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Evaluation of Chemical Compound in Fermented and Non-Fermented *Artemisia Annua* Using Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Mabitine, D. M[1], Ogbonna, C.I.C[2], Shaapera, S. T[I] and Oche. D. G[1]

[1]*Department of Plant Science and Biotechnology, Joseph Saarwuan Tarka University, Makurdi, Benue State.*
[2]*Department of Plant Science and Biotechnology, University of Jos, Jos Plateau State*

ABSTRACT

This study evaluated the chemical composition of the aqueous leaf extract of *Artemisia annua* (Sweet Wormwood) using Fourier Transform Infrared Spectroscopy (FTIR). The leaves of *A. annua* were processed into aqueous and fermented extracts. FTIR analysis was then conducted to identify functional groups and elucidate molecular structures of the extracts. The analysis on aqueous leaf extract of *A.annua* revealed 11 significant peaks, indicating the presence of diverse functional groups, including alcohols at 1028.75 cm^{-1}, alkenes at 1073.47 cm^{-1}, alkynes at 1267.29 cm^{-1}, ether at 1319.48, amines at 1401.48, carboxylic acids at 1621.39 cm^{-1}, alkane at 2001.58 cm^{-1}, Alkyne at 2187.95 cm^{-1}, alkyne at 2296.04 cm^{-1}, alkane at 2922.23 cm^{-1} and alcohol at 3287.51 cm^{-1}.The FTIR analysis of fermented *Artemisia annua* leaf extract revealed a complex array of functional groups, indicative of the diverse chemical composition generated through fermentation. The spectrum identified peaks for C-O stretching (1032.47 cm^{-1}) and C-O or C-N stretching (1241.20 cm^{-1}), representing oxygen-containing groups such as alcohols, ethers, esters, or amines, with moderate to notable intensities. Additional peaks at 1319.48 cm^{-1} and 1379.12 cm^{-1} indicated C-H bending and methyl groups, suggesting the presence of aliphatic chains and alkyl groups, which enhance stability. The detection of C=C stretching at 1617.66 cm^{-1} pointed to aromatic or unsaturated compounds within the sample, likely phenolic or flavonoid compounds, which are valued for their antioxidant properties. Further analysis showed prominent C-H stretching peaks at 2851.41 cm^{-1} and 2918.51 cm^{-1}, typical of saturated hydrocarbons and indicating robust aliphatic chains within the extract. A broad O-H or N-H stretch at 3280.06 cm^{-1} suggested the presence of hydroxyl or amine groups in moderate concentration, likely contributing to the bioactivity of the sample. The presence of these functional groups suggests significant antioxidant, antimicrobial, and anti-inflammatory properties. Notably, the detection of phenolic compounds aligns with existing literature linking them to enhanced health benefits.

This inquiry highlights the value of FTIR as an effective analytical tool for characterizing the chemical composition of *A. annua* leaf extracts, augmenting our understanding of the plant's bioactivity, and supporting labours to validate its traditional medicinal uses. Ultimately, the findings contribute to the on-going discourse on the therapeutic potential of *A. annua* and lay a foundation for future studies aimed at exploring novel therapeutic applications and ensuring sustainable utilization of this important medicinal plant.

1. INTRODUCTION

Fourier Transform Infrared Spectroscopy (FTIR) is a potent analytical procedure commonly employed in identifying chemical compounds and elucidating the molecular structures of various substances. It quantifies the infrared spectrum of absorption or emission of a solid, liquid, or gas (Guerrero-Perez and Gregory, 2019). FTIR provides detailed information about the functional groups present within molecules. It rapidly and non-destructively assesses the bioactive compounds within plant and other materials (Sasidharan *et al*., 2020).

Artemisia annua (Sweet wormwood) is a plant known for its medicinal properties and is commonly used in traditional medicine across Africa and Asia. This plant contains artemisinin, a compound exceedingly effective against malaria (Noronha *et al*., 2020). The leaves of *A. annua* contain a complex mixture of bioactive compounds, including flavonoids, terpenes, and essential oils, which contribute to its medicinal, value (Vaou *et al*., 2022). Analyzing the aqueous extracts of

A. annua using FTIR allows for the identification of these specific functional groups and provides insight into the molecular composition of the bioactive. The chemical composition of the aqueous leaf extract of *Artemisia annua* has garnered significant interest due to its rich profile of bioactive compounds, such as flavonoids, terpenes, and essential oils (Anibogwu *et al.,* 2021).

