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Phytochemical Screening, GC-MS Study, FTIR analysis and antimicrobial activities of ethanol extracts of *Tetrapleura tetraptera* fruit

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Abstract

Introduction: Antimicrobial and phytochemical properties have been associated with the therapeutic effects of plants. This study aimed to qualitatively assess the *Tetrapleura tetraptera* fruit ethanol extract for phytoconstituents.

Method: The extract was subjected to GC-MS and FTIR analysis to identify bioactive components and functional groups. The Mycelial Inhibition Method was employed to evaluate the extract's antifungal properties against selected microorganisms. In the phytochemical screening, the ethanol extract exhibited the presence of all examined phytochemicals.

Results: GC-MS analysis revealed 15 compounds from 6 recognized classes, with Ethyl Oleate (48.21%), 9-Octadecenoic acid, (E)- (19.18%), 9-Oxabicyclo [6.1.0] nonane, cis- (8.86%), Hexadecanoic acid, ethyl ester (6.24%), and 9,12-Octadecadienoic acid, ethyl ester (4.46%) being the principal chemical components. Results from FTIR analysis indicated significant chemical functional groups in the ethanol extract. Moreover, the extract demonstrated enhanced antimicrobial activity compared to the control, suggesting its potential therapeutic applications. Conclusion: These findings imply that *Tetrapleura tetraptera* could serve as a source of bioactive substances.

Keywords: *Tetrapleural tetraptera*; FTIR; Phytochemical screening; antimicrobial activity; GC-MS

1. Introduction

Medicinal plants are a substantial constituent of the plant kingdom and exhibit a broad global distribution[1]. According to[2], the pharmacological assessment of plant-derived chemicals is a well-established approach for the identification of compounds, hence facilitating the creation of innovative and secure therapeutic agents. These botanical specimens possess distinct organic substances known as phytochemicals, which elicit various physiological effects within the human body. Various bioactive compounds, including as tannins, alkaloids, terpenoids, steroids, and flavonoids, have been identified[3]. In order to further our comprehension of the biological activities and phytoconstituents associated with medicinal plants, it is imperative to conduct thorough investigations[4]. Moreover, it is important to emphasize the necessity for comprehensive inquiries in this domain due to the scarcity of medicinal plant species that have been

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subjected to comprehensive scientific examination[5]. *Tetrapleura tetraptera,* a widely recognized medicinal plant classified under the Fabaceae family (previously Leguminosae: Mimosoideae), is alternatively referred to as "Aridan" or "Aidan" within the Yoruba ethnic community residing in Southwestern Nigeria, "Abogolo" within the Igala community residing in North-central Nigeria, and "Dawo" within the Hausa community residing in Northern Nigeria. The species is commonly observed in the lowland woods of numerous tropical African nations. It is recognized for its fruits, which are composed of a mushy pulp and small, brownish-black seeds. These fruits exude a distinctive fragrance and pungent perfume[6]. The optimal habitat for *Tetrapleura tetraptera* is the rainforest, where it can attain heights ranging from 20 to 25 meters and a girth of between 1.2 to 3 meters. Additionally, it can be observed in riverine forests, the southern savannah woodland, and the forest outliers within the African plains. The stem bark exhibits a somewhat level, grayish-brown texture and is slender. The foliage exhibits a sessile nature, bearing a glabrous or minutely hairy appearance. It possesses a common stalk measuring roughly 15-30 cm in length, which is gently channeled on its top side. According to[6-7], the fruit is characterized as enduring, suspended at the branches' ends on sturdy stalks measuring approximately 25 cm in length.

