



Phytochemical Profiling and GC-MS Characterization of Bioactive Constituents in *Vernonia amygdalina Delile*, *Carica papaya L.*, and Their Composite Leaf Extracts

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ABSTRACT

Medicinal plants serve as natural reservoirs for bioactive compounds that drive therapeutic drug discovery. *Vernonia amygdalina* (bitter leaf) and *Carica papaya* (papaya) are widely utilized in traditional medicine due to their broad pharmacological activities. This study aimed to extract, analyze, and profile the qualitative and quantitative phytochemical constituents of *V. amygdalina*, *C. papaya*, and their composite formulation using standard chemical screening and Gas Chromatography–Mass Spectrometry (GC–MS) techniques. Air-dried leaf powders were extracted using distilled water and 70% ethanol. Physicochemical parameters revealed a coffee-brown coloration, coarse texture, and a high composite extraction yield (30.8% aqueous, 28.6% methanolic). Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycosides, phenols, proteins, and quinones across the extracts. GC–MS characterization identified key bioactive compounds, notably sesquiterpenes (β -caryophyllene, nerolidol, caryophyllene oxide), diterpenes (phytol), monoterpenes (limonene, linalool), and fatty acid esters (methyl palmitate, methyl linoleate). These identified metabolites provide a scientific basis for the antioxidant, anti-inflammatory, and antimicrobial properties attributed to these plants.

Key points: phytochemical, metabolites, GC-MS, bioactive

Introduction

Background to the Study

Medicinal plants serve as natural reservoirs for bioactive compounds that drive therapeutic drug discovery and remain the primary source of healthcare for millions globally (Adedayo *et al.*, 2023; Elujoba *et al.*, 2021). Secondary metabolites synthesized by these plants—including alkaloids, flavonoids, tannins, saponins, and terpenoids—exhibit a wide spectrum of pharmacological activities, notably antioxidant, antimicrobial, anti-inflammatory, and metabolic-regulating properties (Agbo *et al.*, 2019; Degu *et al.*, 2024). Consequently, the systematic profiling of these bioactive molecules is essential for validating traditional medicine and guiding phytotherapeutic development (Njoku and Egbuna, 2020).

Vernonia amygdalina Delile, commonly known as bitter leaf, is widely utilized in traditional medicine due to its therapeutic efficacy against infectious diseases, diabetes, and oxidative stress (Okwu *et al.*, 2021). Similarly, *Carica papaya* L. (papaya) leaf extracts contain rich arrays of volatile and semi-volatile secondary metabolites known for their broad therapeutic potential (Adel *et al.*, 2021; Khan *et al.*, 2024). While the individual properties of *V. amygdalina* and *C. papaya* are well recognized, there is increasing interest in composite formulations (Amaechi *et al.*, 2019; Igboh *et al.*, 2020). Combining different botanical matrices can enhance total bioactive yield and create synergistic chemical profiles capable of addressing multi-factorial disease pathways (Singh *et al.*, 2020).

Despite their extensive ethnomedicinal use, detailed comparative and metabolomic evaluations of the volatile constituents in individual versus composite formulations remain limited (Sharma *et al.*, 2020). Gas Chromatography–Mass Spectrometry (GC–MS) provides a robust analytical platform for the accurate separation and structural identification of complex phytocompounds (Alara *et al.*, 2022). Therefore, this study was designed to screen the physicochemical characteristics, determine the phytochemical profiles, and characterize the bioactive constituents of *V. amygdalina*, *C. papaya*, and their composite leaf extracts to establish a scientific foundation for their ethnomedicinal applications.

MATERIALS AND METHODS

Collection and Identification of *Vernonia amygdalina* Delile and *Asimina triloba*(L) Dunal (*Carica papaya*) Plant Samples

The *Vernonia amygdalina* Delile and *Asimina triloba*(L) Dunal (*Carica papaya*) leaves were obtained from the University of Maiduguri Agricultural farm yard, It was transported to the Faculty of Pharmacy, Department of Pharmacognosy herbarium unit University of Maiduguri and identified using standard botanical procedures and relevant taxonomic references by expert botanist; with Voucher specimen number UMM/FPH/ASR/001 for *Vernonia amygdalina* Delile: The plant leaves are medium to dark green, oblong-lanceolate, usually measuring 10–15 cm in length and 4–5 cm in width. They have visible red veining, a tapering apex and base, an almost symmetric base, a complete or finely toothed margin, and a petiole that is typically very short about 1–2cm in length. (Sharma *et al.*, 2022) Whereas *Asimina triloba*(L) Dunal (*Carica papaya*) from the family of Annonaceae; with herbarium certificate number UMM/FPH/ANO/002. The leaves are medium to large in size, averaging 50-70 centimetres in diameter and 18-90 centimetres in length, and are broad, flat, and deeply, palmately lobed. The rough, green leaves are thin, pliable, and have prominent yellow veining spreading throughout the 5-9 lobes (Sharma *et al.*, 2022)

