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Comparative Phytochemicals and GC-MS Analysis of *Tetrapleura tetraptera's* Stem and Leaf Ethanolic Extracts

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Abstract

Tetrapleura tetraptera **(Fabaceae) is an important herb in African traditional medicine. It is renowned for its many therapeutic features, including its antibacterial, anti-inflammatory, and antidiabetic activities. This study investigated and contrasted the volatile compounds and phytochemicals detected in the bark of the stem and leaves of *Tetrapleura tetraptera* ethanolic extract. To show that there were multiple phytoconstituents, both qualitative and quantitative phytochemical tests were conducted using normal laboratory methods. We employed Gas Chromatography–Mass Spectrometry (GC-MS) to develop a list of volatile chemicals. The phytochemical screening showed that both portions of the plant had flavonoids, tannins, saponins, phenols, and steroids. Quantitative research indicated that the stem exhibited greater levels of flavonoids and phenolics when compared to those of the leaf, with values of 460.91 ± 0.09 µg/mL and 457.38 ± 1.43 µg/mL respectively. Flavonoids were the predominant components in the leaf and stem bark extracts. On the other hand, the leaf extracts contained the lowest number of tannins (85.06 ± 0.12 µg/mL) and the stem bark extracts had the lowest quantity of phlobatanins (44.86 ± 0.40 µg/mL). The GC-MS research revealed the following bioactive compounds: 4-(pyridin-2-yl) piperazine-1-carbaldehyde, n-hexadecanoic acid (palmitic acid), kolavelool, D-limonene, and bicyclo[3.1.0]hexan-3-ol derivatives. The stem bark extracts had the most n-hexadecanoic acid, which made up 24.53% of the area. The leaf extracts had the highest bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-, which made up 38.43% of the area. The stem and leaf revealed large differences in compound variety and abundance, suggesting a part-specific chemical composition. These findings reveal the medicinal potential of *Tetrapleura tetraptera* and show that the stem bark and leaf have diverse phytochemical compositions, which means that they have various effects on different organs. Consequently, these plant extracts may be employed to treat and manage infections and tackle antimicrobial resistance. Biotechnologically, these compounds show promising natural therapeutic potential that could be used as antibacterial, anti-inflammatory, antidiabetic medicines and to tackle antimicrobial resistance.**

Keywords: *Tetrapleura tetraptera*, GC-MS, bioactive compounds, phytochemicals, ethnomedicine, stem bark and leaf extract.
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Introduction

For many years, medicinal herbs have been used by people to prevent and cure a diverse range of diseases and infections. Traditional knowledge has facilitated scientific investigation and development of modern pharmaceuticals sourced from plants (Mammari et al., 2023, Onyeukwu et al., 2024). Despite a lack of thorough clinical testing and a lack of scientific understanding of their activities, medicinal plants continue to be a key source of healthcare in many nations, including Nigeria and other regions of Africa. Plants have been exploited medicinally to cure a multitude of disorders, according to previous studies (Adeyemi et al., 2020, Chijindu et al., 2020, Lokwutor et al., 2024, Onakurhefe et al., 2022).

The native plant *Tetrapluera tetraptera*, usually referred to as the Aidan tree, offers several health benefits (Mensah et al., 2024). This perennial and deciduous blooming plant, which is a member of the Fabaceae family, has evergreen leaves that are dark, base that are woody, branches that are spread and sturdy. The plant is mainly found in West Africa and is extensively spread throughout Equatorial Africa. According to Sharanya et al. (2023), common names include Aridan (Yoruba), Dawo (Hausa), Uyayak (Igbo), Ighimiakia (Bini), Aidan tree/fruit (English), and Prekese (Ghana). It is used to improve taste to soups and is believed to reinforce the immune system. In local healthcare systems where access to synthetic drugs may be limited, its extensive use suggests its effectiveness. According to Adusei et al. (2019), *Tetrapleura tetraptera* has long been used to treat bronchitis, coughs, asthma, and symptoms that resemble those of tuberculosis. To produce teas or infusions that are consumed the fruit is cooked. According to Mensah et al. (2024), the plant's antimicrobial characteristics aid postpartum moms prevent infections and inflammation. In many societies, it has been used for spiritual purposes. *Tetrapleura tetraptera* is widely renowned for its many medicinal properties, which are mostly associated with its rich phytochemicals, which improves its nutritional and therapeutic features. These substances are distributed in the plant, including the bark of the stem, its leaf, pulp, seeds, as well as the entire fruits.

