Once completely dried, the leaves were milled into a fine powder using a mechanized grinder. The total yield of dried powdered plant material obtained for extraction was 4171.823 g.

3.2 Materials

Analytical-grade reagents were used for all procedures to maintain reproducibility and purity. These included:

- Absolute ethanol (99.9%)
- n-Hexane
- Deionised/distilled water
- GC-MS grade solvents
- High-purity helium gas (99.999%) for GC–MS carrier flow

3.3 Equipment

The major instruments and laboratory apparatus used in this study included:

- Analytical weighing balance
- Standard glassware (beakers, cylinders, funnels, stirring rods)
- Glass separatory funnel
- Rotary evaporator
- Freeze dryer
- Muslin cloth and aluminium foil
- Airtight containers for extract storage
- Agilent GC–MS system equipped with MassHunter software

3.4 Preparation of Plant Extract

Extraction was performed using a modified maceration technique adapted from Guillaume *et al*. (2011). A portion of the powdered leaves (2500 g) was placed in a clean extraction vessel and submerged in 10 L of absolute ethanol, maintaining a plant-to-solvent ratio of 1:4 (w/v). The mixture was macerated for 72 hours and agitated intermittently to facilitate efficient solute diffusion into the solvent.

After maceration, the mixture was filtered through muslin cloth to remove plant residues. The ethanol filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator. The semi-solid concentrate was subsequently freeze-dried to obtain a dry ethanolic crude extract (CEE). The total mass of dried CEE obtained was 155 g, which was stored in airtight containers at 4 °C until required for fractionation.

3.5 Hexane Fractionation of Crude Extract

The crude ethanolic extract was fractionated using standard liquid–liquid partitioning based on a modified procedure of Jamil *et al.* (2012). Exactly 50 g of CEE was dissolved in 250 mL of ethanol (0.2 g/mL; 20% w/v) to achieve a uniform solution. The solution was transferred into a separatory funnel, followed by the addition of 250 mL of *n*-hexane. The mixture was shaken vigorously with intermittent venting to release pressure, allowing non-polar compounds to partition preferentially into the *n*-hexane phase. The funnel was allowed to stand until two distinct layers formed. The upper hexane layer was carefully collected.

This procedure was repeated eight times, each with fresh portions of *n*-hexane, to ensure exhaustive extraction of non-polar constituents. All hexane layers were pooled, filtered, and concentrated under reduced pressure at 40 °C. The resulting hexane fraction was stored at 4 °C pending GC-MS analysis.

Figure 3.1: Fractionation of Crude Extract of L*onchocarpus cyanescens* using Hexane, Acetone, and Distilled Water

3.6 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The hexane fraction of *Lonchocarpus cyanescens* was analysed using an Agilent GC–MS system equipped with MassHunter software and the NIST14.L spectral library. Before injection, the dried sample was dissolved in an appropriate GC-grade solvent to obtain a clear solution suitable for analysis. A 1.0 µL portion of this solution was introduced into the injection port in splitless mode to ensure that the maximum amount of analyte entered the column.

The chromatographic separation was carried out on an HP-5MS capillary column measuring 30 m × 0.25 mm with a film thickness of 0.25 µm. Helium gas with a purity of 99.999% served as the mobile phase and was maintained at a constant flow rate of 1.0 mL/min throughout the run. The injector temperature was adjusted to promote complete vaporisation of the sample. The oven program was set to begin at 60 °C, where the temperature was held for two minutes before being increased at a rate of 10 °C per minute until reaching 280 °C. The final temperature was held for ten minutes, producing a total run time of approximately twenty minutes. This temperature programming allowed compounds of different volatilities to elute in an orderly manner.

As each component exited the GC column, it entered the mass spectrometer, where ionisation occurred through electron impact at 70 eV. The ionised molecules fragmented into characteristic ions which were then separated based on their mass-to-charge ratios. The system operated in scan mode so that spectra were continuously acquired for each eluting compound. Each spectrum generated contained both molecular ions and fragment ions, producing a unique fingerprint for each compound.

Identification of the constituents in the sample was carried out by comparing the obtained mass spectra and retention data with those contained in the NIST14.L mass spectral library

incorporated into the MassHunter software. Only compounds with acceptable spectral similarity indices and reliable match factors were recorded as identified constituents. The relative abundance of each compound was automatically determined from the percentage peak areas generated by the analytical software. These peak area values provided an estimate of how much each compound contributed to the total composition of the hexane fraction.