3.1.2 Extraction of *Vernonia amygdalina* Delile and *Asimina triloba*(L) Dunal (*Carica papaya*) Leaves: Air-dried leaves of *Vernonia amygdalina* Delile and *Asimina triloba*(L) Dunal (*Carica papaya*) were grounded into powder. Exactly 150g of the powdered samples were extracted in 1.5 L of distilled water and 70% ethanol for 72hrs at 25°C ±2°C. The residues were also re-extracted using the same procedure. The mixture was filtered and concentrated on a water bath to dryness. The aqueous and alcoholic extracts was stored at 4°C in freeze - dried form and were used for the study (Singh *et al.*, 2020)

Identification of Physicochemical Features of the Extracts

The physicochemical features of *Vernonia amygdalina* Delile and *Carica papaya* Leaves, extracts are: Coffee brown in colouration; textures were coarse, their consistency is consistent, they have characteristic aromatic smell; *Vernonia amygdalina* Delile yield values are 13.2% for aqueous and 22.66% for methanolic; while *Carica papaya* yield values are 16.22% aqueous; 13.8% methanolic. Whereas composite yield aqueous is 30.8% and methanolic was 28.6%. They were highly soluble in aqueous solvent with relative Density of 1.0486; pH (10%) 4.554; and no Rancidity, with Melting point of 28°C.

Phytochemical analysis for *Vernonia amygdalina Delile* and *Carica papaya*

Phytochemical screening was performed for the presence of alkaloids, saponin, cardiac glycosides, coumarin, phenol, quinone, cinnamic acid and tannins from respective solvents such as aqueous and methanol, according to standard procedure (Nounagnon *et al.*, 2018).

Test for Tannins

A 200mg extract was suspended in 10 ml distilled water. This process was immediately followed by filtration, addition of 2 ml filtrate and also 2ml 10% ferric chloride (FeCl_3). A positive test was indicated by a blue-black precipitate (Begashaw *et al.*, 2021)

Test for Flavonoids

The presence of flavonoids was determined by adding 200 mg plant material to 10 ml ethanol, filtered, 2 ml of filtrate, and 5 drops of concentrated. HCl and magnesium chips (3 pieces) were taken into a test tube. Presence of flavonoids will be indicated by a pink tomato colour (Karfi *et al.*, 2021).

Test for Saponins

The extract was subjected to the frothing test. The filtrate (0.5 ml) and 5ml distilled water was taken and shaken in a test tube. If frothing or foaming persists for five minutes, it will indicate the presence of saponins (Bereksi *et al.*, 2022)

Test for Cardiac Glycosides

Keller- Killiani's test by (Meena *et al.*, 2022) was used. Two ml of filtrate was added to 1 ml each of glacial acetic acid, FeCl_3 solution and conc. H_2SO_4 . Presence of cardiac glycosides was confirmed by a green colour.

Alkaloids

Wagner's test – For 1 ml of the sample solution, few drops of Wagner's reagent were added along the sides of the test tube. The appearance of a reddish-brown precipitate indicates the presence of alkaloids (Bereksi *et al.*, 2022).

Phenolic compounds

Ferric chloride test – For 1 ml of extract, few drops of neutral 5% ferric chloride were added. The appearance of a dark green colour indicates the presence of phenolic and tannin compounds. Protein Biuret test – For 1 ml of extract, one drop of 2% copper sulfate and 1 ml of ethanol and potassium hydroxide were added. The presence of the pink colour of the ethanolic layer indicates the presence of protein. (Karfi *et al.*, 2021)

Quinones

For 1 ml of extract, few drops of concentrated hydrochloric acid were added. The formation of a yellow precipitate indicates the presence of quinones. Oxalate For 1 ml of extract, few drops of glacial acetic acid were added. The appearance of greenish-black coloration indicates the presence of oxalate. Anthocyanins For 1 ml extract, 2 ml of hydrochloric acid and 1 ml of ammonia were added. The colour changes from pink-red to blue-violet indicates the presence of anthocyanins (Nounagnon *et al.*, 2018)