Natural bioactive compounds termed phytochemicals have protective and disease-preventive characteristics that make them valuable in pharmacology and medicine (Egharevba et al., 2019, Onyeukwu et al., 2025). Adusei et al. (2019) purported that these substances were linked to a variety of pharmacotherapy actions, such as inflammation reducer, antibacterial, antioxidant, anti-hyperglycemic, hepatoprotective, immunomodulatory, and antimalarial activities.

Even with its extensive use, there is still a dearth of rigorous and consistent phytochemical investigation of the leaves and stem bark. Most previous investigations concentrated on individual compounds or crude extracts without thoroughly comparing or measuring leaves and stem bark. The accurate evaluation of these bioactive compounds provided by contemporary analytical methods like GC-MS is essential for confirming traditional applications, guaranteeing quality control, and creating potent herbal formulations (Ranjan et al., 2023, Muley et al., 2026).

The phytochemical composition of *Tetrapleura tetraptera* extracts is greatly impacted by the extraction technique and solvent selection. According to studies, ethanol works better than aqueous solvents at removing bioactive chemicals from the fruit, stem bark, and leaves, among other plant parts. The potential of ethanol to dissolve hydrophilic and hydrophobic molecules and pass through plant cell membranes may stimulate the production of phytochemicals like flavonoids, steroids, saponins, phenols, tannins and terpenoids. Thus, this study employed GC-MS to carry out a full phytochemical analysis of *Tetrapleura tetraptera's* leaves and stem bark to support its medical usage with scientific proof and to enable the production of standardized herbal products easier.

Materials and Methods

Plant Collection and Authentication

Fresh *T. tetraptera's* leaves and bark of the stem were obtained in March 2025 from New Site Ewulu, Aniocha South Local Government Area, Delta State, Nigeria (6°03'50.6"N 6°33'18.9"E). The samples were conveyed in clean, sterile polythene bags to prevent contamination. Upon reaching the laboratory, it was validated in the University of Benin, Department of Plant Biology and Biotechnology. Voucher specimens (UBH-T472) were deposited for future reference.

Plant Sample Preparation

To get rid of unnecessary items like stalks, dirt bits, and other debris, the collected samples were washed. The stem barks were also chopped into tiny junks. To get rid of surface flaws, they were meticulously cleaned with water first, and then washed with sterile water. To prevent the loss of thermolabile and photosensitive phytochemicals, the cleaned leaves and stem barks were parched for six weeks (25-28°C) without being exposed to direct sunlight (Fatile et al., 2021). The samples were dried, then blended into powder with an electric grinder, sifted to achieve consistent texture, and stored at room temperature closed jar until they were required for extraction and analysis.

Plant Sample Extraction

A 150g crushed sample was weighed and placed into bottles. The samples were submerged in 500 mL of 100% ethanol, agitated three times a day, and then stored in a dark room for seventy-two hours. A rotary evaporator was utilized to vaporize the solvent and cheesecloth was used to decant the supernatant. After being weighed, the dried extracts were stored for a period at -4˚C in sterile universal vials for the freezer. For the purpose of screening for phytochemicals, 10% of the crude extract was redissolved in ethanol and used for the analysis.

Qualitative Phytochemical Assessment of Tetrapleura tetraptera

Phytochemical screening was evaluated on the ethanolic extracts of *Tetrapleura tetraptera*' leaf and stem bark using the procedures outlined previously by Onyeukwu et al. (2025) for the identification of cardiac glycosides, steroids, terpenoids, phenol and phlobatanins and Maheshwaran et al. (2024) for the identification of flavonoids, saponins, tannins, coumarins, alkaloids and anthraquinone.

Flavonoids

Ethanolic filtrate of 1 mL was mixed with 5 mL of 10% NH_3 followed by 1 mL of H_2SO_4. Flavonoids was detected by the appearance of yellow colour.

Tannins

Exactly 2 mL of water and and 1 mL of extract (0.5 g/5 mL) was boiled in a test tube, before being filtered with the addition of three drops of 0.1% ferric chloride, and brownish green to blue-black appearance shows a positive test.

Cardiac glycosides (Keller-Killiani test)

One drop of ferric chloride was added to glacial acetic acid and was mixed with 1 mL of 0.5 grams/5 mL of plant extract. A slow application of 1 mL of H_2SO_4 was made. A browning at the surface reveals that cardenolides, which have a deoxysugar component, were present.

