

Isolation and Characterization of Methanolic Stem Bark Extracts of *Polyalthia longifolia* and Root Extract of *Annona senegalensis* Plants

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Abstract

The growing global concern over antimicrobial resistance has prompted intensified research into plant-derived natural products as alternative therapeutic agents. This study aimed to isolate and characterize the bioactive constituents from the methanolic stem bark extract of *Polyalthia longifolia* and the root extract of *Annona senegalensis*, two plants widely used in West African ethnomedicine. The crude extracts were subjected to fractionation using column chromatography, employing solvent systems of increasing polarity. The obtained fractions were analysed by Thin Layer Chromatography (TLC) to determine the number of constituent compounds and their retention factor (R_f) values. Characterization of the most active fractions was carried out using Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC-MS). The

crude extracts were successfully fractionated resulting in six fractions from each plant. Fractions F₁ and F₅ from *P. longifolia* and F₃ and F₄ from *A. senegalensis* were selected for detailed phytochemical investigation based on preliminary thin-layer chromatography (TLC) and bioactivity screening. The FTIR spectroscopy revealed the presence of hydroxyl (O-H), carbonyl (C=O), alkyl (C-H), ester (C-O), and aromatic (C=C) groups—consistent with alcohols, flavonoids, phenolics, glycosides, and fatty acids. Gas Chromatography–Mass Spectrometry (GC-MS) analysis further identified a wide range of bioactive compounds. For *P. longifolia*, major constituents included 2,4-di-tert-butylphenol, methyl palmitate, n-hexadecanoic acid, methyl stearate, and squalene—compounds reported in literature to possess significant antimicrobial, anti-inflammatory, and antioxidant activities. Similarly, the GC-MS profile of *A. senegalensis* fractions revealed the presence of (+)-roemerine, trans-4-chlorochalcone, hexadecanoic acid, and 9-octadecenoic acid, among others, which have also been associated with broad-spectrum antimicrobial effects. The results validate the ethnomedicinal use of these plants and support their potential as sources of new antimicrobial agents. The combination of FTIR and GC-MS provided a reliable approach to profiling and identifying phytoconstituents in complex plant matrices. These findings not only expand the phytochemical knowledge of *P. longifolia* and *A. senegalensis* but also contribute to ongoing efforts in natural product-based drug discovery.

Introduction

Medicinal plants have served as therapeutic agents and sources of novel bioactive compounds for centuries. According to the World Health

Organization (WHO, 2022), nearly 80% of the world's population, particularly in developing countries, relies on traditional herbal medicine for their primary healthcare needs. This widespread reliance has prompted increased scientific inquiry into medicinal plants as reservoirs of bioactive secondary metabolites, especially in the face of rising antimicrobial resistance. Natural products and their derivatives currently represent over 50% of drugs in clinical use, and their continued exploration is crucial for addressing pressing global health challenges (Atanasov *et al.*, 2021).

The *Annonaceae* family is one of the most prominent in tropical ethnomedicine, with species such as *Polyalthia longifolia* and *Annona senegalensis* being traditionally used for a wide range of ailments. *Polyalthia longifolia*, commonly known as the “false ashoka” or “Indian mast tree,” is native to the Indian subcontinent but is also widely cultivated in tropical Africa, including Nigeria. It has been traditionally employed in the treatment of fever, skin diseases, microbial infections, and hypertension (El-Mahmood *et al.*, 2010; Ramesh *et al.*, 2011). Phytochemical investigations have revealed that *P. longifolia* is rich in biologically active compounds such as alkaloids (e.g., liriodenine), flavonoids, saponins, and diterpenoids, all of which have demonstrated pharmacological activities including antimicrobial, cytotoxic, antioxidant, and anti-inflammatory effects (Oyedemi *et al.*, 2018; Sudjaroen, 2020).

Similarly, *Annona senegalensis*, known locally in many parts of Africa as “wild custard apple,” is a deciduous shrub or small tree widely used in folk medicine across West and Central Africa. Traditionally, various parts of the plant—especially the root, stem bark, and leaves—are used in the treatment of fever, diarrhea, respiratory ailments, urinary tract infections, and skin diseases (Ngueguim *et al.*, 2019). Several studies have reported that extracts of *A. senegalensis* exhibit antibacterial, antifungal, anti-inflammatory, and antioxidant properties (Yakubu *et al.*, 2021; Oyedemi *et al.*, 2018). Phytochemical screening of this species has identified a rich profile of flavonoids, tannins, phenols, glycosides, and alkaloids, with compounds such as roemerine and quercetin being well-documented for their therapeutic potential (Jain *et al.*, 2021; Babalola *et al.*, 2021).

Despite the well-documented traditional uses of these plants, there is a need for systematic scientific validation of their phytochemical constituents and pharmacological properties through advanced analytical techniques. Isolation and identification of active principles in plant extracts require bioactivity-

guided fractionation and sophisticated spectroscopic and chromatographic tools such as column chromatography, Fourier Transform Infrared (FTIR) spectroscopy, and Gas Chromatography-Mass Spectrometry (GC-MS). These methods allow for the elucidation of functional groups, compound structures, and potential pharmacophores within plant matrices, enabling a deeper understanding of the biological mechanisms and therapeutic relevance of the isolated constituents (Sasidharan *et al.*, 2011; Desbois and Smith, 2010).

In this study, the methanolic extracts of *P. longifolia* stem bark and *A. senegalensis* root were subjected to column chromatography, and selected fractions (F1 and F5 for *P. longifolia*; F3 and F4 for *A. senegalensis*) were analyzed using FTIR and GC-MS techniques. These specific fractions were chosen based on preliminary TLC analysis and bioassays, which indicated the presence of multiple bioactive components. By identifying and characterizing the chemical constituents of these fractions, the study aims to validate traditional claims, uncover potential therapeutic agents, and contribute to the growing compendium of plant-derived drug candidates.

Ultimately, the findings from this work are expected to contribute to the rational development of plant-based therapeutics, bridge traditional and modern medicine, and support sustainable utilization of indigenous botanical resources. Furthermore, the outcomes will provide a scientific framework for the standardization and quality control of herbal medicines derived from *P. longifolia* and *A. senegalensis*.

Materials and Methods

Plant Collection and Extraction Procedures

Fresh stem bark of *Polyalthia longifolia* and root of *Annona senegalensis* were collected from wild-growing mature plants in Mubi North, Adamawa State, Nigeria, and authenticated by Dr. John Kagana Dagze at the Department of Biological Sciences, Federal Polytechnic Mubi. The plant materials were air-dried under shade to prevent photodegradation and ground into fine powder using a laboratory mill. A total of 200 grams of each powdered sample were subjected to Soxhlet extraction using 95% methanol for 8 hours. The methanol extracts were concentrated under reduced pressure using a rotary evaporator at 40 °C to remove the solvent, and the crude extracts were stored in amber bottles at 4 °C until further analysis.