The fruit has a green coloration at its tender stage, but undergoes a transformation into a shiny, glabrous, and dark-purple-brown appearance as it matures and ripens. Its dimensions range from roughly 15-25 cm in length and 4-5 cm in width, depending on its size. Additionally, the fruit possesses four longitudinal, wing-like ridges that span nearly 2.5-3.0 cm in width. There are two woody wings and two soft wings, which are utilized for food, beverages, and medicine. According to[8], the seeds, which are small, black, hard, and flat, are hidden within the pods. The exceptional therapeutic properties of *Tetrapleura tetraptera* have attracted considerable interest in the fields of phytochemical and pharmacological research. The substance exhibits a high concentration of phytochemicals and demonstrates antioxidant and antibacterial properties, hence providing evidence for its traditional therapeutic use [8-9]. Furthermore, previous studies have revealed that it possesses several therapeutic properties, including anti-diabetic, anti-hypolipidemic, anti-convulsant, central nervous system-depressant, analgesic, anxiolytic, anti-inflammatory, antioxidant, and antibacterial effects [8, 10, 11]. Convulsions, discomfort, microbiological infections, hypertension, malaria, feverish situations, and weakened immune systems are commonly treated with this treatment modality. Additionally, it is employed in the culinary industry as a tonic, flavor enhancer, and seasoning. Furthermore, it serves as a valuable plant in the production of soap and the creation of Prekese bitters, syrup, and spices[12].

The medicinal properties of *Tetrapleura tetraptera* are attributed to its flavonoid derivatives and other secondary metabolites such as alkaloids and terpenoids. Efforts have been made by some authors to determine the phytochemical constituents of various parts of *Tetrapleura tetraptera* [13, 14]. Moreover, many medicinal plants serve as sources of antioxidants, which play a role in managing degenerative diseases caused by oxidative damage [15]. However, there is still a dearth of information regarding the antioxidant ability as well as the phytochemical constituents of this plant. Therefore, this work aims to conduct phytochemical screening, GC-MS study, FTIR Analysis, and antimicrobial activities of Ethanol Extracts of crude Extracts of *Tetrapleura tetraptera* fruit.

Figure 1 Image of *Tetrapleura tetraptera* fruit

2. Materials and Methods

2.1. Plant Collection and Authentication

The fruits of *Tetrapleura tetraptera* were collected in Ijebu Itele, Ogun State, in the southwest region of Nigeria, precisely in July, 2023. The plant samples were taken to the Medicinal Plant Section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, for identification and authentication

2.2. Plant Sample Preparation

The plant material's fruit was meticulously washed with distilled water, air-dried for 60 days, and finely ground into powder using an electric Kinelco blender. Subsequently, the powder was stored in an airtight container for further studies.

2.3. Reagents Used

The analytical-grade chemicals utilized were procured from Bristol Scientific Company, an authorized distributor of Sigma-Aldrich products in Nigeria.

2.4. Plant Sample Extraction

A complete maceration using 5000 mL of ethanol was utilized on 400 g of finely ground plant material. A thorough extraction and dissolution of compounds was done by agitation with an ultrasonic sonicator for an hour at room temperature.

2.5. Qualitative Phytochemical Screening of Plant extracts

A standardized methodology outlined by[16] was employed to assess the phytochemical components of *Tetrapleura tetraptera* extract. Various phytochemicals were investigated, including phenols, anthraquinones, reducing sugars, alkaloids, glycosides, flavonoids, tannins, steroids, and terpenoids.

2.5.1. Alkaloids

A solution of 1% hydrochloric acid (HCl) in water was used to acidify 4 mL of test extracts in a steam bath. After adding a drop of Meyer's reagent, each test tube was filled with 1 mL of the acidified solution. The development of a creamy white precipitate confirmed the presence of alkaloids.

2.5.2. Tannins

Five (5) drops of Ferric chloride ($FeCl_3$) was added to each 2 mL test solution. Subsequently, a murky green precipitate was observed, indicating the presence of tannins.

2.5.3. Anthraquinones

In a separate investigation, five milliliters of each test solution were boiled in a water bath with a 10% hydrochloric acid (HCl) solution for ten minutes at 100 °C. The filtrate was then cooled and mixed with an equal volume of trichloromethane ($CHCl_3$). Each solution was heated, and 10% ammonia (NH_3) solution was added. The emergence of a rose-pink solution provided evidence of the existence of anthraquinones.

2.5.4. Glycosides

A 50% sulfuric acid concentration (5 mL) was added to each test extract in individual test tubes. The mixture was then heated in boiling water for fifteen minutes. After adding Fehling's reagent, the resulting mixture was heated until it reached the boiling point. A brick-red precipitate confirmed the presence of glycosides.

2.5.5. Reducing Sugars

Each extract was mixed with distilled water in a test tube, agitated vigorously, and filtered. Subsequently, Fehling's solutions A and B were introduced, followed by a boiling period of ten minutes for the filtrate. The presence of reducing sugars was confirmed when an orange-red precipitate formed.