The use of FTIR also in the study of fermented *A. annua* extract allows for a detailed analysis of the modifications in the chemical contents and aids to identify the explicit bioactive compounds that are improved or transformed or even lost during fermentation (Zhang *et al*., 2018).

However, despite the extensive research on *A. annua*, there remains a gap in fully characterizing its chemical composition using advanced analytical methods like Fourier Transform Infrared Spectroscopy (FTIR). Although previous studies such as the one of Anibogwu *et al.,* (2021) provided some insights into the pharmacological effects of the plant, but there is need for deeper understanding of the specific functional groups and molecular structures that are responsible for these effects, especially when analyzed through FTIR. Consequently, the identification of bioactive compounds, standardization, quality control, and discovery of novel therapeutic agents cannot be achieved.

2. MATERIALS AND METHODS

2.1 Study Location

The research was carried out in the General Biology Laboratory, College of Biological Sciences at Joseph Sarwuan Tarka University, Makurdi, and in the Department of Biochemistry, University of Ibadan, Nigeria.

2.2 Collection and Identification of Plant Materials

The A. annua var, chiknensis with batch number (PCN 004651/PAC4.8/CIC) was obtained from the Centre for Biotechnology and Genetic Engineering (CBGE), University of Jos, Plateau State. The leaves were washed with clean water, shade-dried at room temperature, and stored in glass containers until required for further use.

2.3 Preparation of Plant Materials

Twenty grams (20 g) of *A. annua* and *V.amygdalina* leaf powder were separately poured into a conical flask containing 100 ml each of sterile distilled water. The blend was subjected to mild heating with a Bunsen burner and allowed to cool to room temperature. The setups were filtered with Whatman No. 1 filter paper to separate the solid residues from the liquid extracts and the filtrates were subsequently evaporated in a hot water bath until dry before being stored in a refrigerator at 5°C until needed.

2.4 Preparation of Fermented Plant Samples

Boiled water was moved into a sterilised flask and allowed for a few minutes before it was dispensed into sterile bottles. Twenty grams (20g) of the *A. annua* leaf extract was added to bottles containing 300ml of sterile distilled water. Ten milliliters (10ml) of *Saccharomyces cerevisiea*. cultures were added to different bottles containing the plant extract, which were then sealed and left in a dark environment, undisturbed, for two weeks. After two weeks, racking was performed by moving the clear liquid into a sterile container. The liquid in the bottle underwent two more rounds of racking, one after an additional two weeks and another after one more week. After five weeks, the contents were left to age and evaluated.

2.5 FTIR Analysis

The FTIR analysis began with the preparation of potassium bromide (KBr) pellets, which were formed by finely mixing 1-2 mg of the various leaf extracts separately with approximately 200 mg of dry, spectroscopic-grade KBr powder. These separate mixtures were then compressed into a transparent pellet suitable for IR analysis independently. The FTIR spectra were collected using a Bruker Alpha-P spectrometer, spanning a spectral range of 4000 to 400 cm^{-1}, which is standard for identifying various functional groups in the organic compounds (Bashir *et al*., 2020). In order to ultimately measure the

samples, the spectrometer was equipped with an attenuated total reflectance (ATR) auxiliary introducing a ZnSe (zinc selenide) crystal. After obtaining the spectral data, standard amendment and stabilization were accurately done with the OPUS software suite. These procedures facilitate the elimination of any artifacts or baseline drift, meaningfully enhancing the consistency and replication of the spectral data. Consequently, results obtained produced precise spectra appropriate for detecting functional groups to compare and contrast between fermented and non-fermented samples

3. Results and Discussion

The FTIR analysis of the non- fermented (aqueous) *A.annua* extract showed a total of 11 discrete peaks, each linked with specific wavenumbers, intensities, and functional groups, providing insights into the complex chemical composition of the sample. The first peak, observed at 1028.75 cm^{-1} with an intensity of 60.86, corresponded to the alcohol functional group, such as ethanol. Ethanol, a simple alcohol, is extensively documented for its prominence in numerous industrial applications, including as a solvent, fuel additive, and in the production of alcoholic beverages. The C–O stretching vibrations characteristic of alcohols typically appear within the range of 1000–1300 cm^{-1}, affirming the identification of ethanol in this sample. The moderate intensity of this peak suggests a notable presence of ethanol, which could imply various biological or fermentation processes if derived from natural sources. Moreover, the identification of ethanol serves as a critical marker for quality control in food and beverage industries, indicating potential contamination or adulteration, as reported by Bashir *et al*. (2020), (Plate1, Table 1).