Column Chromatography and Thin Layer Chromatography (TLC)

Five grams of the dried methanolic extract were pre-adsorbed onto silica gel and loaded onto a column packed with silica gel (60–120 mesh). Elution was performed with increasing polarity gradients of hexane:ethyl acetate and ethyl acetate:methanol. Fractions were collected in volumes of 20–40 mL and monitored via TLC using various solvent systems. Spots were visualized under UV light at 254 and 365 nm and by spraying with iodine and vanillin-sulfuric acid spray reagents. The R_f values were calculated to monitor compound separation (Hameed *et al.*, 2023).

Antimicrobial Activity of Isolated Fractions of the Plants

The antimicrobial activity of the isolated fractions of the plants were evaluated using Agar Well Diffusion Method for assessing antimicrobial activity, (Balouri *et al.*, 2016).

A well was punched into the agar plate, and the plant extract was placed directly into the well.

- Molten nutrient agar was poured into sterile petri dishes and allow to solidify.
- The surface of the agar was inoculated with the microbial suspension (using swab).
- A well (6 mm in diameter) was punched into the agar plates using a sterile cork borer.
- Each well was filled with 50, 75, and 100 mg/mL of the isolated fractions of the plants.
- The plates were incubated at 37°C for 24 hours.
- The diameter of the zone of inhibition in mm around each well were measured.

The results were compared with a standard antibiotic (Gentamycin) for reference.

Fourier Transform Infrared (FTIR) Spectroscopy

Fractions showing promising activity were subjected to FTIR analysis. KBr pellet method was used for preparing the samples, and spectra were recorded over the range 4000–400 cm⁻¹ using a PerkinElmer FTIR spectrometer. Functional groups were identified based on standard IR absorption frequencies (Ogunleye *et al.*, 2021)

Gas Chromatography–Mass Spectrometry (GC-MS)

Active fractions were analyzed using an Agilent 7890A GC system coupled with an Agilent 5975C mass selective detector. An HP-5MS capillary column was used, with helium as the carrier gas. Injection volume was 1 μ L in split mode (1:10). The temperature program was: initial 60 °C for 3 minutes, ramped to 300 °C at 10 °C/min. Compounds were identified based on retention time and spectral matching with the NIST14 MS library (Halilu *et al.*, 2023).

Results and Discussion

The methanolic stem bark extract of *Polyalthia longifolia* was successfully fractionated into six fractions using column chromatography with increasing polarity gradients as shown in Table 1. Among these, Fractions F1 and F5 demonstrated the most significant bioactivity and were selected for detailed characterization. Fraction F1, obtained with Hexane/Ethyl acetate (90:10), showed a single prominent TLC spot with an R_f value of 0.77 and a yellowish appearance, indicative of a moderately polar compound. In contrast, Fraction F5, eluted with Ethyl acetate/Methanol (90:10), showed three distinct TLC spots (R_f = 0.21, 0.43, 0.67) and strong preliminary inhibition zones, suggesting a complex mixture of bioactive constituents.

The antimicrobial effectiveness of F1 and F5 may result from the combined action of these compounds on bacterial membrane integrity, protein synthesis inhibition, and oxidative stress modulation (Atanasov *et al.*, 2021; Desbois & Smith, 2010). The presence of multiple bioactive components further underscores the relevance of *Polyalthia longifolia* as a potential source of lead compounds in antimicrobial therapy.

The methanolic root extract of *Annona senegalensis* was similarly fractionated, yielding six fractions Table 2. Fractions F3 and F4 exhibited the most promising bioactivity. Fraction F3 (Hexane/Ethyl acetate 70:30) revealed two TLC spots (R_f = 0.46, 0.64) and a light green coloration, suggesting moderate polarity and compound diversity. Fraction F4 (Ethyl acetate/Methanol 60:40) produced strong antimicrobial inhibition zones, with two distinct spots (R_f = 0.23, 0.52), indicating potent phytochemical constituents.

Table 1: Column Chromatography Fractionation of Methanolic Extract of *P. longifolia* Stem Bark

Fraction Code	Solvent System Used	Volume Collected (mL)	TLC Observation (Rf value)	Color	Preliminary Bioactivity
F1	Hexane:Ethyl acetate (90:10)	20	One spot (Rf = 0.77)	Yellowish	Moderate activity
F2	Hexane:Ethyl acetate (80:20)	30	Two spots (Rf = 0.36, 0.63)	Pale brown	Mild inhibition zone
F3	Hexane:Ethyl acetate (70:30)	25	One prominent spot (Rf = 0.51)	Light green	No activity
F4	Ethyl acetate:Methanol (60:40)	35	One diffuse spot (Rf = 0.30)	Colourless	Mild inhibition zone
F5	Ethyl acetate:Methanol (90:10)	25	Three spots (Rf = 0.21, 0.43, 0.67)	Brownish	Strong inhibition zone
F6	Methanol	30	One diffuse spot (Rf = 0.28)	Dark brown	Mild inhibition zone

Note: TLC carried out on silica gel plates using UV light and iodine vapor for spot detection. Antibacterial activity was determined by agar well diffusion method.

Table 2: Column Chromatography Fractionation of Methanolic Extract of *Annona senegalensis* Root

Fraction Code	Solvent System Used	Volume Collected (mL)	TLC Observation (Rf value)	Color	Preliminary Bioactivity
F1	Hexane:Ethyl acetate (90:10)	25	One spot (Rf = 0.67)	Yellowish	Less or no activity
F2	Hexane:Ethyl acetate (80:20)	40	One prominent spot (Rf = 0.54)	Pale brown	Mild inhibition zone
F3	Hexane:Ethyl acetate (70:30)	35	Two spots (Rf = 0.46, 0.64)	Light green	Moderate activity
F4	Ethyl acetate:Methanol (60:40)	30	Two spots (Rf = 0.23, 0.52)	Sea foam green	Strong activity

F5	Ethyl acetate (90:10)	40	Two spots ($R_f = 0.24, 0.58$)	Brownish	Mild inhibition zone
F6	Methanol	20	One diffuse spot ($R_f = 0.25$)	Dark brown	Mild inhibition zone

Note: TLC was performed on silica gel plates with visualization under UV light and iodine vapor. Preliminary bioactivity was assessed using the agar well diffusion method against test organisms.