2.5.6. Saponins

Five (5) milliliters of distilled water were mixed with two milliliters of the extracts, agitated well, and heated to boiling. The presence of saponins was confirmed by the appearance of foaming, resembling creamy milk with tiny air bubbles.

2.5.7. Flavonoids

Four (4) milliliters of sodium hydroxide solution (NaOH) in water were added to each of the approximately two-milliliter extracts in separate test tubes. The presence of flavonoids was confirmed by a yellow solution that turned colorless when hydrochloric acid (HCl) was added.

2.5.8. Phlobatanins

Five (5) milliliters of the extracts were mixed with distilled water and filtered. The resulting filtrate was then boiled with a 2% hydrochloric acid (HCl) solution. The presence of phlobatanin was confirmed by observing a crimson residue in the mixture.

2.5.9. Steroids

The confirmation of the presence of steroids was achieved through the observation of a brownish-to-red coloration with the addition of five drops of concentrated sulfuric acid (H_2SO_4) to each millilitre of the individual test extract solution.

2.5.10. Terpenoids

A layered solution was prepared by combining 2 mL of chloroform ($CHCl_3$) with 3 mL of strong sulfuric acid (H_2SO_2) for each 2 mL extract. The observed contact exhibited a reddish-brown hue, so providing evidence for the existence of terpenoids.

2.5.11. Phenolic

Phenols were confirmed by the development of bluish-black coloration in 5 milliliters of extracts when precisely four drops of ferric chloride were added.

2.6. Antimicrobial Assays

2.6.1. Collection and Maintenance of Test Microbes

In this study, fungi (*Aspergillus fumigatus, Aspergillus niger, Aspergillus flavu*s) were utilized. The microorganisms were sourced from the Department of Microbiology, LAUTECH Teaching Hospital, Ogbomoso, Oyo State, Nigeria, originating from established stock cultures. Maintenance involved agar slants in Mc - Cartrey bottles stored in a refrigerator.

2.7. Antifungal Activity of Crude Extracts of *T. tetraptera* Extract

The Mycelial Inhibition Method[17] was employed to assess the antifungal properties of the plant extract and nanoparticles. This involved adding nanoparticles with varying concentrations (150 µg/ml) to potato dextrose agar plates. The plates were subsequently inoculated with 6 mm agar plugs derived from 48-hour-old cultures of As*pergillus niger, Aspergillus flavus, and Aspergillus fumigatus.* The control studies involved inoculating fungal plugs on PDA plates without including the plant extract and silver nanoparticles. The plates were incubated at a temperature of 28 ± 2 °C for a duration of 72 hours. In order to ascertain the percentage of growth inhibitions, the diameters of fungal growths in all the plates were measured and subsequently utilized.

2.8. Fourier Transform Infrared Analysis

The infrared (IR) spectra were obtained using a Fourier transform infrared spectrophotometer (FT-IR) equipped with a universal attenuated total reflector (ATR) sampling accessory, manufactured by Perkin Elmer Spectrum 100. The functional groups in each percent of separated samples were identified using Fourier transform infrared spectroscopy (FTIR) with the Spectra 100 equipment manufactured by Perkin Elmer in the United States.

2.9. Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography-mass spectrometry (GC-MS) was employed for the analysis using a unique equipment (QP-2010 Ultra Shimadzu, Japan) and a DB-5MS capillary column (30 m length, 0.25 mm internal diameter, and 0.25 mm film thickness) operating in SCAN mode. The carrier gas used in this study was helium, which had a flow rate of 0.72 mL/min and a total flow of 31.8 mL/sec. This flow rate was characterized by a specific linear velocity. Additionally, a purge flow of 3.0 mL/min was applied. The temperatures of the injection port and detector were kept at 250°C and 300°C, respectively. The oven temperature programme began by setting it to 60°C for 0 minutes, then gradually increasing it to 300°C at a rate of 10°C per minute, and finally stabilizing it for 16 minutes. A splitless injection mode was employed, with a 1 µL injection volume.