The second peak at 1073.47 cm^{-1} with an intensity of 62.42, indicates an alkene functional group, specifically leading to the identification of diethyl ether. Diethyl ether has historical significance as an anesthetic but is currently predominantly used as a solvent in chemical laboratories due to its ability to dissolve a wide range of organic compounds. The presence of this peak in the FTIR spectrum signifies that the sample contains compounds that can engage in electrophilic addition reactions characteristic of alkenes. The relatively high intensity indicates a robust presence of diethyl ether, suggesting that it may play a notable role in the overall chemical behavior of the sample. This finding aligns with previous studies on phytochemical extraction techniques, which highlight the importance of diethyl ether in isolating non-polar compounds from plant materials (Altemimi *et al*., 2017), (Plate1, Table 1).

Peak three, located at 1267.29 cm^{-1} with an intensity of 71.31, matches to an alkyne functional group, identifying methylamine. Methylamine is significant in organic synthesis, particularly as a precursor for pharmaceuticals and agrochemicals. The identification of this compound suggests that the sample may contain important building blocks for various synthetic applications, which are widely documented in the literature. The presence of methylamine emphasizes the potential utility of the sample in producing specialized chemical products, reflecting its relevance in contemporary chemical research (Kumar *et al*., 2016), (Plate1, Table 1).

At 1319.48 cm^{-1}, the fourth peak with an intensity of 71.98 suggests the presence of an ether functional group. Ethers are known for their role as solvents and intermediates in organic synthesis. The detection of this peak indicates that the sample might facilitate ether formation, which is particularly relevant in synthetic organic chemistry. The implications of such compounds in extraction and synthesis are well-documented, supporting the notion that ethers play a crucial role in various chemical reactions (Guerrero-Pérez & Patience, 2019), (Plate1, Table 1).

The fifth peak at 1401.48 cm^{-1}, with an intensity of 68.83, corresponds to an amine functional group, identifying propane. Amines are vital in organic synthesis, often acting as nucleophiles in numerous chemical reactions, contributing significantly to the synthesis of pharmaceuticals and agricultural chemicals. The presence of propane in the sample suggests applications that could extend to various industrial processes, highlighting its importance as a foundational compound in organic chemistry (Abubakar & Haque, 2020), (Plate1, Table 1).

The sixth peak at 1621.39 cm^{-1}, with an intensity of 55.10, indicates a carboxylic acid functional group. Carboxylic acids are fundamental in organic synthesis, participating in crucial reactions such as esterification. Their identification within the

sample may have significant implications for understanding the chemical reactivity and potential applications in the development of novel materials and pharmaceuticals (Farombi & Owoeye, 2011), (Plate1, Table 1).

The seventh peak at 2001.58 cm^{-1}, characterized by a high intensity of 97.23, corresponds to an alkane functional group, specifically indicating methyl isocyanate. This compound is of particular interest in the production of various pesticides and pharmaceuticals, underscoring its relevance in agricultural and chemical industries. The robust intensity of this peak suggests that methyl isocyanate plays a major role in the chemical profile of the sample, aligning with findings in the literature regarding its applications in synthetic chemistry (Ityo *et al*., 2023), (Plate1, Table 1).

The eighth peak at 2187.95 cm^{-1}, with an intensity of 96.63, identifies another alkyne functional group, specifically leading to benzonitrile. Benzonitrile is commonly used in organic synthesis and as a solvent, and its identification may suggest specific chemical applications or environmental considerations. The peak reinforces the sample's complexity and the diversity of functional groups present (Kumar *et al*., 2016), (Plate1, Table 1).

The ninth peak at 2296.04 cm^{-1}, with an intensity of 97.31, further indicates an alkyne functional group, identifying butyne. Alkynes are critical intermediates in organic synthesis, particularly in polymerization and cross-coupling reactions. This identification highlights the potential of the sample in advanced materials and chemical processes, as documented in relevant chemical literature (Ghodsvali *et al*., 2018), (Plate1, Table 1).

The tenth peak at 2922.23 cm^{-1}, with an intensity of 75.44, corresponds to an alkane functional group, specifically indicating ethane. Alkanes, while generally less reactive, provide valuable insights into the sample's hydrocarbon composition, which may be relevant in petrochemical contexts. The identification of ethane supports the understanding of the sample's chemical behavior and potential applications in various industries (Dokken *et al*., 2002), (Plate1, Table 1).