FTIR spectral analysis of these two fractions of *P. longifolia* stem bark revealed prominent bands corresponding to O–H stretching vibrations ($\sim 3395\text{ cm}^{-1}$), indicative of alcohols and phenolic compounds known for their antioxidant and antimicrobial properties as shown in Figure 1 and 2. C–H stretching ($\sim 2926\text{ cm}^{-1}$) and C=O vibrations ($\sim 1634\text{ cm}^{-1}$) further confirmed the presence of long-chain fatty acids and esters. C–O stretches around 1110 cm^{-1} and C–N bands at 1030 cm^{-1} corroborated the existence of glycosidic and alkaloidal compounds, respectively. These findings align with phytochemical data and reinforce the ethnopharmacological relevance of this species (Sasidharan *et al.*, 2011; Edeoga *et al.*, 2005).

FTIR analysis similar two fractions of *A. senegalensis* root confirmed the presence of hydroxyl ($\sim 3320\text{ cm}^{-1}$), carbonyl ($\sim 1725\text{ cm}^{-1}$), and aromatic ($\sim 1600\text{ cm}^{-1}$) groups, which are consistent with phenolic acids, flavonoids, and alkaloids. C–O stretching ($\sim 1250\text{--}1050\text{ cm}^{-1}$) and N–H bending confirmed the presence of amines, glycosides, and esters as shown in Figure 3 and 4. These functional groups corroborate the antimicrobial potential of *Annona senegalensis*, as supported by phytochemical screenings and previous literature (Ngueguim *et al.*, 2019; Oyedemi *et al.*, 2018).

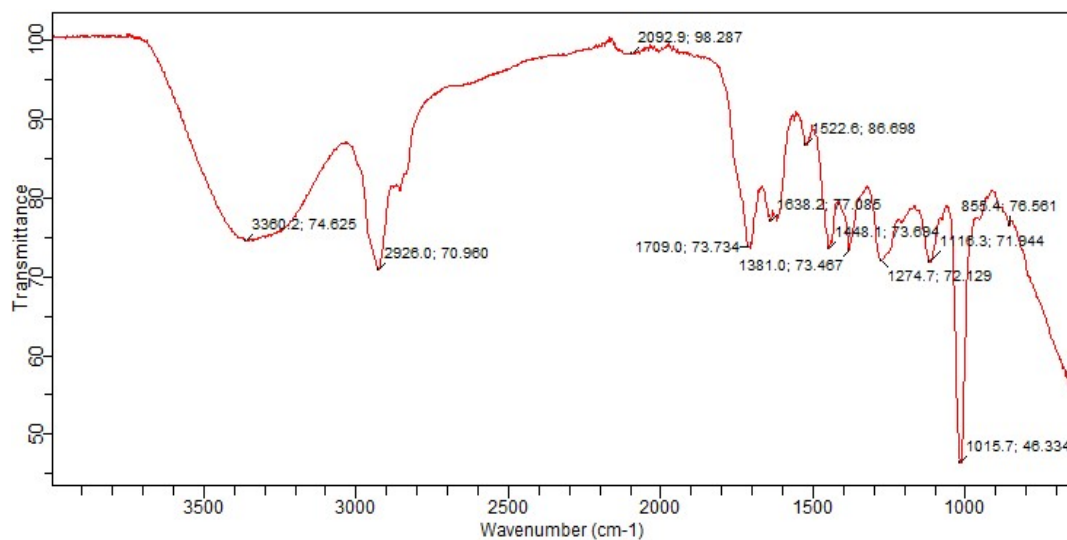


Figure 1: FTIR Spectrum of Polyalthia longifolia Stem Bark Fraction 1 (F1)

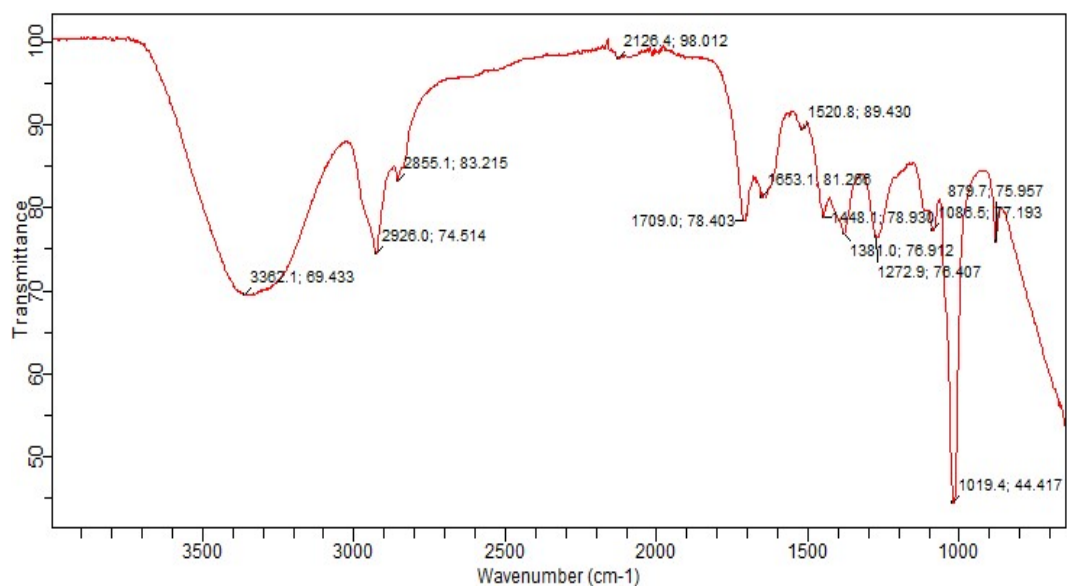


Figure 2: FTIR Spectrum of Polyalthia longifolia Stem Bark Fraction 5 (F5)

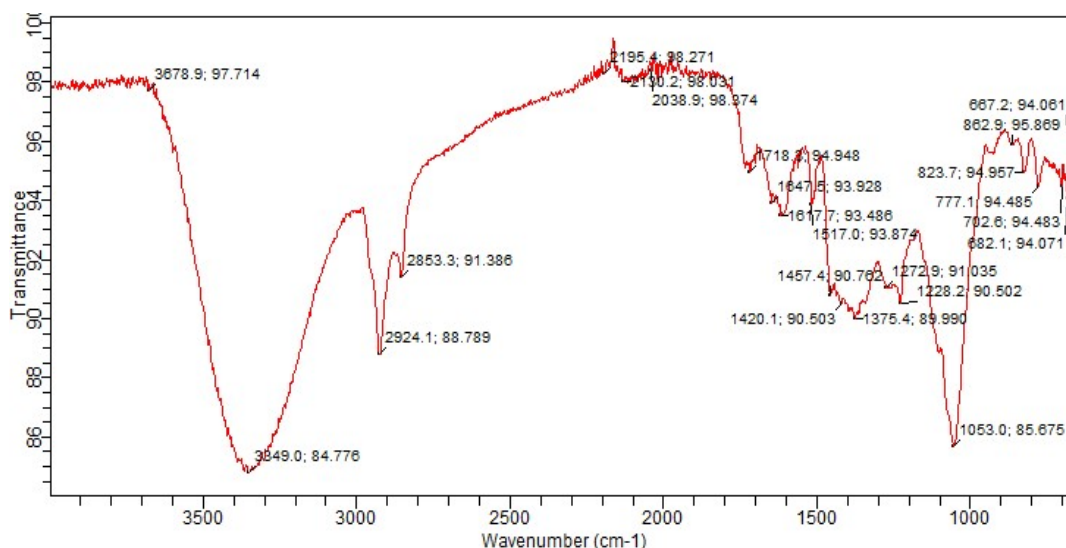


Figure 3: FTIR Spectrum of *Annona senegalensis* Fraction 3 (F3)