Test for Saponin (Frothing test)

The capacity of saponins to foam in aqueous solution shows a positive test. 5 mL and 1 mL of distilled water and extract (0.5 g/5 mL) respectively was mixed and shaken to create a stable froth. This was further confirmed by observing emulsion formation after adding olive oil in three drops and then mixed properly.

Test for Steroids

Each sample was extracted using 0.5 mL of ethanol (0.5 g/5 mL) and 2 mL of concentrated sulphuric acid, followed by 2 mL of concentrated acetic anhydride. Steroids were found when the colouration altered from violet to blue or green.

Test for Terpenoids (Salkowski test)

2 mL and 3 mL of concentrated chloroform and H_2SO_4 respectively were combined with 1 mL of the extract in a test tube. The interface's reddish brown hue verified that terpenoids were present.

Test for Phenols

A test tube containing 5 mL of (0.5g/5mL) ethanol extract was mixed with 3 drops of aqueous 10% $FeCl_3$ solution. The development of green or blue colour suggested the existence of phenols.

Test for Phlobatanins

2 mL of 1% HCl was added to 3 mL of (0.5g/5mL) extract and was boiled. A red precipitate's deposition was interpreted as proof that phlobatanins were present.

Test for Coumarin

5 mL of 0.5 g/5 mL ethanol extract was split into two portions after being diluted in 2 mL of hot distilled water. Half of the volume was used as a control, and the other half received 0.5 mL of 10% NaOH in addition to chloroform. A yellow colour indicate positive test.

Test for Alkaloids

3 drops of Mayer's reagent were combined with 1 mL of (0.5 grams/5 milliliters) ethanol extract. Positive test for alkaloids was established by the production of a cream-colored precipitate.

Test for Anthraquinone

1 mL of (0.5g/5mL) extracts and 5 mL of benzene were mixded in a test tube and quickly shaken in 2.5 mL of pure NH_3. A pink-red colouration that formed at the lower phase depicted free anthraquinone presence.

Quantitative Phytochemicals Analysis

The total amounts of flavonoids, saponins, and steroids were estimated using the Madhu et al. (2016) approach. The total amounts of cardiac glycosides and phenols were estimated using the Tofighi et al. (2016) while the methods of Ameen et al. (2021), Sharma et al. (2021), and Komala (2022) were used to estimate the total numbers of tannins and terpenoids, respectively.

Estimation of Flavonoids

Exactly 1 mL and 4 mL of the test sample and water respectively was mixed in a 10 mL volumetric flask in addition to 0.3 mL $NaNO_2$ and 0.3 mL $AlCl_3$ after 5mins. This was followed by adding of 2 mL of 1 M NaOH after six minutes incubation at room temperature. It was made up to 10 mL with distilled water. The spectrophotometry was read at 510 nm while using quercetin as a reference.

Saponin Estimaton

Exactly 1 mL of the samples in addition to 2 mL of vanillin, followed by 2 mL of a 72 % H_2SO_4 solution were mixed and heated for 10 minutes at 60°C in a water bath. This was read at 544 nm, using Diosgenin as a reference.

Estimation of Steroids

Exactly 1 mL of the sample was added into 10 mL of volumetric flask, followed by 2 mL 4 N H_2SO_4 and 2 mL $FeCl_3$ (0.5 % w/v), 0.5 mL $K_3[Fe(CN)_6]$ (0.5 % w/v). The mixture was heated and periodically shaken before being diluted with water to the appropriate consistency. This was read at 780 nm against a blank while stigmasterol was employed as standard.

Estimation of Terpenoids

In a test tube, 500 µL of 99.5% sulphuric acid, 250 µL of vanillin solution (50 mg/ml), and 75 µL of plant extract were added. This was placed in an ice bath and 2500 µL of 99.5% acetic acid was added after it had been heated for 20 minutes at 60°C in a water bath. After 20 minutes of cooling, the absorbance at 548 nm was measured. The standard employed was beta-sitosterol.

Estimation of Phenols

Ethanol solution of each sample (0.2–100 µg/mL) was mixed with Folin-Ciocalteu reagent (2 ml, diluted 1:10 with distilled H_2O). After five minutes, 1.5 mL of 60 g/L in distilled water saturated $NaHCO_3$ solution was added. Following a 90-minute period at room temperature, it was read at 725 nm. The process was repeated using different concentrations of gallic acid solution (0.2-1.0 µg/mL).