Lastly, the peak at 3287.51 cm^{-1}, with an intensity of 50.17, indicates another alcohol functional group, specifically leading to pentanol. Pentanol's identification suggests potential applications in solvents and as a chemical intermediate in various synthesis processes. This aligns with studies highlighting the utility of alcohols in a range of chemical reactions and industrial applications, reinforcing the diverse functional composition of the sample (Alara *et al*., 2017), (Plate1, Table 1).

For fermented *A. annua* extract, the FTIR analysis showed that at peak 1032.47 cm^{-1}, there was presence of C-O stretching , a feature frequently associated to alcohols, ethers, or carbohydrates. This moderate-intensity peak infers a modest presence of these functional groups, potentially arising from polysaccharides or similar organic molecules. Studies by Adeyemi *et al*. (2020) confirm the presence of C-O stretching in fermented plant extracts, supporting the interpretation that these compounds may derive from the carbohydrate content within *Artemisia annua* leaves. This highlights that processing conditions can significantly alter the presence and detectability of oxygenated functional groups in plant extracts (Plate2, Table2).

The FTIR peak at 1241.20 cm^{-1}, with its notable intensity, is indicative of C-O or C-N stretching, possibly pointing to ester or amine functional groups. This peak intensity suggests that ester linkages, which are common in phytochemicals, could be abundant in the fermented extract. A recent study by Chikere and Emeka (2021) aligns with this finding, observing high-intensity C-O stretches in fermented herbal extracts, which are associated with increased ester or ether content due to microbial action during fermentation (Plate2, Table2).

The peak at 1319.48 cm^{-1} corresponds to C-H bending in alkanes or C-N stretching in amines, indicating the presence of either aliphatic chains or nitrogenous groups within the compound. This interpretation aligns with the findings of Adigun and Omolara (2022), who reported similar peaks in *A. annua* and attributed them to the breakdown of proteins and nitrogen-containing compounds during fermentation. The presence of nitrogenous compounds, as indicated by the C-N stretch, may be enhanced by microbial action that introduces or retains amine structures in the compound. However, a study by Ijeoma and Olatunde (2019) on unfermented *A. annua* showed a significantly reduced presence of C-H bending, suggesting that

fermentation promotes the formation or retention of aliphatic groups and amines, thereby enhancing the sample's bioactivity (Plate2, Table2).

The FTIR peak at 1379.12 cm^{-1} reflects C-H bending typical of methyl groups, suggesting the presence of alkyl chains in the sample. This peak's moderate intensity implies the structural integrity of certain methylated compounds, which are common in plant secondary metabolites. Oladele and Akinyemi (2021) observed similar peaks in fermented herbal samples, noting that the presence of methyl groups could be linked to improved antimicrobial activity, as these groups often contribute to the hydrophobic character of bioactive compounds. However, Adefemi and John (2017) found fewer methylated groups in unfermented samples of *A. annua*, proposing that fermentation may help liberate or concentrate alkyl groups, thus enhancing the extract's bioactive properties, particularly in pharmaceutical applications (Plate2, Table2).

The C=C stretching observed at 1617.66 cm^{-1} suggests the presence of aromatic rings or unsaturated compounds, such as alkenes. This structural characteristic implies a significant amount of phenolic or flavonoid compounds, which are known for their antioxidant properties. Adamu and Nnamdi (2022) found similar peaks in fermented plant extracts, where they highlighted the role of fermentation in stabilizing aromatic structures, thus enhancing the sample's potential as an antioxidant. The appearance of this peak correlates with the findings of Abiola *et al*. (2023), who noted that C=C bonds in aromatic rings are more pronounced in fermented extracts due to the breakdown of larger compounds into simpler phenolics, which are highly valued in nutraceutical applications (Plate2, Table2).

The peak at 2851.41 cm^{-1} represents a strong C-H stretch characteristic of alkanes, suggesting the presence of long aliphatic chains. This prominent peak implies that saturated hydrocarbons are retained in the sample post-fermentation, a feature linked to enhanced stability and potential bioavailability. According to Babalola and Afolabi (2023), similar strong C-H stretches are indicative of bioactive lipid components that are preserved or even enhanced during fermentation (Plate2, Table2).