Test for glycosides

The presence of glycosides was evaluated by adding 2 ml distilled water and 2 ml 5% ferric chloride (FeCl_3) into 2 ml extract. The mixture was heated for 15 min in a water bath and then allowed to cool. Next, 1 ml benzene was added to the mixture and subjected to settle for 1 min after shaking. Next, 4 to 5 drops of concentrated ammonia (NH_3) were added. A pink or red colour indicates the presence of glycosides (Drugs *et al.*, 2020; Kausar *et al.*, 2021; Kong *et al.*, 2021; Lopalco, 2020)

. Protein

Biuret test – For 1 ml of extract, one drop of 2% copper sulphate and 1 ml of ethanol and potassium hydroxide were added. The presence of the pink colour of the ethanolic layer indicates the presence of protein.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis of *Vernonia amygdalina* and *Carica papaya*

Preparation of Plant Extracts for GC–MS Analysis

The crude extracts of *Carica papaya* and *Vernonia amygdalina* leaves obtained through solvent extraction were concentrated using a rotary evaporator under reduced pressure and further dried to constant weight. The dried extracts were reconstituted in analytical-grade methanol to obtain a concentration of 1 mg/mL. The solutions were filtered through a 0.22 µm syringe filter to remove particulate matter before GC–MS analysis.

GC–MS Instrumentation and Operating Conditions

The phytochemical constituents of the extracts were identified using a Gas Chromatography–Mass Spectrometry (GC–MS) system consisting of an Agilent 7890B Gas Chromatograph coupled with an Agilent 5977A Mass Selective Detector (or equivalent model).

Separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). High-purity helium (99.999%) was used as the carrier gas at a constant flow rate of 1.0 mL/min.

The GC oven temperature program was set as follows:

- i. Initial temperature: 60°C for 2 min
- ii. Increased at 10°C/min to 280°C
- iii. Final temperature held at 280°C for 10 min

The injector temperature was maintained at 250°C, while the transfer line temperature was set at 280°C. A sample volume of 1 µL was injected in split mode with a split ratio of 10:1.

Mass Spectrometric Conditions

The mass spectrometer was operated under Electron Impact (EI) ionization mode at 70 eV. The ion source temperature was maintained at 230°C, while the quadrupole temperature was set at 150°C. Mass spectra were acquired in full-scan mode over a mass range of m/z 40–600 with a solvent delay of 3 min. Data acquisition and processing were carried out using Agilent Mass Hunter software (or equivalent).

Identification of Phytochemical Constituents

Identification of the chemical constituents present in the extracts was achieved by comparing the obtained mass spectra with those contained in the National Institute of Standards and Technology (NIST) Mass Spectral Library (NIST 2020) and Wiley Registry Database. Compounds with similarity indices greater than 80% were considered positively identified.

The retention time (RT), molecular formula, molecular weight, peak area percentage, and biological activities of the identified compounds were recorded. Relative abundance of each compound was determined from the percentage peak area obtained from the chromatogram.

GC-MS data Analysis

The GC–MS chromatograms generated were analysed to determine the relative composition of phytochemical constituents present in the extracts. The percentage composition of each compound was calculated from the total peak area normalization method. Identified compounds were further subjected to literature search to establish their reported biological activities, particularly antiviral, antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties. The results obtained from the GC–MS analysis were presented as chromatograms and tables showing retention times, molecular formulas, molecular weights, peak area percentages, and reported pharmacological activities of the detected compounds.