Estimation of Cardiac glycosides

10 mL of freshly made Baljet's reagent was added to 10 % extract. Following an hour, distilled water of 20 mL was added to dilute the mixture, and the absorbance was read at 495 nm. The standard used was securidaside.

Estimation of Tannins

Tannins were determined using the Folin-Ciocalteu technique. Distilled water and sample extract of 10.7 mL and 0.1 mL respectively were put in volumetric flask. After stirring, it was let to stand 30 minutes at room temperature. Following the same technique as for the sample extract, tannic acid was employed as reference at 700 nm.

GC-MS Analysis

This was carried out using Agilent 7890B GC system coupled to a 5977A Mass Selective Detector, which was operated under standard settings acceptable for phytochemical investigation. The injection part and detector were retained at 250°C, and the oven temperature program started at 60°C (hold for 2 minutes), rapidly rose to 150°C at 10°C/min, hold for 2 minutes, and then ramped to 280°C at 5°C/min, hold for 10 minutes. A splitless Injection Mode was adopted, with an injection volume of 1 µL. chemical contents were identified based on mass spectra of those in the National Institute of Standards and Technology (NIST) Mass Spectral Library, using the Agilent ChemStation software. Compounds were matched based on retention time, molecular ion peaks, and distinctive fragmentation patterns (Onyeukwu et al., 2025, Kind & Fiehn, 2007).

Statistical Analysis

Data was reported as mean ± standard deviation using GraphPad Prism version 10. The differences in the concentration of phytochemicals between leaf and stem bark was analyzed using a non-parametric Mann-Whitney U test. An evaluation of the phytochemical absorbance across various phytochemicals was carried out using kruskal-walis test.

Results

A range of metabolites, such as phenols, terpenoids, flavonoids, tannins, cardiac glycosides, steroids, saponins, and phlobatannins, were found in the ethanolic extracts of *Tetrapleura tetraptera* leaves (Sample A) and stem bark (Sample B), as depicted in Tables 1 and 2. Flavonoids and steroids were the most abundant chemicals in the leaf extract, followed by moderate levels of tannins and saponins and a minimal amount of cardiac glycosides. There were no anthraquinones, terpenoids, phenols, phlobatannins, coumarins, or alkaloids. On the other hand, phenols, steroids, and flavonoids were also strongly abundant in the stem bark extract. Terpenoids and phlobatannins were slightly abundant, although tannins and cardiac glycosides were moderately present. Coumarin, alkaloids, anthraquinones and saponins were were not present in the stem bark. Flavonoids were the most abundant of the eight phytochemicals that were tested; their concentrations in the leaf and stem bark extracts were 460.91 ± 0.09 µg/mL and 457.38 ± 1.43 µg/mL, respectively. In contrast, tannins had the lowest concentration (85.06 ± 0.12 µg/mL) in the leaf extracts, and phenolphthaleins had the lowest concentration (44.86 ± 0.40 µg/mL) in the stem bark extracts.

Table 1: Qualitative Phytochemical Constituents of *Tetrapluera tetratera* Leaves and Stem Bark Ethanolic Extract

PHYTOCHEMICALS	SAMPLE A	SAMPLE B
Flavonoids	+++	+++
Tannins	++	++
Cardiac glycosides	+	++

Saponins	++	-
Steroids	+++	+++
Terpenoids	-	+
Phenols	-	+++
Phlobatanins	-	+
Coumarin	-	-
Alkaloids	-	-
Anthroquinone	-	-

KEY: Sample A = *Tetrapleura tetraptera* (Leaf), Sample B = *Tetrapleura tetraptera* (Stem bark), (+++) = Presence is high (++) = Presence is moderate (+) = Present slightly, (-) = Absence.