3. Results and Discussion

3.1. Qualitative Phytochemical Screening Result

Table 1 shows the results of the phytochemical screening conducted on an ethanol extract of the fruit of *Tetrapleura tetraptera*. The screening confirmed the presence of alkaloids, glycoside, flavonoids, saponins, tannins, anthraquinones, reducing sugar, phenols, steroids, phobatananis, and terpenoids. This finding is consistent with the review by[11], which found comparable phytoconstituents in *Tetrapleura tetraptera* extracts.

Table 1 Phytochemical composition of Ethanolic extract of *Tetrapleura tetraptera*

Phytoconstituents	EtOH extract
Alkaloids	++
Glycosides	++
Flavonoids	++
Tannins	++
Saponins	++
Terpenoids	++
Phenols	++
Steroids	++
Anthraquinones	++
Reducing Sugar	++
Phlobatanins	++

Key: ++ represent (Abundant Reached in Phyto constituents)

3.2. Gas Chromatography-Mass Spectrometry Analysis Result

Table 2 Phytoconstituents of the extract of ethanol stem bark of *Tetrapleura tetraptera*

S/N	RT	Area %	Organic compound	MF	WM	Class
1	5.691	1.25	Naphthalene	$C_{10}H_8$	128.0	Aromatic hydrocarbon
2	8.008	1.08	6-Tetradecene, (Z)-	$C_{14}H_{28}$	196.0	Alkenes
3	10.091	1.17	5-Octadecene, (E)-	$C_{14}H_{28}$	196.37	Alkenes
4	11.956	0.78	5-Eicosene, (E)-	$C_{20}H_{40}$	280.53	Alkenes
5	13.478	2.66	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Fatty acid
6	13.656	6.24	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48	Fatty acid
7	14.938	19.18	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.46	Fatty acid
8	14.972	4.46	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308.50	Fatty acid
9	15.029	48.21	Ethyl Oleate	$C_{20}H_{38}O_2$	310.50	Ester
10	15.195	2.19	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.53	Ester
11	16.008	1.21	Palmitoyl chloride	$C_{16}H_{31}ClO$	274.90	Acyl chlorides
12	17.295	1.18	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.45	Aldehyde
13	17.856	8.86	9-Oxabicyclo [6.1.0]nonane, cis-	$C_8H_{14}O$	126.20	Ether
14	18.142	0.69	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.46	Fatty acid
15	20.631	0.85	5,5-Dimethyl-1,3-dioxane-2-ethanol	$C_8H_{16}O_3$	160.21	Ether

According to GC-MS analysis, fifteen compounds belonging to six known families of chemicals were discovered in the ethanol extract of *Tetrapleura tetraptera* fruit. These compounds encompass aromatic hydrocarbons, alkenes, fatty acids, esters, aldehydes, and acyl chlorides. The primary chemical constituents identified were Ethyl Oleate (48.21%), 9-Octadecenoic acid, (E) - (19.18%), 9-Oxabicyclo [6.1.0] nonane, cis- (8.86%), Hexadecanoic acid, ethyl ester (6.24%), and 9, 12-Octadecadienoic acid, ethyl ester (4.46%). Figure 3 illustrates the chromatogram of the GC-MS analysis, Figure 4 shows the chemical structure of compounds, while Table 2 provides a comprehensive list of the compounds, including their retention times, percentage peak areas, molecular weights, and respective chemical classes.