3.7 Data Analysis and Quantification

The GC-MS software automatically generated data, including retention times and percentage peak areas. The percentage peak area was used to estimate the relative abundance of each compound. Descriptive statistics produced mean retention time values (±1.52 min) and mean peak area percentages (±3.42%). Identified compounds were grouped into chemical classes to improve the interpretation of dominant lipophilic constituents in the hexane fraction.

CHAPTER 4

4.0 RESULTS

Table 4.1: GC-MS spectral analysis of hexane extract of L*onchocarpus cyanescens*

No	Compound (Library ID)	Molecular Formula	Molecular weight	Retention Time (min)	Peak Area (%)
1	1-Ethyl-2-methylbenzene	C_9H_{12}	120.19	3.179	6.63
2	(1S,3R)-(+)-m-Menthane	$C_{10}H_{20}$	140.27	3.213	3.37
3	1,2,4-Trimethylbenzene (Mesitylene)	C_9H_{12}	120.19	3.362	33.50
4	n-Butylbenzene	$C_{10}H_{14}$	134.22	3.494	1.79
5	4-Methyl-decane	$C_{11}H_{24}$	156.31	3.608	2.41
6	p-Cymene	$C_{10}H_{14}$	134.22	3.665	2.38
7	Mesitylene / 1-Ethyl-3-methylbenzene	C_9H_{12}	120.19	3.705	5.76
8	Butyl-cyclohexane	$C_{10}H_{20}$	140,27	3.768	2.19
9	1-Methyl-3-propylbenzene	$C_{10}H_{14}$	134.22	4.031	2.30
10	o-Cymene / 1-Ethyl-3,5-dimethylbenzene	$C_{10}H_{14}$	134.22	4.123	6.42
11	o-Cymene (duplicate peak)	$C_{10}H_{14}$	134.22	4.398	1.80
12	4-Ethyl-1,2-dimethylbenzene	$C_{10}H_{14}$	134.22	4.478	2.32
13	Undecane	$C_{11}H_{24}$	156.31	4.592	4.45
14	Methyl dodecanoate (methyl laurate)	$C_{13}H_{26}O_2$	214.34	9.582	5.11
15	Methyl tetradecanoate (methyl myristate)	$C_{15}H_{30}O_2$	242.4	11.522	1.92

16	Methyl hexadecanoate (methyl palmitate)	$C_{17}H_{34}O_2$	270.46	13.278	3.13
17	Methyl oleate (9-octadecenoic acid methyl ester)	$C_{19}H_{36}O_2$	296.5	14.692	3.43
18	1,3-Dioctanoin	$C_{19}H_{36}O_4$	328.49	15.063	6.23
19	Bis(2-ethylhexyl) phthalate (DEHP)	$C_{24}H_{38}O_4$		19.309	4.86

Peak numbering corresponds to the order of elution and to the peaks annotated on the total ion chromatogram (TIC) shown in Figure 4.1. *NIST (2017) NIST 14 Mass Spectral Library. Gaithersburg: National Institute of Standards and Technology.*