Table 2: Quantitative Phytochemical Composition of *Tetrapluera tetraptera* Leaf and Stem Bark Ethanolic Extracts

Phytochemicals (µg/mL)	**Sample A**	**Sample B**
Flavonoids	460.91 ± 0.09[a]	457.38 ± 1.43[a]
Tannins	85.06 ± 0.12[b]	76.91 ± 0.13[b]
Phenols	–	66.10 ± 0.27[c]
Terpenoids	–	126.44 ± 0.08[d]
Cardiac glycosides	175.57 ± 0.10[e]	176.25 ± 0.19[e]
Steroids	160.60 ± 0.15[f]	162.65 ± 0.15[f]
Saponins	133.96 ± 0.52[g]	–
Phlobatannins	–	44.86 ± 0.40[h]

Results are shown as Mean ± SD. Sample A = *Tetrapleura tetraptera* (Leaf), Sample B = *Tetrapleura tetraptera* (Stem bark). Same letter across rows are not significantly different and different letters across column are significantly different ($p < 0.05$)

GC-MS Analysis of Ethanolic Leaf and Stem Bark Extract of Tetrapleura tetraptera

GC-MS analysis of ethanolic *Tetrapleura tetraptera's* leaf and stem bark extract are shown in Tables 3 and 4 while Figures 2 and 3 show the gas chromatograms. The ethanolic leaf extract exhibited 19 absorption peaks in which two peaks are unknown. 5-Hydrazinocarbonyl-1H-imidazole-4-carboxylic acid, (3-methoxy-phenyl)-amide ($C_{12}H_{12}N_6O_4$) with RT (14.806) has a peak area of (1.48%), Benzamide, 4-fluoro-N-(2,4-dimethoxyphenyl)- ($C_{15}H_{14}FNO_3$) with RT (15) has a peak area of (4.65%). Additional chemicals include Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)-, Kolavelool, 4-(Pyridin-2-yl) piperazine-1-carbaldehyde, Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)- etc. In the leaf extract, Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)- had the maximum peak area whereas Naphthalene, octahydro-7-methyl-4-methylene-1-(1-methylethyl)- had the lowest peak area. In the stem bark extracts of *Tetrapleura tetraptera*, 19 absorption peaks were identified. The principal elements discovered were D-limonene ($C_{10}H_{16}$) with RT (4.472) and a peak area of (6.11%), 2-propenoic acid esters ($C_{13}H_{20}O_2$) with RT (8.975) and peak area of (5.34%), Tetradecane ($C_{14}H_{30}$) with RT (9.17) and

peak area of (1.05). etc. In the stem bark extract, n-hexadecanoic acid (palmitic acid) had the highest peak area (24.53%) however, 22-Methyl-tricosanoic acid, pyrrolidide had the lowest peak area.

Table 3: (GC-MS) Identified Compounds Present in the Ethanolic Leaves Extract of *Tetrapluera tetraptera*

Peak #	RT (min)	Area %	Top Compound	Molecular Formula	Molecular Weight (g/mol)
1	14.806	1.48	5-Hydrazinocarbonyl-1H-imidazole-4-carboxylic acid, (3-methoxy-phenyl)-amide	$C_{12}H_{12}N_6O_4$	304.27
2	15	4.65	Benzamide, 4-fluoro-N-(2,4-dimethoxyphenyl)-	$C_{15}H_{14}FNO_3$	275.28
3	15.081	1.47	Benzamide, 4-fluoro-N-(2,4-dimethoxyphenyl)-	$C_{15}H_{14}FNO_3$	275.28
4	15.103	1.46	Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)-	$C_{15}H_{24}$	204.35
5	15.172	3.58	Benzamide, 4-fluoro-N-(2,4-dimethoxyphenyl)-	$C_{15}H_{14}FNO_3$	275.28
6	15.304	1.3	Kolavelool	$C_{20}H_{32}O_2$	304.47
7	15.349	1.5	Bornyl acetate	$C_{12}H_{20}O_2$	196.29
8	15.452	2.14	Kolavelool	$C_{20}H_{32}O_2$	304.47
9	15.498	5.22	Kolavelool	$C_{20}H_{32}O_2$	304.47
10	17.015	1.58	2a,5,5,10b-Tetramethyl-hexahydro-2ah-2-oxa-10a-aza-benzo[g]cyclopenta[cd]azulen-1-one	$C_{17}H_{25}NO_2$	275.39
11	17.272	11.74	4-(Pyridin-2-yl)piperazine-1-carbaldehyde	$C_{10}H_{13}N_3O$	191.23
12	17.621	23.18	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{18}O$	154.25
13	17.741	7.75	4-(Pyridin-2-yl)piperazine-1-carbaldehyde	$C_{10}H_{13}N_3O$	191.23
14	17.901	15.25	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{18}O$	154.25
15	17.936	5.48	Guaia-1(10),11-diene	$C_{15}H_{22}$	202.33
16	19.933	4.85	Naphthalene, octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	$C_{15}H_{24}$	204.35
17	20.345	1.24	Naphthalene, octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	$C_{15}H_{24}$	204.35
18	20.791	3.63	Unknown	_	_
19	20.808	2.49	Unknown	_	_