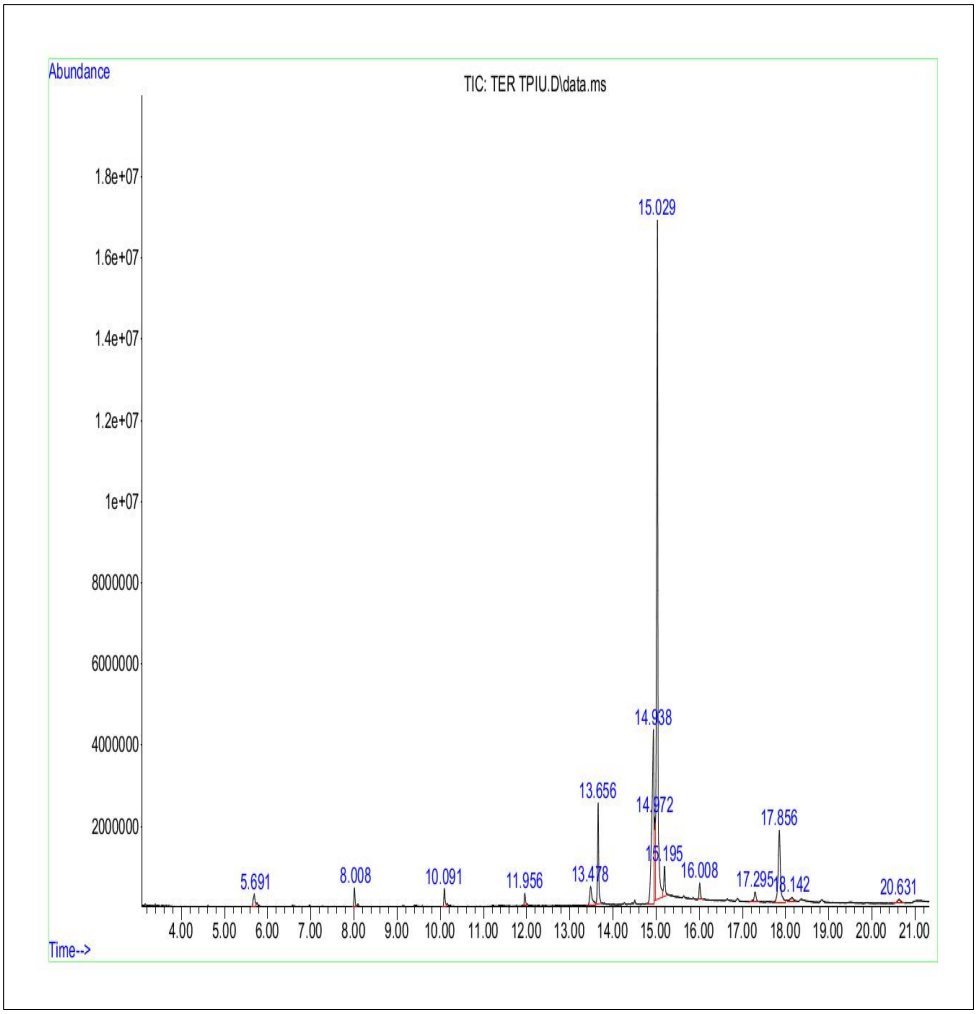

Figure 2 The chromatogram of phytoconstituents in ethanol fruit extract of *Tetrapleura Tetrapetra*