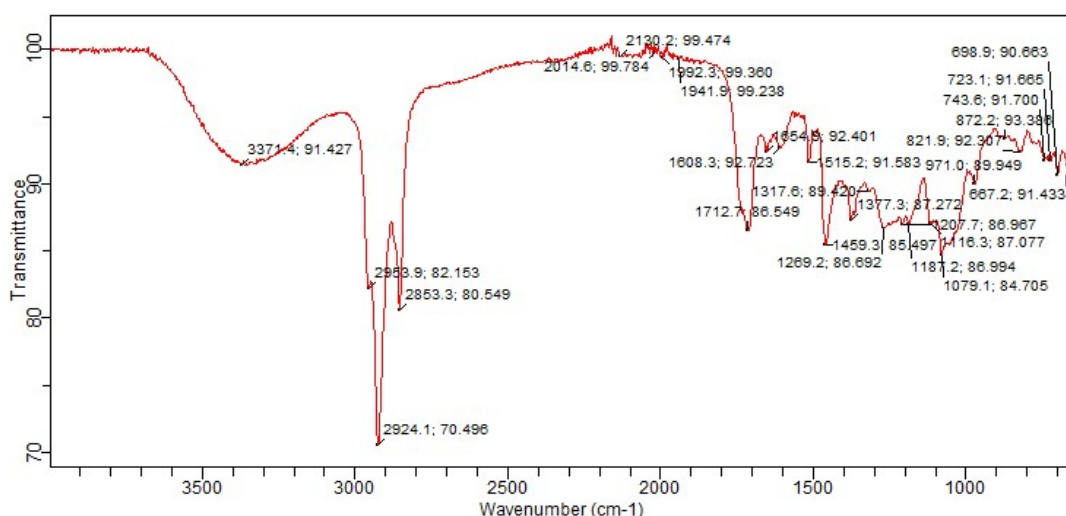


Figure 4: FTIR Spectrum of *Annona senegalensis* Fraction 4 (F4)

GC-MS analysis of Fraction F1 and F5 of *P. longifolia* stem bark identified multiple bioactive constituents as shown in figure 3 and 4. Notably, 2,4-di-tert-butylphenol, a potent antimicrobial and antioxidant compound, and fatty acid derivatives such as methyl palmitate (hexadecanoic acid methyl ester), methyl stearate, and n-hexadecanoic acid were abundant. The presence of squalene—a known antioxidant and membrane stabilizer—alongside (+)-roemerine, an aporphine alkaloid with cytotoxic activity, suggests synergistic mechanisms of bioactivity. These findings are supported by recent studies (Yakubu *et al.*, 2021; Jain *et al.*, 2021; Bouyahya *et al.*, 2019) that have documented the therapeutic

significance of these compounds, especially in antimicrobial and anti-inflammatory drug development.

GC-MS profiling of Fractions F₃ and F₄ of *A. senegalensis* root revealed a complex mixture of fatty acid esters and alkaloids. Key bioactive compounds included (+)-roemerine, trans-4-chlorochalcone, n-hexadecanoic acid, methyl stearate, oleic acid (9-octadecenoic acid), and squalene. These compounds have demonstrated a wide range of antimicrobial, antifungal, and anti-inflammatory activities in both in vitro and in vivo models (Mahfouz *et al.*, 2021; Sudjaroen, 2020).

Notably, (+)-roemerine is an isoquinoline alkaloid known for its antifungal and apoptotic effects, while chlorochalcones possess antibacterial properties through disruption of microbial DNA replication (Jain *et al.*, 2021). The fatty acids identified (e.g., hexadecanoic acid, oleic acid) are known to alter microbial membrane permeability, ultimately leading to cell lysis. The detection of squalene in both plants suggests a shared mechanism involving free radical scavenging and lipid membrane stabilization.

Overall, the chromatographic separation and subsequent characterization of these fractions support the long-standing traditional use of *A. senegalensis* in the management of infections and inflammatory conditions. The diversity of pharmacophores identified also underscores its potential for drug discovery and warrants further pharmacological and toxicological evaluation.

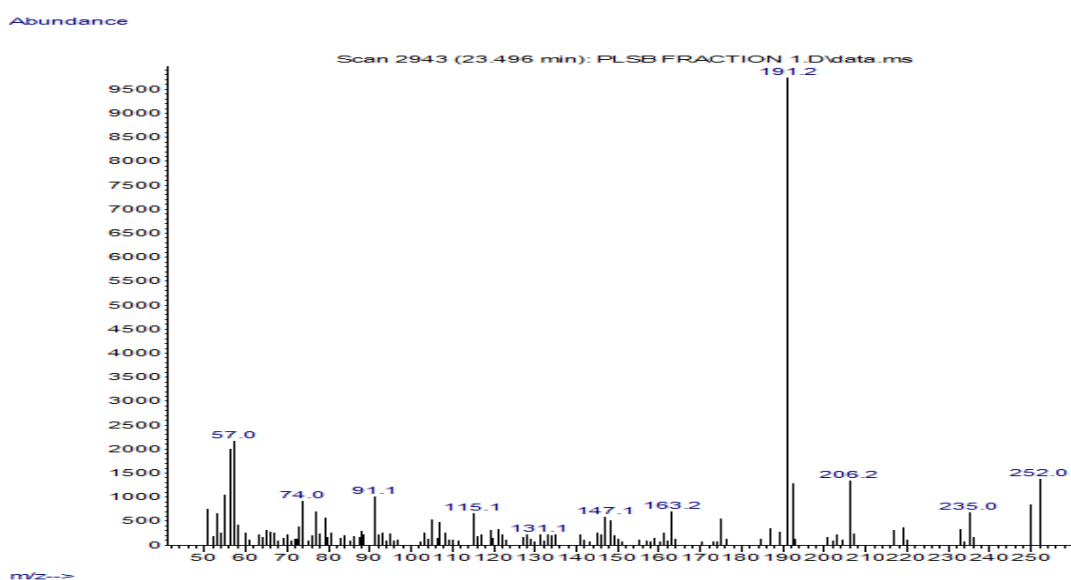
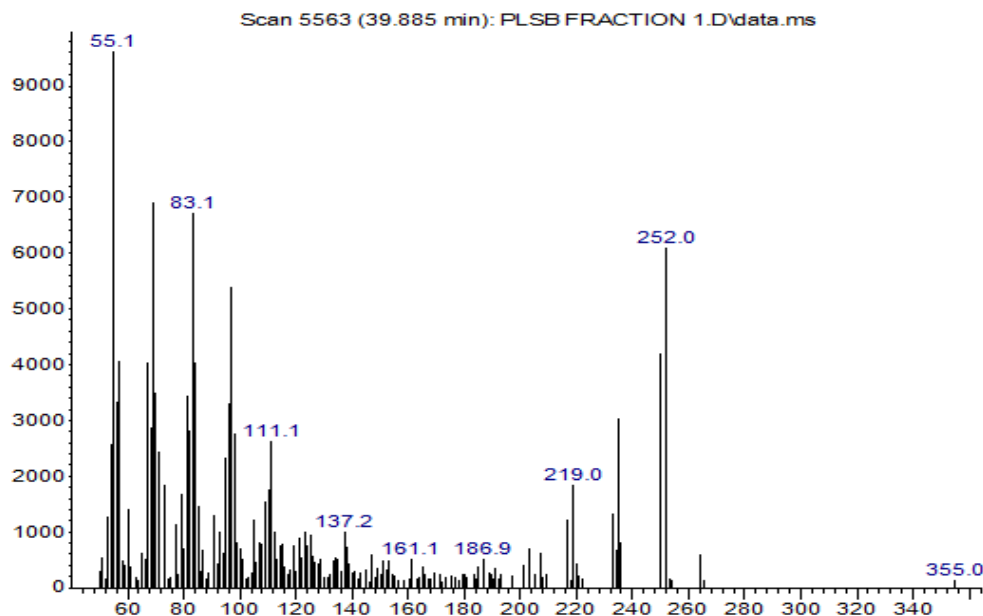