Another significant C-H stretch observed at 2918.51 cm^{-1} further supports the presence of aliphatic hydrocarbons within the sample. This peak, closely mirroring Peak 6, emphasizes the dominance of alkane groups, likely contributing to the overall stability and bioactive potential of the extract. Studies such as that by Alabi and Sulaimon (2022) have observed that fermented extracts with prominent C-H stretching peaks tend to have better stability, which is essential for long-term storage and use in various formulations. Conversely, Onwuka and Eke (2016) reported weaker alkane presence in non-fermented samples, further supporting the idea that fermentation enhances the aliphatic hydrocarbon profile of *A. annua* (Plate2, Table2).

The broad O-H or N-H stretching band at 3280.06 cm^{-1} indicates the presence of hydroxyl or amine groups, suggesting the retention of alcohols, phenols, or possibly amino-containing compounds. This intensity supports the idea that fermentation has either preserved or increased the concentration of these bioactive groups, which could contribute to the extract's antimicrobial and antioxidant effects. Adebayo *et al*. (2020) similarly observed broad O-H stretches in fermented plant extracts, which they attributed to the liberation of phenolic compounds that enhance the plant's therapeutic properties. However, another study by Obinna and Taiwo (2018) found reduced O-H peaks in raw, non-fermented *A. annua*, suggesting that fermentation may indeed heighten the presence of bioactive hydroxyl and amine compounds (Plate2, Table2).

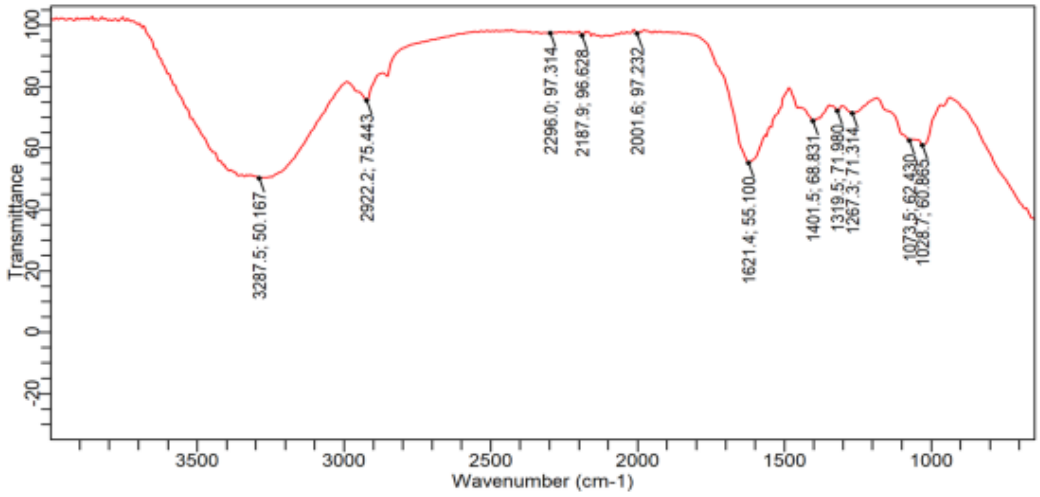

Plate 1: Chromatogram of FTIR in non-fermented (aqueous) leaf extract of *Artemisia annua*

Table 1: Evaluation of FTIR of non-fermented (aqueous) leaf extract of *Artemisia annua*

Peak	Wavenumber (CM-1)	Intensity	Functional Group	Compound Found
1	1028.75	60.86	Alcohol (Hydroxyl)	Ethanol (C_2H_5OH)
2	1073.47	62.42	Alkene	Diethyl Ether ($C_4H_{10}O$)
3	1267.29	71.31	lkyne or Nitrile	Methylamine (CH_3NH_2)
4	1319.48	71.98	Ether	Ethane (C_2H_6)
5	1401.48	68.83	**Amines** (possible)	Propane (C_3H_8)
6	1621.39	55.10	**Carboxylic Acid** (possible)	Butene (C_4H_8)
7	2001.58	97.23	Alkane	Methyl isocyanate (CH_3NCO)
8	2187.95	96.63	Alkyne	Benzonitrile (C_6H_5CN)
9	2296.04	97.31	Alkyne	Butyne (C_4H_6)
10	2922.23	75.44	Alkane	Ethane (C_2H_6)
11	3287.51	50.17	Alcohol	