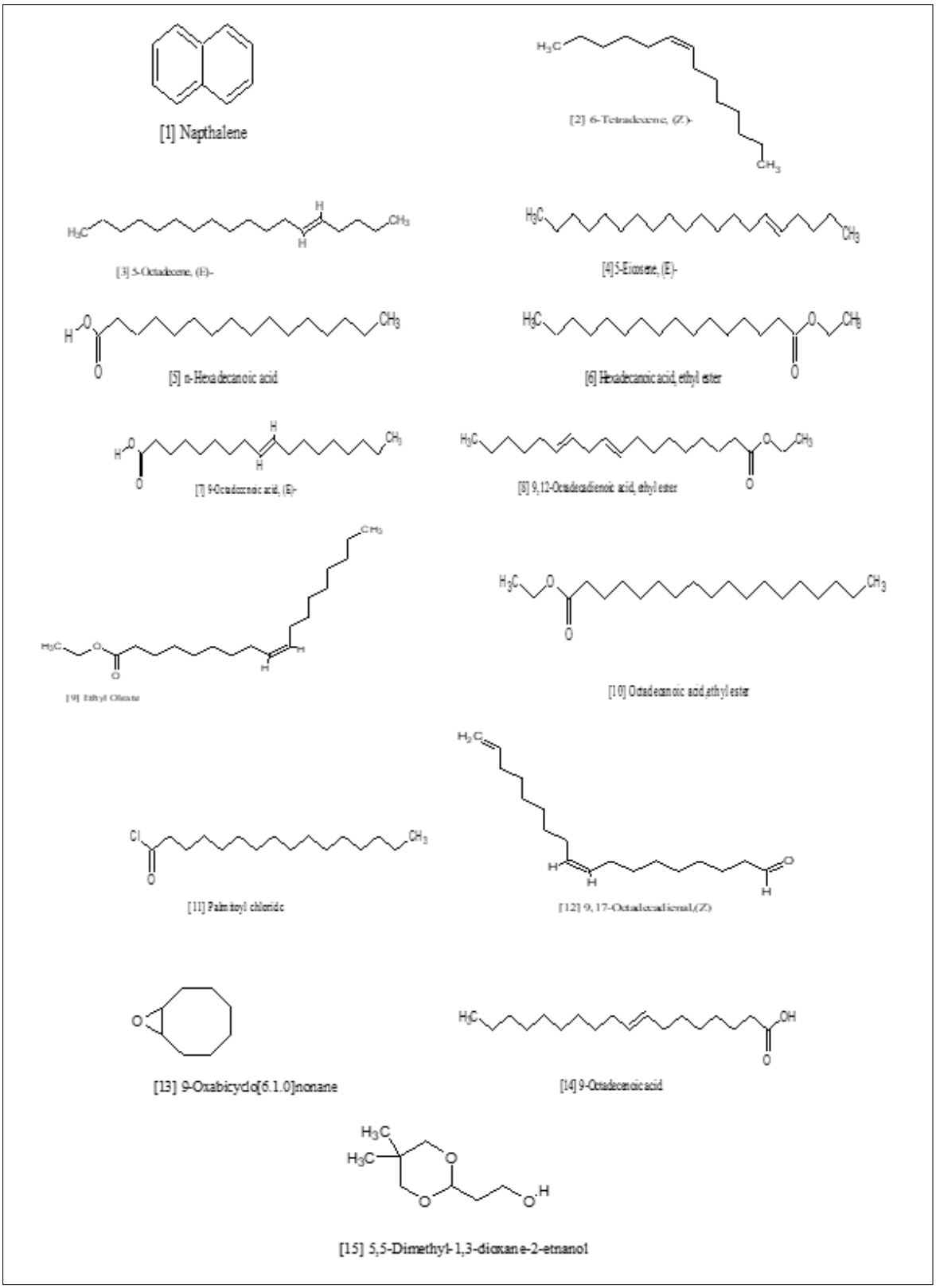

Figure 3 Chemical structure of compounds from GC-MS analysis of ethanol fruit extract of *Tetrapleura tetraptera*