Figure 5: GC-MS Profile of *P. longifolia* Stem Bark Fraction 1 (2,4-Di-tert-butylphenol)

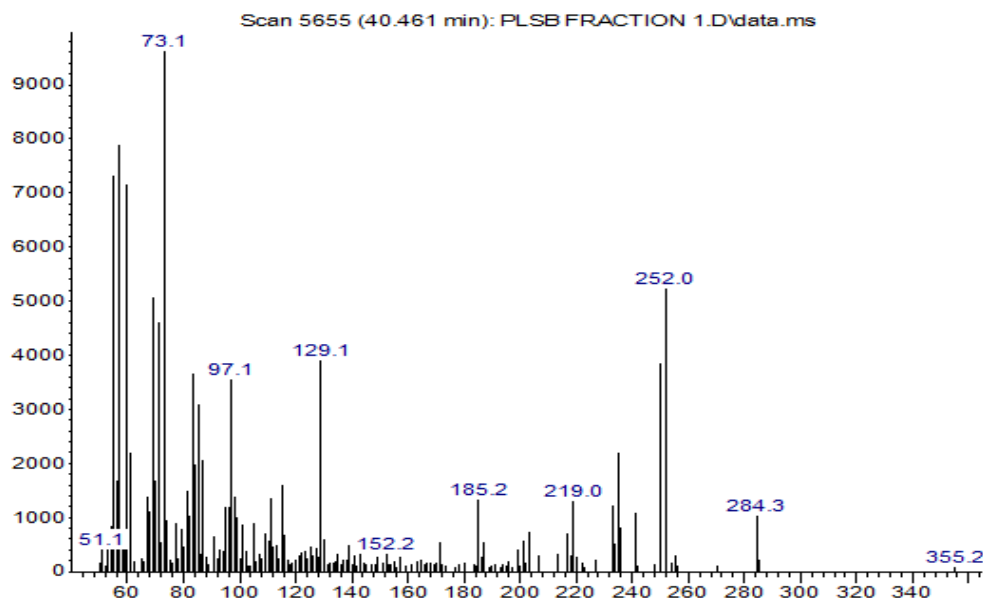
Abundance



m/z-->

Figure 6: GC-MS Profile of *P. longifolia* Stem Bark Fraction 1 [9-Octadecenoic acid (Oleic Acid)]

Abundance



m/z-->

Figure 7: GC-MS Profile of *P. longifolia* Stem Bark Fraction 1 [Octadecanoic Acid (Stearic Acid)]

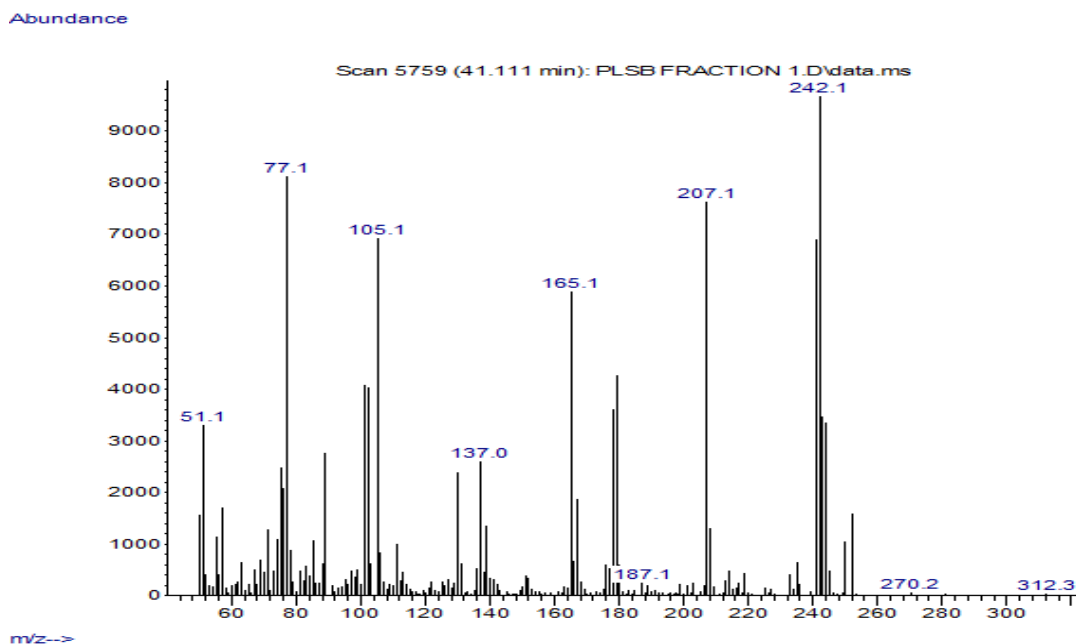


Figure 8: GC-MS Profile of *P. longifolia* Stem Bark Fraction 1 (*trans*-4-Chlorochalcone)