RT: Retention time

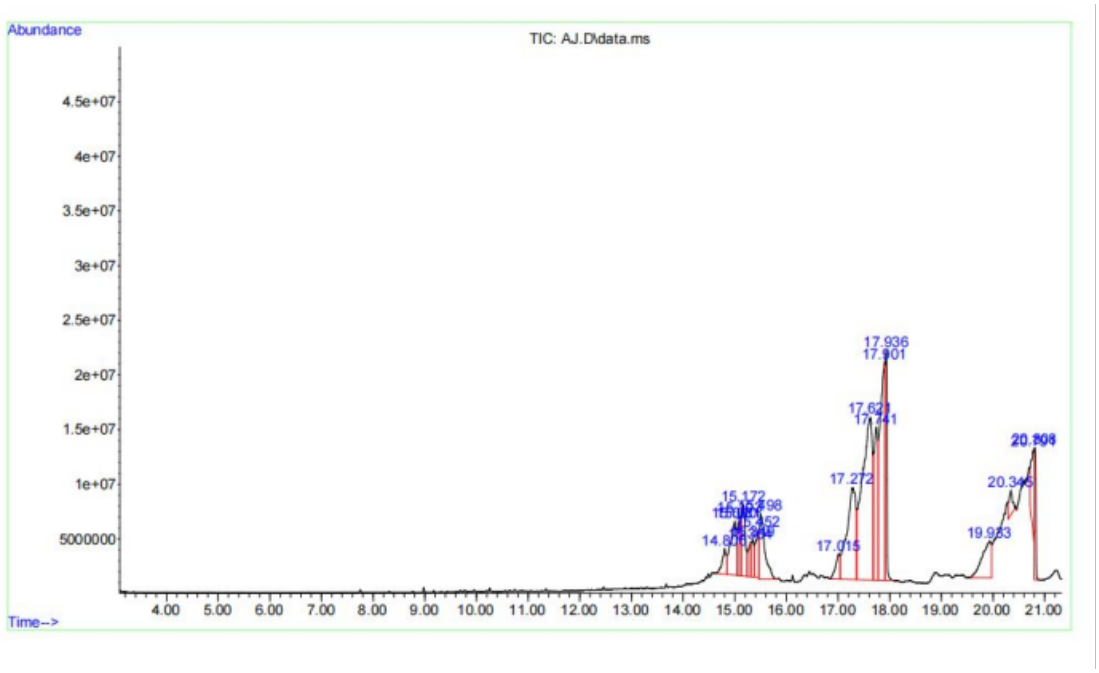


Figure 1: Gas Chromatograph of Ethanolic Extract of *Tetrapluera tetraptera* Leaves.

Table 4: (GC-MS) Identified Compounds Present in Ethanolic Stem Bark Extract of *Tetrapluera tetraptera*

Peak #	RT (min)	Area %	Top Compound	Molecular Formula	Molecular Weight (g/mol)
1	3.167	1.18	3-Hexene, 2,5-dimethyl-, (E)-	C_8H_{14}	110.20
2	4.472	6.11	D-Limonene	$C_{10}H_{16}$	136.24
3	7.751	1.16	Decane, 2,3,7-trimethyl-	$C_{13}H_{28}$	184.36
4	8.975	5.34	2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester	$C_{13}H_{20}O_2$	208.30
5	9.17	1.05	Tetradecane	$C_{14}H_{30}$	198.39
6	10.251	1.67	Eicosane	$C_{20}H_{42}$	282.55
7	11.338	1.11	Hexadecane	$C_{16}H_{34}$	226.44
8	12.649	1.2	Silane, dichlorodiisopropyl-	$C_6H_{16}Cl_2Si$	187.18
9	13.908	1.09	1-Propene, 3-chloro-1,1,3,3-tetrafluoro-	$C_4H_3ClF_4$	162.51
10	14.44	1.96	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{16}H_{32}O_2$	256.42
11	14.474	1.68	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-	$C_{20}H_{28}O_3$	316.44