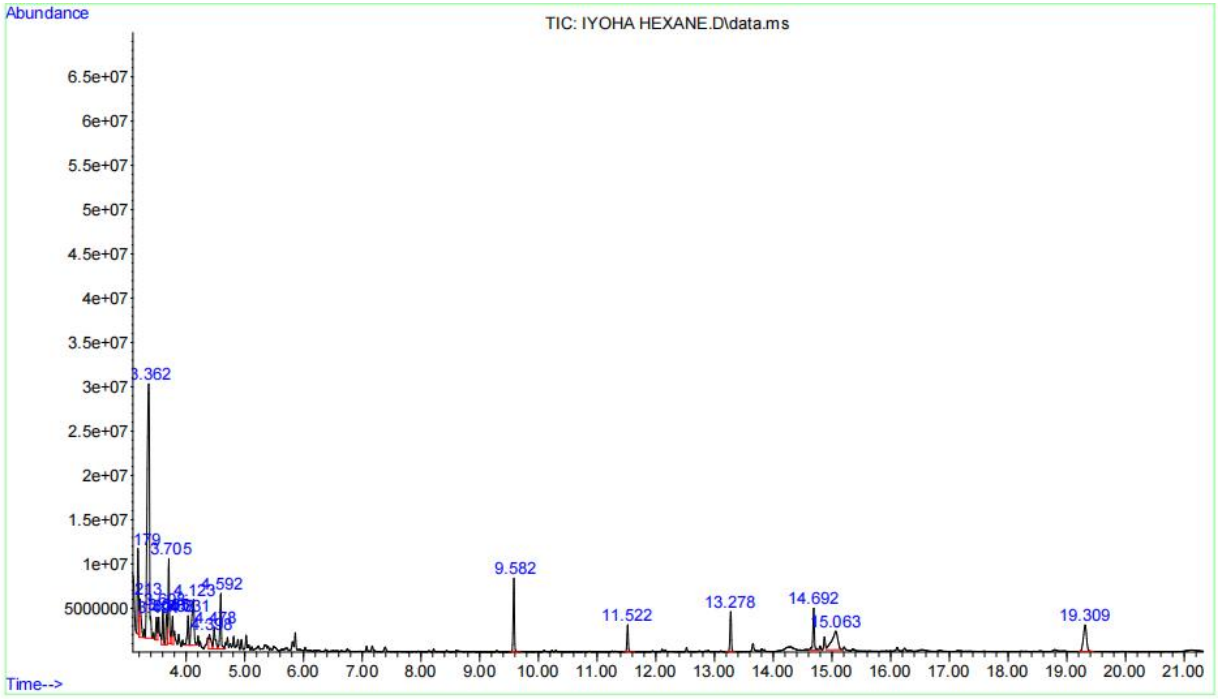

Figure 4.1: Gas Chromatography- Mass spectrum of hexane extract of L*onchocarpus cyanescens*

Table 4.2: Chemical characteristics of the compounds identified in the hexane fraction of L*onchocrpus cyanescens* by GC-MS

Phytochemical Class	Subclass	Compounds Identified
Aromatic Hydrocarbons	Benzenes	1,2,4-Trimethylbenzene (Mesitylene), 1-Ethyl-2-methylbenzene, n-Butylbenzene, 1-Methyl-3-propylbenzene, 4-Ethyl-1,2-dimethylbenzene
	Aromatic-like (Hybrid)	Mesitylene / 1-Ethyl-3-methylbenzene
Terpenoids	Monoterpene Hydrocarbons	(1S,3R)-(+)-m-Menthane, p-Cymene, o-Cymene, o-Cymene (duplicate peak)
Lipids and Derivatives	Fatty-Acid Methyl Esters (FAMEs)	Methyl dodecanoate (Methyl laurate), Methyl tetradecanoate (Methyl myristate), Methyl hexadecanoate (Methyl palmitate), Methyl oleate (9-Octadecenoic acid methyl ester)
	Glycerides (Diesters)	1,3-Dioctanoin (Glyceryl dioctanoate)
Aliphatic and Cycloalkane Hydrocarbons	Straight-Chain Alkanes	Undecane
	Branched Alkanes	4-Methyl-decane
	Cycloalkanes	Butyl-cyclohexane

CHAPTER 5

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

Medicinal plants continue to serve as vital reservoirs of structurally diverse bioactive compounds, underpinning both traditional remedies and modern pharmaceuticals. *Lonchocarpus cyanescens* (Schumach. and Thonn.) Benth., commonly known as Yoruba indigo, has a longstanding ethnomedicinal history in West and Central Africa, particularly in the management of inflammation, arthritis, diarrhoeal diseases, and neuropsychiatric disorders. Previous studies have predominantly focused on polar extracts rich in flavonoids, triterpenoids, and phenolic acids, leaving the plant's non-polar constituents largely unexplored. To address this gap, the present study employed Gas Chromatography–Mass Spectrometry (GC–MS) to profile the n-hexane fraction, revealing the lipophilic metabolites that may complement the pharmacological activities of polar compounds.

The GC–MS analysis of the hexane fraction revealed nineteen compounds spanning aromatic hydrocarbons, monoterpenes, aliphatic hydrocarbons, fatty acid methyl esters, and glycerides. This confirms that *L. cyanescens* leaves contain a chemically diverse set of non-polar metabolites with potential pharmacological relevance. The major compounds identified, in order of abundance, were 1,2,4-trimethylbenzene (33.50%), 1-ethyl-2-methylbenzene (6.63%), o-cymene (6.42%), and 1,3-dioctanoin (6.23%). Other compounds, such as methyl laurate, methyl palmitate, and methyl oleate, were present at lower concentrations but contribute to the overall bioactivity profile of the fraction.