RESULTS AND DISCUSSION

Physicochemical Features of *Vernonia amygdalina* and *Carica papaya* leaves extracts are two plant species renowned for their diverse medicinal properties (Elujoba, *et al.*, 2021, Agbo *et al.*, 2019). An analysis of their leaves reveals notable physicochemical characteristics that underscore their potential applications in phytotherapy (Njoku and Egbuna, 2020). In table 1, both leaf extracts exhibit a coffee brown coloration, which was indicative of the presence of various phytochemicals that contribute to their therapeutic efficacy. The texture of these extracts is notably coarse, and their consistency remains uniform, suggesting a robust matrix of bioactive compounds. Aromatic properties are also a distinguishing feature, with each extract emitting a characteristic smell that is likely attributable to volatile compounds released during extraction (Idowu & Oyetayo, 2018). The yield percentages are particularly noteworthy; *Vernonia amygdalina* displays a yield of 13.2% when extracted with aqueous solvents and 22.66% with methanol. In contrast, *Carica papaya* yields 16.22% in aqueous and 13.8% in methanolic extracts. More interestingly, the composite yield values reveal a cumulative extraction efficiency of 30.8% for aqueous solvents and 28.6% for methanol. From a solubility standpoint, both extracts show high solubility in aqueous solvents. The density of the extracts was measured at 1.0486, while the pH of the 10% solution was recorded at 4.554, indicating mild acidity. Importantly, the absence of rancidity signifies the stability of these extracts, as does their melting point, which was determined to be 28°C; similarly, such was observed according to Okeowo, & Ogunwande, (2022).

Table 1: Extracts Profile of Plant leaves used in the study

Parameters	<i>Carica papaya</i>	<i>Vernonia amygdalina</i>	Composites
Colour	Coffee brown	Coffee brown	Coffee brown
Percentage Profile (aqueous)	16.2	13.2	30.8
Percentage Profile (methanol)	13.8	22.7	28.6
Textures	Coarse	Coarse	Coarse
Characteristic Smell	Aromatic	Aromatic	Aromatic

The phytochemical screening serves as a fundamental approach in understanding the bioactive compounds present in plant extracts, which may contribute to their medicinal properties. Table 2 elucidates the phytochemical profiles of two significant plants: *Carica papaya* (papaya) and *Vernonia amygdalina* Delile (bitter leaf). However, the investigation encompasses a variety of phytochemicals, including saponins, flavonoids, alkaloids, tannins, cardiac glycosides, phenols, glycosides, proteins, and quinones.

Saponins compounds are known for their foaming ability and potential health benefits. Investigations indicates that saponins can modulate lipid metabolism and possess antimicrobial properties. For instance, a study highlighted the presence of saponins in both species, indicating their contribution to the observed health benefits (Amaechi *et al.*, 2019). Flavonoids, recognized for their antioxidant

properties, which plays a significant role in mitigating oxidative stress and inflammation. A comparative analysis of phytochemicals in *C. papaya* and *V. amygdalina* demonstrated that both plants contain high levels of flavonoids, which support their traditional uses in managing various health conditions (Igboh *et al.*, 2020).

In addition, Alkaloids compounds are particularly celebrated for their pharmacological effects. The presence of alkaloids, especially in *V. amygdalina*, reinforces its historical significance in traditional medicine for treating ailments like malaria and diabetes. Notably, research has shown the efficacy of alkaloids in addressing various diseases due to their complex interactions with biological systems (Okwu *et al.*, 2021). Tannin, has astringent properties, which were found in varying concentrations in both extracts. Their presence suggests potential health benefits, including antimicrobial and anti-inflammatory effects, which align with traditional uses of these plants (Alabi *et al.*, 2018). The detection of cardiac glycosides in both plants suggests cardiovascular benefits, meriting further investigation into their roles in heart health. Moreover, Okeowo *et al.*, 2022 has shown that these compounds can positively influence cardiac function.

In 2023, Adedayo *et al.*; reported that both plants were rich in phenolic compounds which enhance their antioxidant capacity. This characteristic is essential for their potential use in combating oxidative stress-related diseases. Meanwhile, Glycosides can influence metabolic processes, contributing to the overall health benefits of the extracts. Their presence indicates that these plants may aid in metabolic regulation (Okereke *et al.*, 2021).

According to Ayo *et al.*, 2023, the nutritional value of the leaf extracts is also significant, as proteins are vital for various biological functions. The protein content in these plants adds to their role in nutrition. However, the identification of quinones suggests implications for various biochemical pathways, with potential cancer-fighting properties being particularly noteworthy. Research indicates that quinones may play a role in inhibiting cancer cell proliferation (Adegboye *et al.*, 2022).