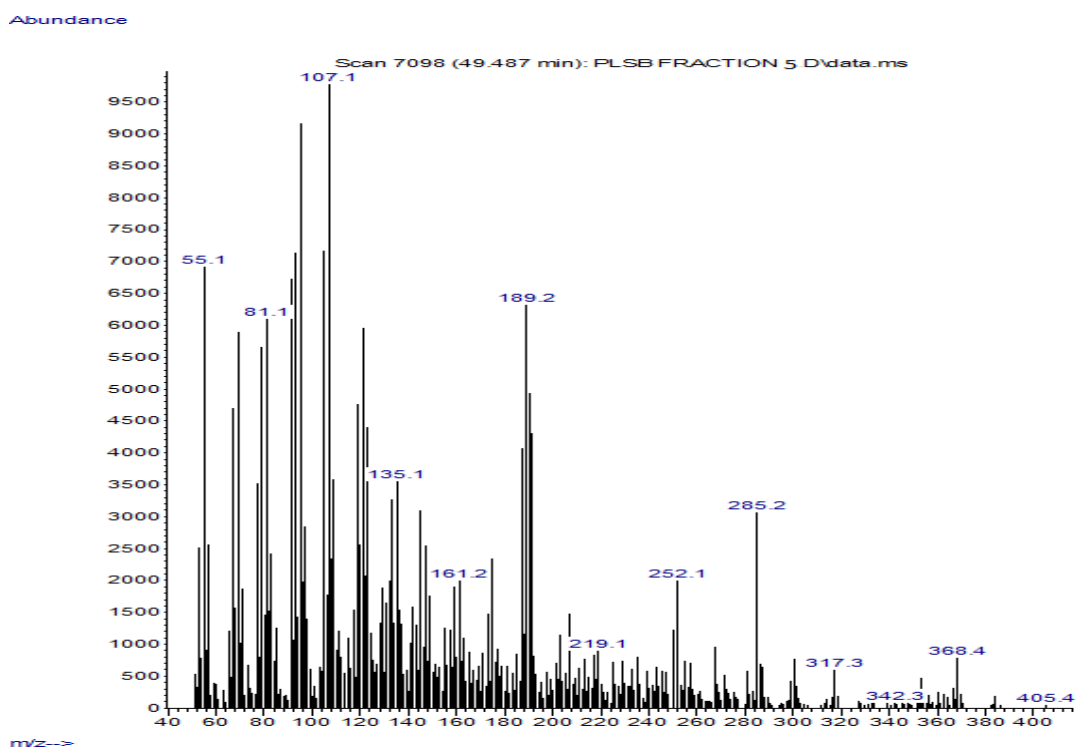


Figure 9: GC-MS Profile of *Polyalthia longifolia* Stem Bark Fraction 5 (*Methyl kolavenate*)

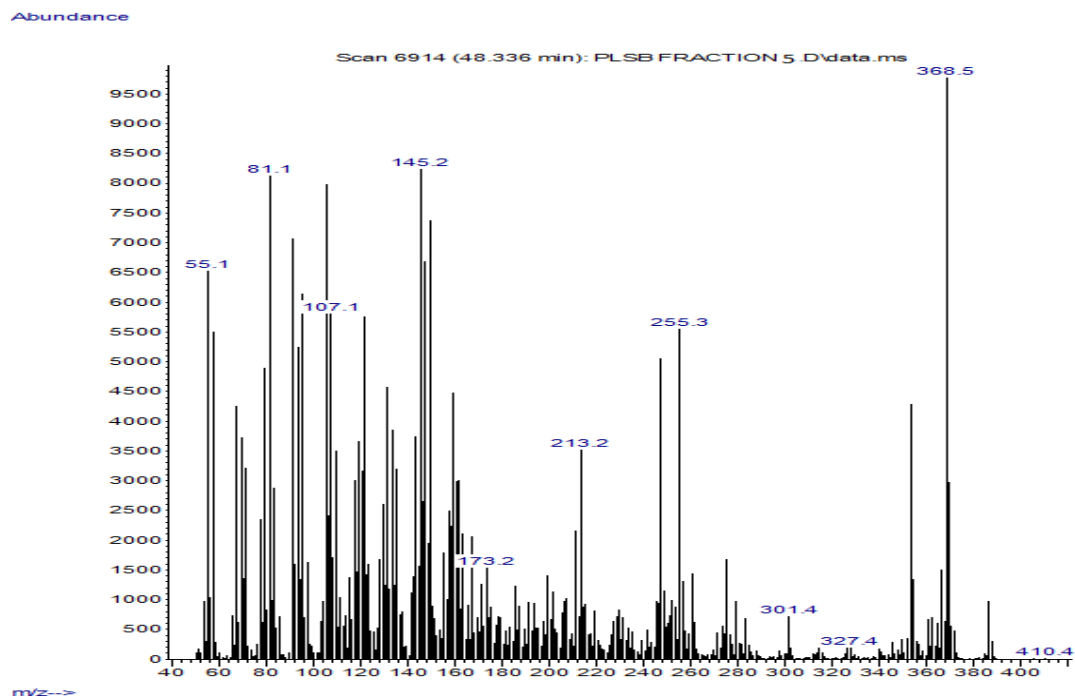


Figure 10: GC-MS Profile of *Polyalthia longifolia* Stem Bark Fraction 5 (Cholesta-3,5-diene)