			2,8-dione		
12	14.806	24.53	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
13	15.195	1.3	Anteiso-pentacosanoic acid, pyrrolidide	$C_{29}H_{57}NO$	435.77
14	15.481	1.82	Anteiso-pentacosanoic acid, pyrrolidide	$C_{29}H_{57}NO$	435.77
15	15.538	1.29	2-Thiophenecarboxylic acid, 5-heptadecyl-4-methyl-, methyl ester	$C_{20}H_{32}O_2S$	336.53
16	15.561	1.58	2-Thiophenecarboxylic acid, 5-heptadecyl-4-methyl-, methyl ester	$C_{20}H_{32}O_2S$	336.53
17	15.99	1.04	22-Methyl-tricosanoic acid, pyrrolidide	$C_{26}H_{52}NO$	394.69
18	16.38	4.33	1,4-Cyclohexanedimethanamine	$C_8H_{18}N_2$	142.24
19	16.425	11.12	5,8,11,14,17-Eicosapentaenoic acid, pyrrolidide	$C_{25}H_{41}NO_2$	387.61

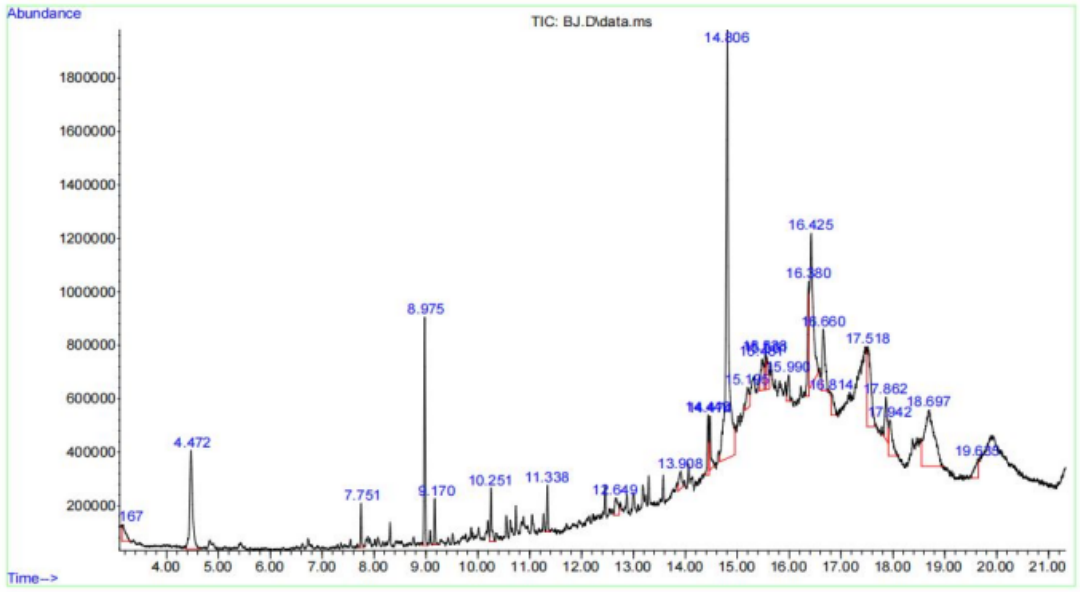


Figure 2: Gas Chromatograph of Ethanolic Extract of *Tetrapluera tetraptera* Stem Bark

Discussion

From Tables 1 and 2, the ethanolic extracts of *Tetrapleura tetraptera* leaves and stem bark were discovered to contain a range of secondary metabolites. Flavonoids were the most prevalent in both the leaf and stem bark extracts which are recognized as potent antioxidants with antibacterial and anti-inflammatory potentials (Larbie et al., 2020). This corroborates recent studies (Chijindu et al., 2024, Njideaka et al., 2024) that have reported the abundance of flavonoids in plants. Flavonoids are groups of phytochemicals present in plant which contributes to the growth of plant and its