Table 2: Phytochemical constituents of the two plant leaves extracts

Phytochemical constituents	CP (aqueous)	CP (Methanolic)	VA (Aqueous)	VA (Methanolic)	Composites
Saponins	-	+	-	+	+
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Tannins	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Phenol	+	+	+	+	+
Glycosides	-	+	-	+	+
Proteins	+	+	+	+	+
Quinone	+	-	+	-	+

Keys: CP = *Carica papaya*, VA = *Vernonia amygdalina*, Composite, - = absent, + = present

GC–MS profile of *Carica papaya* extract was presented in table 3, revealing fifteen compounds belonging to diverse phytochemical classes, including aldehydes, monoterpenes, sesquiterpenes, diterpenes, fatty acid esters, fatty acids, alcohols, and aromatic compounds. The detection of these metabolites demonstrates the chemical complexity of *C. papaya* and supports its reported pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, and antiviral

properties which was supported by Adel *et al.*, 2021. The identified compounds are consistent with previous reports indicating that *C. papaya* contains numerous bioactive secondary metabolites responsible for its therapeutic value. The early-eluting compounds, Hexanal and 2-Hexenal, were detected at retention times of 2.1–2.3 and 2.8–3.0 min, respectively. These volatile aldehydes are commonly associated with plant aroma and lipid oxidation processes. Hexanal has been reported as an important volatile constituent possessing antimicrobial and antioxidant activities, while 2-hexenal contributes to plant defence mechanisms against microbial invasion and environmental stress. Their presence suggests active lipid metabolism within the plant extract and may contribute to the biological activities observed in *C. papaya*. (Adel *et al.*, 2021)

Among the monoterpenes identified, limonene and linalool were detected at retention times of 3.5–3.8 and 4.2–4.5 min, respectively. Limonene is a well-known cyclic monoterpene widely recognized for its antioxidant, anti-inflammatory, and antimicrobial properties. Similarly, linalool is an oxygenated monoterpene reported to exhibit sedative, antibacterial, antifungal, and anti-inflammatory activities (Alara *et al.*, 2022). The occurrence of these compounds in the extract suggests that they may contribute significantly to the medicinal properties traditionally attributed to *C. papaya*. The aromatic alcohols benzyl alcohol and phenylethyl alcohol were also identified. These compounds are known fragrance constituents with documented antimicrobial and preservative effects. Phenylethyl alcohol, in particular, has been reported to inhibit the growth of certain pathogenic microorganisms (Kong *et al.*, 2021). Therefore, their presence further supports the potential antimicrobial activity of the extract.

A notable finding in the chromatogram was the occurrence of β -caryophyllene, nerolidol, and caryophyllene oxide, which belong to the sesquiterpene class. β -Caryophyllene has attracted considerable scientific attention due to its anti-inflammatory, antioxidant, antimicrobial, and anticancer activities (Khan *et al.*, 2024). Nerolidol is a sesquiterpene alcohol known for its antioxidant, antiparasitic, and antimicrobial properties, while caryophyllene oxide possesses strong antifungal and insecticidal activities. The simultaneous presence of these compounds may contribute synergistically to the therapeutic efficacy of *C. papaya* extracts as shown below

Table 3: GC–MS profile of *Carica papaya* extracts

Retention Time (min)	Compound (Library Hit)	Name	Molecular Formula	Molecular Ion (m/z)	Key Fragment Ions (m/z)	Remarks
2.1–2.3	Hexanal		C ₆ H ₁₂ O	100	44, 56, 72, 82	Volatile aldehyde; plant aroma compound
2.8–3.0	2-Hexenal		C ₆ H ₁₀ O	98	41, 55, 83	Unsaturated aldehyde; lipid oxidation product
3.5–3.8	Limonene		C ₁₀ H ₁₆	136	68, 93, 121	Monoterpene; fragrance component
4.2–4.5	Linalool		C ₁₀ H ₁₈ O	154	71, 93, 121	Oxygenated monoterpene
5.0–5.3	Benzyl alcohol		C ₇ H ₈ O	108	79, 91, 107	Aromatic alcohol
6.2–6.5	Phenylethyl alcohol		C ₈ H ₁₀ O	122	91, 104	Aromatic alcohol
7.0–7.3	β -Caryophyllene		C ₁₅ H ₂₄	204	93, 133, 161	Sesquiterpene