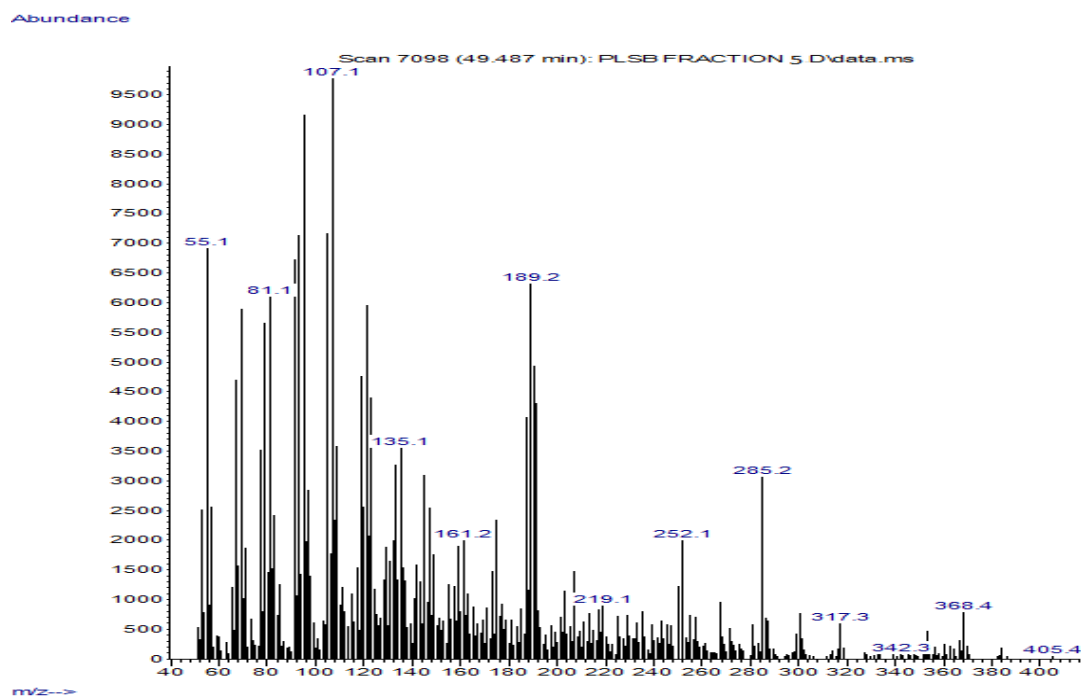


Figure 11: GC-MS Profile of *Polyalthia longifolia* Stem Bark Fraction 5 (Methyl kolavenate)

Abundance

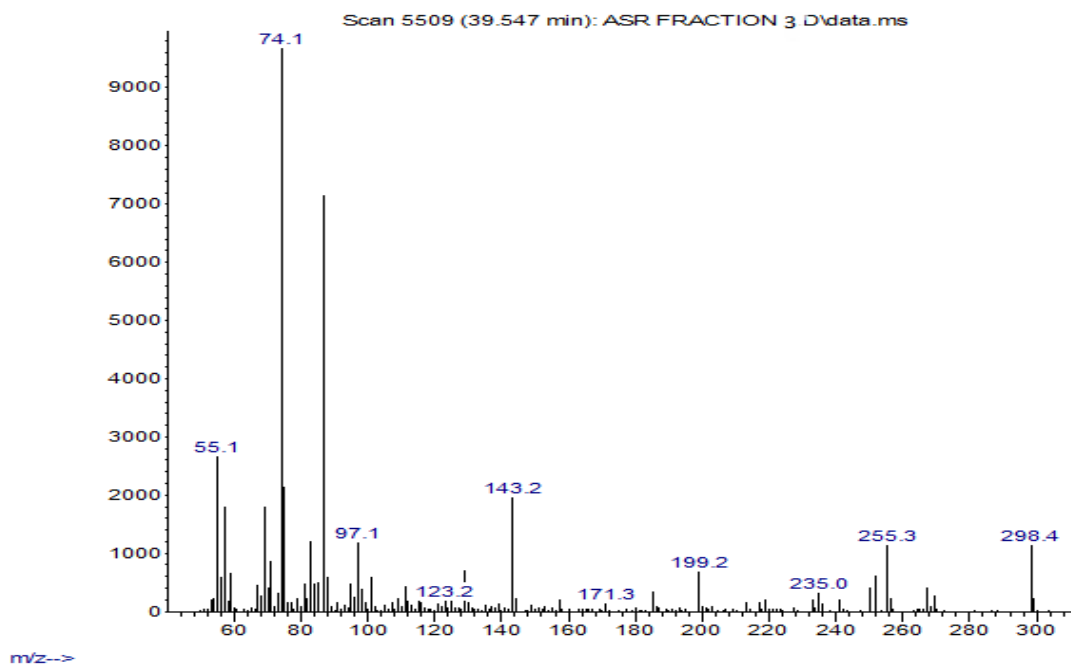


Figure 12: GC-MS Profile of *Annona senegalensis* Root Fraction 3 (Methyl stearate)

Abundance

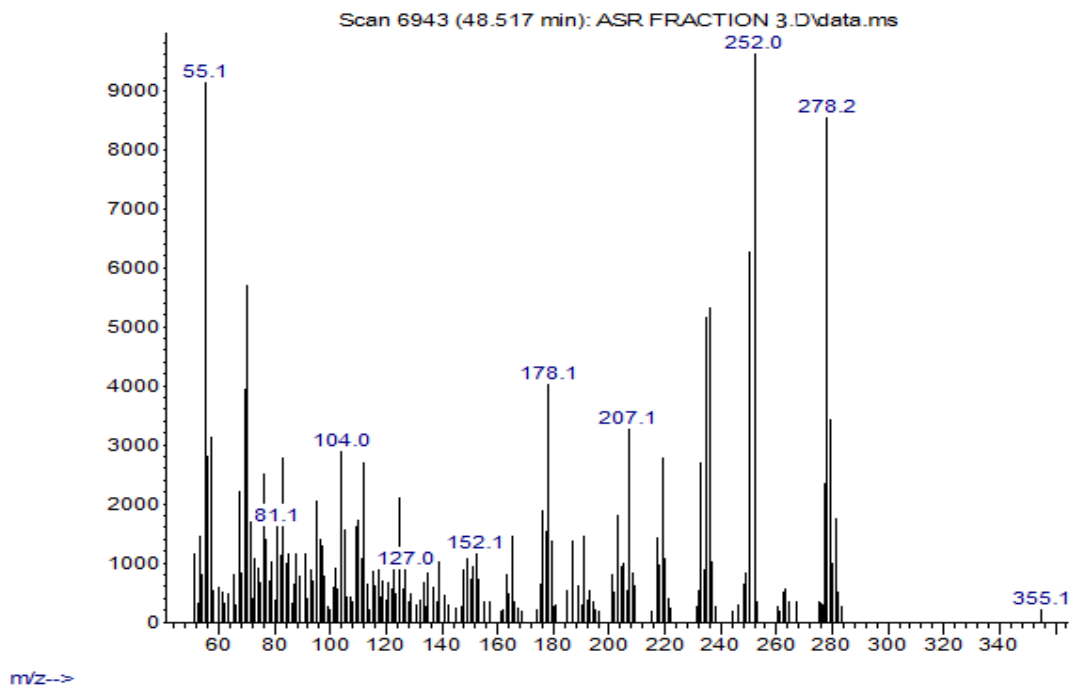


Figure 13: GC-MS Profile of *Annona senegalensis* Root Fraction 3 [(+)-Roemerine]