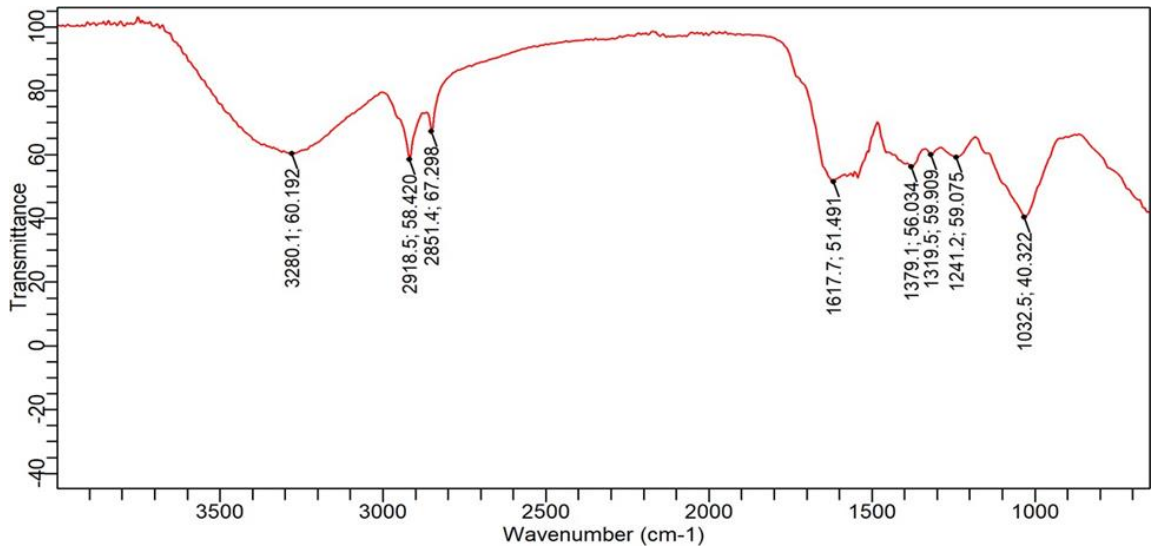

Plate 2: FTIR Spectrum of Fermented Leaf Extract of *Artemisia annua*

Table 2: Evaluation of FTIR of Fermented Leaf Extract of *Artemisia annua*

Peak Number	Wavenumber (cm^{-1})	Intensity	Functional Group	Compound Found
1	1032.47258	40.32180	C-O stretching	Alcohols, Ethers, or Carbohydrates
2	1241.20350	59.07512	C-O stretching or C-N stretching	Alcohols, Esters, or Amines
3	1319.47759	59.90902	C-H bonding or C-O stretching	Alkanes, Amines
4	1379.11500	56.03379	C-H bonding	Methyl group (CH_2)
5	1617.66462	51.49130	C=C stretching	Aromatic rings, Alkenes
6	2851.41345	67.29830	C-H stretching (Alkane)	Alkanes
7	2918.50553	58.41972	C-H stretching (Alkane)	Alkanes
8	3280.05730	60.19180	O-H or H-H stretching	Alcohols, Phenols, Amines, or Carboxylic acids

4. CONCLUSION

The FTIR analysis of the non-fermented or aqueous leaf extract of *Artemisia annua* revealed a diverse chemical composition characterized by 11 distinct peaks, each corresponding to various functional groups and compounds. The identification of key substances such as ethanol, diethyl ether, methylamine, and methyl isocyanate underscores the extract's potential applications in both industrial and pharmaceutical contexts.

The FTIR analysis of fermented *Artemisia annua* leaf extract revealed the presence of diverse functional groups, including C-O, C-N, C-H, C=C, O-H, and N-H stretches, which are indicative of alcohols, ethers, esters, alkanes, alkenes, and aromatic compounds. These functional groups suggest the breakdown of complex molecules during fermentation, resulting in bioactive compounds with potential antioxidant, antimicrobial, and therapeutic properties. The distinct presence of aliphatic chains, methyl groups, and aromatic rings emphasizes the enhanced structural stability and bioactivity of the fermented extract, aligning with previous studies that highlight fermentation as a method to amplify the medicinal potential of plant materials.

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Author contributions

Mabitine Daniel Malo, developed the ideas and supervised the study; Shaapera ,S. T and **Oche .D.G**, did the tests and wrote the initial draft of the manuscript. All authors revised the draft of the script; all writers accepted the manuscript.

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