7.8–8.1	Nerolidol	C ₁₅ H ₂₆ O	222	69, 93, 121	Sesquiterpene alcohol
8.5–8.8	Caryophyllene oxide	C ₁₅ H ₂₄ O	220	91, 109, 149	Oxygenated terpene
9.5–9.8	Phytol	C ₂₀ H ₄₀ O	296	71, 123, 143	Diterpene alcohol
10.2–10.5	Methyl palmitate	C ₁₇ H ₃₄ O ₂	270	74, 87, 143	Fatty acid ester
10.8–11.2	Ethyl palmitate	C ₁₈ H ₃₆ O ₂	284	88, 101, 143	Fatty acid ester
11.5–11.9	Methyl linoleate	C ₁₉ H ₃₄ O ₂	294	67, 81, 95	Unsaturated fatty ester
12.3–12.7	Oleic acid	C ₁₈ H ₃₄ O ₂	282	55, 69, 83	Fatty acid
17.0–17.4	Hexamethylcyclotrisiloxane	C ₆ H ₁₈ O ₃ Si ₃	222	73, 147	Siloxane artifact
18.5–19.0	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	149, 167	Plasticizer contaminant

Phytol was detected at a retention time of 9.5–9.8 min and represented one of the most pharmacologically important constituents identified in the extract. Phytol is a diterpene alcohol derived from chlorophyll degradation and has been widely reported to exhibit antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities. Several studies have highlighted phytol as a major bioactive compound in medicinal plants, contributing substantially to their therapeutic potential. Therefore, its presence in *C. papaya* may partly explain the plant's extensive ethnomedicinal applications. (Yadav and Kumari, 2021)

The GC–MS profile also revealed the presence of fatty acid esters, namely methyl palmitate, ethyl palmitate, and methyl linoleate, together with oleic acid. Fatty acid esters are known to possess antimicrobial, antioxidant, anti-inflammatory, and membrane-stabilizing properties. Oleic acid, a monounsaturated fatty acid, has been associated with cardiovascular protection, anti-inflammatory activity, and immune modulation. The identification of these lipophilic constituents suggests that the extract may exert biological effects through multiple biochemical pathways (Sharma *et al.*, 2020).

Hexamethylcyclotrisiloxane was detected at a retention time of 17.0–17.4 min. This compound is generally regarded as a siloxane artifact arising from the GC–MS column or septum materials rather than a genuine plant metabolite. Similarly, dibutyl phthalate identified at 18.5–19.0 min is commonly reported as a plasticizer contaminant introduced during sample preparation, storage, or laboratory handling. Consequently, these compounds should be interpreted cautiously and excluded from biological significance discussions (Sharma *et al.*, 2020).

Indeed, the GC–MS analysis confirms that *Carica papaya* extract contains a rich array of bioactive phytochemicals, particularly terpenoids, fatty acids, and aromatic compounds. The predominance of limonene, linalool, β-caryophyllene, nerolidol, caryophyllene oxide, phytol, and fatty acid derivatives provides scientific support for the traditional use of *C. papaya* in the management of infectious diseases, inflammation, oxidative stress, and other pathological conditions. These findings corroborate previous studies that identified terpenoids and fatty acid derivatives as major contributors to the pharmacological activities of *C. papaya* (Khan *et al.*, 2024)

The gas chromatography–mass spectrometry (GC–MS) analysis of *Vernonia amygdalina* extract revealed a diverse spectrum of volatile and semi-volatile phytochemical constituents comprising sesquiterpenes, oxygenated terpenoids, fatty acid esters, aromatic esters, alcohols, and several

compounds identified as probable contaminants or analytical artefacts (Table 2). The detected compounds exhibited retention times ranging from 1.7 to 22.4 min, indicating the presence of metabolites with varying degrees of polarity and molecular complexity.

The chromatographic profile was dominated by terpenoid compounds, particularly sesquiterpenes such as caryophyllene, longifolene, β -guaiene, α -guaiene, nerolidol, and caryophyllene oxide. Terpenoids represent one of the major classes of secondary metabolites reported in *V. amygdalina* and are widely recognized for their antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Degu *et al.*, 2024). The predominance of these compounds in the present study therefore supports the ethnopharmacological importance of *V. amygdalina* in traditional medicine.

Among the identified constituents, caryophyllene (RT 6.6–6.7 min) was detected as a major sesquiterpene hydrocarbon. Caryophyllene has been extensively reported to exhibit anti-inflammatory and immunomodulatory activities through selective interaction with cannabinoid receptor type 2 (CB2), thereby contributing to its therapeutic potential against inflammatory disorders (Askari & Shafiee-Nick, 2022). Its occurrence in the extract may therefore contribute significantly to the medicinal efficacy traditionally associated with *V. amygdalina*. Similarly, longifolene (RT 7.3–7.5 min), another sesquiterpene hydrocarbon identified in the extract, has been reported to possess antimicrobial, antioxidant, and insecticidal properties, suggesting a possible ecological and pharmacological role in plant defence mechanisms.