3.3. Fourier-transform infrared (FTIR) Result

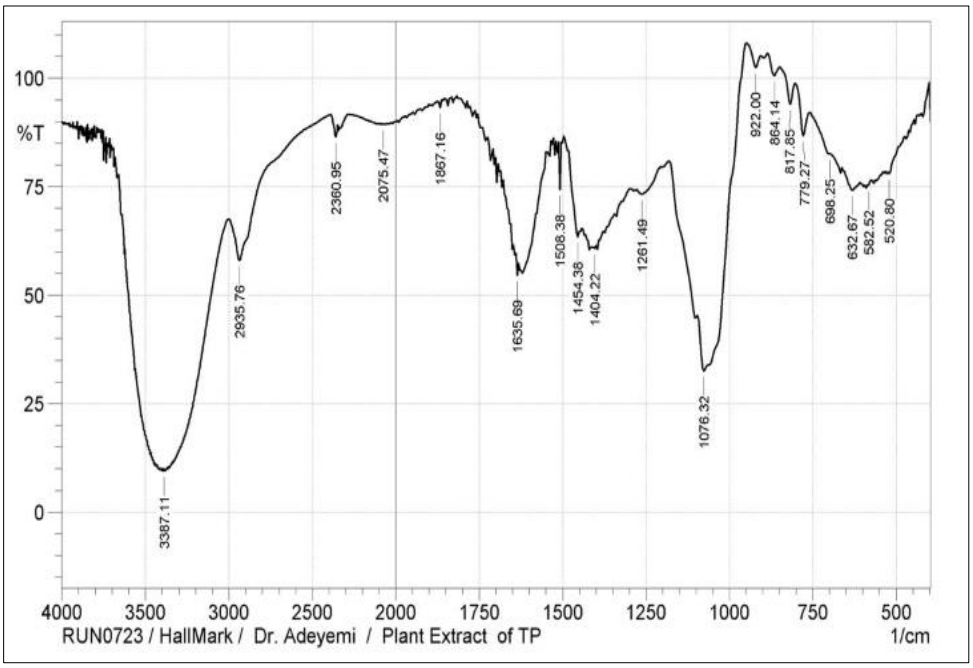

Figure 4 FTIR spectra of ethanol fruit extract of *Tetrapleura tetraptera*

Table 3 Assignments of fundamental peaks or bands

Absoprtion Bands	Types of Vibration	Frequency of Vibration
OH (Hydrogen bonded)	Stretching	3387.11 cm^{-1}
=C-H Alkenes	Stretching	2935.76cm^{-1}
C-H Aliphatic	Stretching	2360.95 cm^{-1}
C=C-C Alkenes	Stretching	1867.16 cm^{-1}
C= O of Ester	Stretching	1635.69 cm^{-1}
CH_3 of methylene	Stretching	1076.32cm^{-1}

The FTIR results of *Tetrapleura tetraptera* ethanol extract display distinct peaks at specific wavenumbers. At 3387.11 cm^{-1}, O-H stretching vibrations imply alcohol or phenol groups, potentially indicating hydroxyl groups in alcohols, phenols, or carboxylic acids. The peak at 2935.76 cm^{-1} corresponds to C-H stretching vibrations, suggesting aliphatic hydrocarbons like those in fatty acids or alkanes. At 2360.95 cm^{-1}, the peak indicates a specific type of C-H stretching, possibly linked to terminal alkynes known for strong absorption due to triple bonds. Another peak at 1867.16 cm^{-1} indicates C≡C stretching vibrations, suggesting alkynes or nitriles. At 1635.69 cm^{-1}, C=O stretching vibrations indicate carbonyl groups, possibly from ketones or aldehydes. Finally, at 1076.32 cm^{-1}, C-O stretching vibrations suggest ethers, esters, or alcohols, implying the presence of esters or ethers. These findings highlight diverse functional groups and chemical bonds in the extract, potentially including alcohols, fatty acids, alkynes, ketones, and esters.

3.4. Antifungal activity of ethanol fruit extract of *Tetrapleura tetraptera*

The antimicrobial potency of *Tetrapleura tetraptera* extract was examined against *Aspergillus flavus, Aspergillus fumigates* and *Aspergillus niger*. The results showed the extent of inhibition of the examined organisms Table 4. As shown in the result, *Tetrapleura tetraptera* extract inhibited the growth of *Aspergillus flavus, Aspergillus fumgatus* and *Aspergillus niger* by 87.07%, 67.45% and 74.73% respectively which were more potent when compared to that of the control 73.50%, 63.30% and 72.80% at 150 µl /ml, this is in accordance with [17] who reported the mycelial inhibition of *Synsepalum dulcificum* against *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger* at the range of 73 to 75%

at 100 µg/ml respectively. These mycelial inhibitory activities could be as a result of extract interaction with the cell membrane of fungalmycelia and disrupting the integrity of the cell membrane [18].

Table 4 Antifungal activities of ethanol fruit extract of *Tetrapleura tetraptera*

Test Organism	Inhibition of control	Extract inhibition
Aspergillus flavus,	73.50	87.07
Aspergillus fumigatus	63.30	67.45
Aspergillus niger	72.80	74.73

4. Conclusion

The primary objective of this work was to determine a range of phytochemical, GC-MS, and FTIR characteristics that could potentially serve as significant indications of economic value for research institutions and pharmaceutical businesses engaged in the production of novel pharmaceuticals. This fundamental information will enable subsequent research endeavors focused on identifying bioactive components, assessing their effectiveness through in vivo experiments, and showcasing their safety and effectiveness in clinical trials.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Author's Deceleration

The authors hereby declare that the work presented in this article is original and that any liability for claim relating to the content of this article will be borne by them.

Author's Contributions

This work was carried out in collaborations among the authors. Author Ezekiel Gbadebo Adeyeni designed the work, supervised it, and prepared the technical arrangement of the manuscript. Author Olusola Nathaniel Majolagbe, Adeekunle Ayodele Alabi and Hakeem Oyebisi Bello also worked in the laboratory with Ezekiel Gbadebo Adeyeni in carrying out the work. The interpretations of the data obtained were done by the four authors (Ezekiel Gbadebo Adeyeni, Majolagbe Nathaniel Olusola, Adekunle Ayodele Alabi and Hakeem Oyebisi Bello). All the authors contributed on the writing and editing of the manuscript. Also, all authors read and approved the final manuscript.

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