maturity and have been applied in food and pharmacology for cardiovascular risk management (Liu et al., 2021, Popiolek-Kalisz & Fornal, 2022). Both samples contain cardiac glycosides, which are extensively explored for their cardiovascular advantages, especially in the monitoring of heart-related illnesses. Both parts have roughly equal levels of steroids. The equal number of steroidal compounds in both extracts suggests broad therapeutic potential, as they play essential roles in controlling immune responses and inflammation. Interestingly, saponins which have antibacterial, cholesterol-lowering, and immune-boosting qualities were only identified in the leaves (Onyeukwu et al., 2025). Their lack in the stem bark confirms prior findings demonstrating saponins are more frequent in the plant's aerial portions (Chijindu et al., 2023, Laaraj *et al*., 2024). Additionally, the investigation found that both the stem bark and the leaves contained tannins, yet the quantities of tannins in the leaves were marginally higher than those in the stem bark. This is congruent with study by Chijindu et al. (2024) and Zhou *et al*. (2025) which demonstrated that tannins have antibacterial and antioxidant characteristics and enable plant extracts have protective advantages. Also, only bark of the stem contained phenols and terpenoids. Terpenoids possesses diverse biologically active pharmacotherapy, such as antibacterial and anti-inflammatory capabilities, whereas phenolic compounds are effective antioxidants (Mbaveng et al., 2021). Part-specific biosynthetic patterns may be represented in their absence in the leaf sample. Also, only the stem bark contains phlobatannins, a subclass of condensed tannins connected to antibacterial action and plant defense systems. Their presence in the bark is compatible with their protective role in woody tissues. Though the makeup varies, *T. tetraptera's* leaves and stem bark both contain beneficial phytochemicals. The functional implications of their individual phytochemical profiles mean that each component can be administered selectively based on the desired therapeutic purpose, even though their overall phytochemical concentration is statistically comparable. A wide array of bioactive compounds with probable therapeutic efficacy were found by GC-MS analysis. Seventeen of the 19 chromatographic peaks obtained in the ethanolic leaf extract were positively identified. Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)- was the most prevalent component, showing up at two retention times and having a considerable proportion to the extract's overall composition (23.18% and 15.25%). Various pharmacological effects, such as antibacterial, anti-inflammatory, and insecticidal characteristics, have been associated to this bicyclic alcohol (Mahato and Sen, 2020). 4-(pyridin-2-yl) piperazine-1-carbaldehyde, a piperazine derivative having broad-spectrum antibacterial and neurological characteristics, was one of the other significant components (Goyal et al., 2021). Additionally, guaia-1(10),11-diene, a sesquiterpene with anti-inflammatory and probably anticancer characteristics, and kolavelool, a diterpenoid alcohol discovered at three separate retention durations, were identified by the inquiry. The anti-inflammatory and antibacterial activities of diterpenoids, such as kolavelool, are well documented, particularly in ethnomedical applications. Other compounds that were identified were saturated polycyclic hydrocarbons and benzamide derivatives, which may have cytotoxic, antiviral, and antioxidant activities. These many components raise the likelihood of synergistic activities that could boost the leaf extract's medical benefits.

A rich phytochemical profile was likewise exhibited by the stem bark extract, with 19 different compounds detected spanning a retention duration of 3.167 to 16.425 minutes. With 24.53% of the total peak area, n-hexadecanoic acid (palmitic acid) was the most prevalent compound. The study by Onyeukwu et al. (2025) that documented the abundance of n-hexadecanoic acid is supported by this. This saturated fatty acid is well-known for its anticancer, lipid-lowering, antioxidant, and antimicrobial characteristics (Carev et al., 2023, Alam et al., 2025, Mufidah et al., 2025). Another interesting molecule was 5,8,11,14,17-eicosapentaenoic acid, pyrrolidide, a derivative of omega-3 fatty acids with recognised cardiovascular and anti-inflammatory characteristics. Along with other aliphatic hydrocarbons as decane and hexadecane, the stem bark also contained D-limonene, a monoterpene with known antioxidant and antibacterial effects (Wojtunik-Kulesza, 2022, de Ávila et al., 2025, Wang et al., 2025). Despite their limited direct bioactivity, these saturated hydrocarbons can boost the extract's solubility and act as carriers of other lipophilic compounds. Additionally, molecules containing nitrogen and sulfur, such

as cyclohexanedimethanamine and derivatives of thiophenecarboxylic acid, were discovered and may have contributed to antioxidative and neuroprotective properties.

Conclusion

The ethanolic extracts of *Tetrapluera tetraptera* stem bark and leaves have been demonstrated to have key phytochemical compounds with distinctive ethnomedicinal effects, according to this study. More phytochemicals were detected in the ethanolic extract of *T. tetraptera's* stem bark than in the ethanolic extracts of the plant's leaves. Therefore, for therapeutic and ethomedical purposes, it is advised to use the ethanolic extract of *T. tetraptera's* stem bark rather than the ethanolic extract of its leaves. Biotechnologically, these compounds show promising natural therapeutic potential that could be used as antibacterial, anti-inflammatory, antidiabetic medicines and also to tackle antimicrobial resistance.

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