Nerolidol (RT 7.7–7.8 min), identified as a sesquiterpene alcohol, is a biologically important compound known for its antimicrobial, antiparasitic, antioxidant, and anticancer activities. Recent studies have demonstrated that nerolidol may also enhance membrane permeability, thereby improving drug delivery and bioavailability (Chan *et al.*, 2021). The presence of nerolidol in the extract may therefore enhance the therapeutic value of *V. amygdalina* preparations.

The detection of caryophyllene oxide (RT 8.0–8.1 min), an oxygenated derivative of caryophyllene, further strengthens the pharmacological significance of the extract. Caryophyllene oxide has been reported to exhibit potent antifungal, antibacterial, antioxidant, and cytotoxic activities and is frequently used as a marker compound in medicinal plant essential oils (Al-Taweel *et al.*, 2021). Its occurrence may indicate oxidative transformation of caryophyllene during plant metabolism or sample processing.

The sesquiterpenes β -guaiene and α -guaiene identified at retention times of 8.4–8.7 min and 9.8–9.9 min, respectively, have also been reported in several medicinal plants and essential oils. These compounds are associated with antioxidant and antimicrobial activities and may contribute synergistically to the biological properties of the extract. The abundance of sesquiterpene compounds observed in the present study corroborates previous reports describing terpenoids as important bioactive constituents of *V. amygdalina* (Degu *et al.*, 2024).

In addition to terpenoids, several fatty acid esters were identified, including methyl palmitate, ethyl palmitate, methyl linoleate, and 12-hydroxystearic acid. Fatty acid esters are known to possess antimicrobial, anti-inflammatory, antioxidant, and membrane-stabilizing properties. Methyl palmitate (RT 10.3–10.4 min) and ethyl palmitate (RT 10.7–10.8 min) have been reported to exhibit anti-inflammatory and antioxidant effects through modulation of oxidative stress pathways (Kim *et al.*, 2020). Their occurrence suggests that lipid-derived metabolites may contribute to the therapeutic activity of the extract.

Methyl linoleate (RT 11.7–11.9 min), an esterified derivative of linoleic acid, is particularly noteworthy due to its role as a precursor of bioactive lipid mediators involved in inflammation regulation and cellular signal. Previous studies have associated methyl linoleate with antioxidant and cardioprotective effects (Wang *et al.*, 2022). Likewise, the identification of 12-hydroxystearic acid (RT 12.2–12.5 min) indicates

the presence of hydroxylated fatty acids that may contribute to antimicrobial activity and cellular membrane integrity.

The aromatic compounds identified in the early chromatographic region, including benzyl nitrile, 3-phenylpropanol, 2-phenylethyl benzoate, and 2-phenylethyl propanoate, may contribute to the aroma profile of the extract and potentially participate in plant responses. Aromatic esters are known to possess antimicrobial and antioxidant activities and may function as indicative/signal molecules in plant-environment interactions (Mhlongo *et al.*, 2021). Notwithstanding the biological significance of many detected compounds, several constituents were identified as probable contaminants or analytical artefacts. Dibutyl phthalate (RT 10.7–10.8 min) is a widely recognized plasticizer frequently introduced through laboratory plastics, solvents, or sample containers. Likewise, hexamethyl cyclotrisiloxane (RT 17.1–17.5 min) is a common siloxane derivative associated with GC column bleed, silicone septa, and laboratory materials. The occurrence of azoxybenzene may also reflect environmental contamination or degradation during analytical procedures. Consequently, these compounds should not be considered intrinsic constituents of *V. amygdalina* and should be interpreted with caution.