The most abundant compound, 1,2,4-trimethylbenzene, is an aromatic hydrocarbon widely used in industrial and pharmaceutical applications due to its lipophilicity and membrane-permeating

properties. In biological systems, compounds of this class can interact with lipid membranes, influencing oxidative processes and cellular protection mechanisms (Moronkola *et al*., 2013). Previous studies have associated aromatic hydrocarbons with antimicrobial and antioxidant activity, suggesting that 1,2,4-trimethylbenzene may contribute to the traditional anti-infective and anti-inflammatory uses of *L. cyanescens*. Its prevalence in the hexane fraction highlights the plant's potential as a source of membrane-active bioactive agents that modulate microbial viability and oxidative stress.

The second most abundant compound, 1-ethyl-2-methylbenzene, is another lipophilic aromatic hydrocarbon with documented antimicrobial and antioxidant potential. Its ability to penetrate lipid bilayers allows it to exert protective effects on cells while contributing to the overall bioactivity of the hexane fraction (Moronkola *et a*l., 2013).

The third most abundant compound, o-cymene, is a monoterpene known for broad pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and analgesic activities (Babatunde *et al*., 2014). Monoterpenes like o-cymene readily penetrate biological membranes, disrupting microbial cell walls and modulating oxidative stress pathways. This aligns with findings in other medicinal plants, where monoterpene-rich fractions exhibited potent anti-inflammatory and cytoprotective effects. The presence of o-cymene in *L. cyanescens* reinforces its ethnomedicinal relevance, particularly in treating infections and inflammatory conditions.

1,3-Dioctanoin, the fourth most abundant compound, is a glyceride with known roles in lipid metabolism and membrane stabilization. Glycerides are increasingly recognized for their mild antimicrobial and antioxidant activity, often acting synergistically with other bioactive

constituents to enhance overall pharmacological effect (Moronkola and Oladosu, 2013). The identification of 1,3-dioctanoin in the hexane fraction suggests potential applications in formulations aimed at modulating lipid-based signalling pathways or supporting cellular protection, complementing the effects of aromatic hydrocarbons and monoterpenes. The combined presence of these four compounds illustrates a chemically and pharmacologically meaningful fraction, likely supporting some of the traditional therapeutic claims associated with *L. cyanescens*.

Compared with previous studies focusing on polar and medium-polar extracts, the present work demonstrates that non-polar fractions contain structurally distinct compounds that may contribute in complementary ways to the plant's medicinal properties. While flavonoids and triterpenoids have been linked to antipsychotic and antidiabetic effects (Onyeka *et al*., 2018; Kazeem and Davies, 2015), lipophilic constituents may enhance antimicrobial, anti-inflammatory, and antioxidant potential. Notably, compounds such as 1,2,4-trimethylbenzene, 1-ethyl-2-methylbenzene, and 1,3-dioctanoin have not been previously reported in *L. cyanescens*, representing novel findings that expand the chemical understanding of the species.

Despite these insights, the study had some limitations. Compound identification relied solely on library matching without verification using authentic standards, and only the hexane fraction was examined. The presence of Bis(2-ethylhexyl) phthalate likely reflects contamination from plastic materials. Additionally, quantitative analysis of each compound was not performed, limiting the ability to correlate relative abundance directly with pharmacological effect.

Future studies should aim to include standard verification, replicate analyses, and complementary analytical techniques such as LC–MS or NMR to confirm compound structures.

Bioassays of isolated compounds are recommended to establish pharmacological activity and potential synergistic effects. Comparative studies across multiple solvent fractions would further elucidate structure–activity relationships, thereby maximizing the ethnomedicinal value of *L. cyanescens*.

5.2 Conclusion

The n-hexane fraction of *Lonchocarpus cyanescens* leaves was successfully characterized using GC–MS, revealing 19 non-polar compounds, including aromatic hydrocarbons, monoterpenes, fatty acid methyl esters, aliphatic hydrocarbons, and glycerides. The major constituents 1,2,4-trimethylbenzene, 1-ethyl-2-methylbenzene, o-cymene, and 1,3-dioctanoin possess documented antimicrobial, anti-inflammatory, antioxidant, and membrane-active properties, supporting some of the plant's traditional medicinal uses. The predominance of volatile and lipophilic compounds demonstrates the efficiency of hexane in selectively extracting nonpolar metabolites. These findings provide a detailed chemical profile of the hexane fraction, thereby expanding the phytochemical knowledge of L. *cyanescens* and laying a foundation for future isolation, characterization and pharmacological evaluation of its bioactive constituents.

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