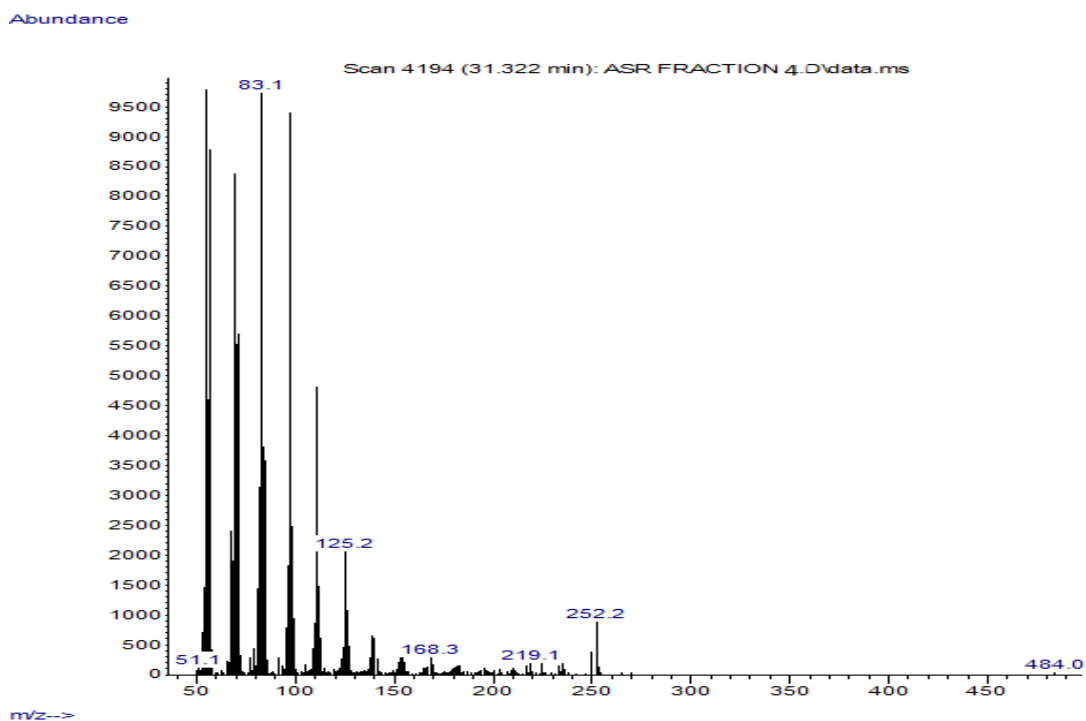


Figure 14: GC-MS Profile of *Annona senegalensis* Root Fraction 4 [5-Octadecene, (E)-]

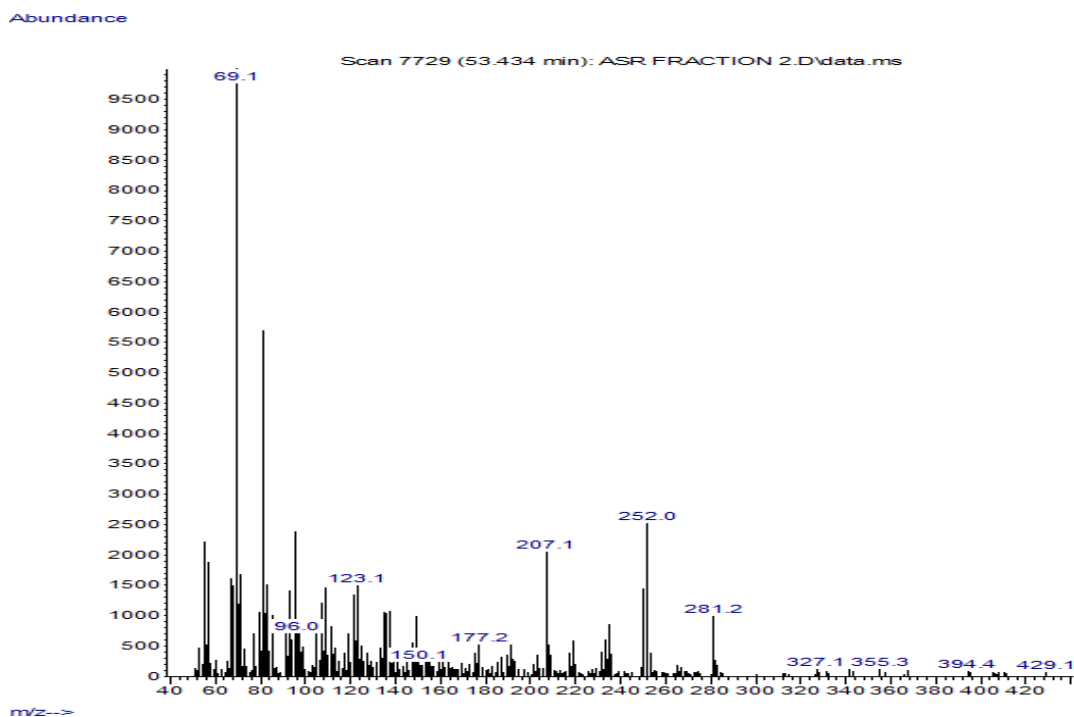


Figure 15: GC-MS Profile of *Annona senegalensis* Root Fraction 4 (Squalene)

Conclusion

This study successfully demonstrated the isolation and characterization of bioactive compounds from the methanolic stem bark extract of *Polyalthia longifolia* and the root extract of *Annona senegalensis* using chromatographic and spectroscopic techniques. Column chromatography enabled the separation of phytochemically rich fractions, particularly F₁ and F₅ from *Polyalthia longifolia* and F₃ and F₄ from *Annona senegalensis*, which exhibited significant antimicrobial activity. The effectiveness of these fractions was confirmed through TLC profiling, FTIR analysis, and GC-MS identification of major phytoconstituents.

FTIR spectroscopy revealed the presence of pharmacologically relevant functional groups including hydroxyl, carbonyl, alkyl, and aromatic moieties. These observations were corroborated by GC-MS, which identified compounds such as 2,4-di-tert-butylphenol, methyl palmitate, squalene, and (+)-roemerine in *Polyalthia longifolia*, and trans-4-chlorochalcone, oleic acid, methyl stearate, and squalene in *Annona senegalensis*. These compounds are known for their antimicrobial, antioxidant, and anti-inflammatory properties, and are believed to act synergistically.

The results validate the ethnomedicinal uses of both plants in treating infections and support their potential as sources of natural antimicrobial agents. The chemical diversity observed among the identified metabolites highlights the importance of integrating traditional medicine with modern analytical tools for drug discovery. Future studies should focus on the *in vivo* evaluation of the most active fractions, their safety profiles, and mechanistic studies to unravel their molecular modes of action. This research contributes valuable data to the growing compendium of bioactive natural products and reinforces the pharmaceutical relevance of indigenous medicinal plants in addressing the global challenge of antimicrobial resistance. Bioassay-Guided Isolation: Future research should focus on isolating individual bioactive compounds from the active fractions (F₁, F₅ of *P. longifolia* and F₃, F₄ of *A. senegalensis*) using bioassay-guided fractionation for precise pharmacological evaluation.

Conflict of interest: The authors declare no competing interest.

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