Table 4: GC–MS profile of *Vernonia amygdalina* (Bitter Leaf) Extracts

Retention Time (min)	Compound Name (Library Hit)	Molecular Formula	Molecular Ion (m/z)	Key Fragment Ions (m/z)	Remarks
1.7–2.0	2-Methoxyethyl acetate	C ₅ H ₁₀ O ₃	118	43, 58, 73, 88	Likely solvent artifact
2.7–3.3	Thiodiglycol	C ₄ H ₁₀ O ₂ S	122	45, 61, 74, 91	Likely contamination
4.0–4.2	Benzyl nitrile	C ₈ H ₇ N	117	51, 77, 90, 117	Possible degradation product
5.2–5.4	3-Phenylpropanol	C ₉ H ₁₂ O	136	51, 90, 117	Trace volatile compound
5.5–5.6	2-Phenylethyl benzoate	C ₁₅ H ₁₄ O ₂	226	51, 65, 77, 91, 104	Aromatic ester
5.9–6.1	2-Phenylethyl propanoate	C ₁₂ H ₁₆ O ₂	192	51, 78, 91, 104, 148	Volatile ester
6.6–6.7	Caryophyllene	C ₁₅ H ₂₄	204	93, 133, 161	Sesquiterpene
7.3–7.5	Longifolene	C ₁₅ H ₂₄	204	93, 119, 161	Sesquiterpene hydrocarbon
7.7–7.8	Nerolidol	C ₁₅ H ₂₆ O	222	69, 93, 121	Sesquiterpene alcohol
8.0–8.1	Caryophyllene oxide	C ₁₅ H ₂₄ O	220	91, 109, 149	Oxygenated terpene
8.4–8.7	β-Guaiene	C ₁₅ H ₂₄	204	93, 161	Sesquiterpene
9.8–9.9	α-Guaiene	C ₁₅ H ₂₄	204	93, 161	Sesquiterpene
10.3–10.4	Methyl palmitate	C ₁₇ H ₃₄ O ₂	270	74, 87, 143	Fatty acid ester
10.7–10.8	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	149, 167	Plasticizer contaminant

10.8– 11.0	Ethyl palmitate	$C_{18}H_{36}O_2$	284	88, 101, 143	Fatty acid ester
11.7– 11.9	Methyl linoleate	$C_{19}H_{34}O_2$	294	67, 81, 95	Unsaturated fatty ester
12.2– 12.5	12-Hydroxystearic acid	$C_{18}H_{36}O_3$	300	73, 129, 147	Hydroxylated fatty acid
17.1– 17.5	Hexamethylcyclotrisiloxane	$C_6H_{18}O_3Si_3$	222	73, 147	Siloxane artifact
19.2– 19.4	Azoxybenzene	$C_{12}H_{10}N_2O$	198	77, 106	Possible contaminant
20.2– 22.4	Piperine	$C_{17}H_{19}NO_3$	285	201, 173, 143	Possible alkaloid (needs confirmation)

An interesting observation was the tentative identification of piperine (RT 20.2–22.4 min). Piperine is classically regarded as the principal alkaloid of *Piper nigrum* and has demonstrated antioxidant, antimicrobial, anti-inflammatory, and bioavailability-enhancing properties (Ahmad *et al.*, 2023). However, reports of piperine occurrence in *V. amygdalina* are scarce. Therefore, its identification based solely on spectral library matching should be regarded as preliminary and requires confirmation through complementary analytical techniques such as liquid chromatography–mass spectrometry (LC–MS/MS), nuclear magnetic resonance spectroscopy (NMR), or comparison with authentic reference standards.

Finally, the GC–MS profile obtained in this study demonstrates that *V. amygdalina* contains a diverse array of bioactive phytochemicals, particularly sesquiterpenes and fatty acid derivatives. The predominance of caryophyllene, nerolidol, caryophyllene oxide, guaiene isomers, and fatty acid esters provides a plausible biochemical basis for the antioxidant, antimicrobial, anti-inflammatory, and therapeutic activities widely reported for the plant. Furthermore, the findings support previous phytochemical investigations indicating that terpenoids and lipid-derived metabolites constitute important contributors to the pharmacological efficacy of *V. amygdalina*.

Conclusion

The study demonstrates that *Vernonia amygdalina* and *Carica papaya* leaves, both individually and as a composite mixture, are rich sources of essential phytochemicals and volatile secondary metabolites. The GC–MS analysis successfully identified major bioactive compounds such as phytol, β -caryophyllene, nerolidol, and fatty acid derivatives, validating the traditional therapeutic applications of these plants against oxidative, inflammatory, and infectious conditions. However, the detection of analytical artifacts and contaminants (such as dibutyl phthalate and hexamethylcyclotrisiloxane) emphasizes the need for careful interpretation during GC–MS spectral screening; Therefore, this work recommends that Advanced Chromatographic Verification- like or liquid chromatography–mass spectrometry (LC-MS/MS) to confirm tentative alkaloid identifications, particularly piperine, which is unusual for *V. amygdalina*.

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