AJBCPS African Journal of Biological, Chemical and Physical Sciences

Vol 4, Issue 1, 2025

ISSN: 2790-9522 (Print) | 2790-9530 (Online)

https://doi.org/10.5281/zenodo.15611170

Website: https://journals.jozacpublishers.com/ajbcps/index

OPEN ACCESS

GC-MS phytochemical profiling, FTIR analyses, and antimicrobial activities of the oily fraction from the ethyl acetate leaf extract of *Pterocarpus osun*

Adewale Elijah Fadeyi[1,3]*, Saheed Akiode[2], Olakunle Ayodeji Fatokun[1], Monday Fenu[2], Oluremi Adeniran[3]

[1]Chemistry Advanced Research Centre, Sheda Science and Technology Complex, FCT, Abuja, Nigeria.

[2]Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, FCT, Abuja, Nigeria.

[3]Chemistry Department, University of Abuja, Nigeria.

*Correspondence: wale.fade@gmail.com

Received: March 07, 2025 | **Accepted:** May 05, 2025 | **Published:** June 06, 2025

Abstract

The Fabaceae family includes *Pterocarpus osun* as one of its flowering plant species. Nigeria, Ghana, and Cameroon are among the West African nations with tropical climates where this plant is native. It has found applications in traditional medicine, where it is used to treat several medical conditions, such as gastrointestinal issues and inflammation. This research aims to determine which compounds in the plant extract provide its medicinal properties. n-hexane, ethyl acetate, and methanol were utilized sequentially to extract the leaves. Using column chromatography, a thick, oily, golden-yellow fraction was obtained from the ethyl acetate crude extract. To ascertain the chemical composition of the isolated viscous oily material, GC-MS and FTIR analyses were conducted. *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* were used as test organisms in an antibacterial test. Numerous chemical components with biological and pharmacological properties, including anti-allergic, antioxidant, anticancer, antibacterial, and antifungal properties, were identified from the isolate's GC-MS studies. The recorded FTIR spectrum reveals the C-H bond present in alkanes (at 2921–2847 cm-1 and 1454–1379 cm-1), the C-O bond of carboxylic acids (1165–1032 cm-1), and the C=O bond in 1728–1709 cm-1. The observed zones of inhibition, especially at the maximum dose (10 mg/mL), demonstrated that the oily isolate was effective against the pathogenic organisms utilized, confirming its therapeutic efficacy in traditional medicine. Thus, the oily isolate from the *P. osun* leaf's ethyl acetate extract can be studied for the development of novel pharmaceuticals.

Keywords: Anti-inflammatory, Ethyl acetate, GC-MS, *Pterocarpus osun*, *Staphylococcus aureus*

1. Introduction

Plants with a variety of phytoconstituents are thought to have medicinal benefits. A wide range of chemicals are present in these plants, such as amino acids, terpenes, alkaloids, phenols, tannins, and flavonoids. Plants get their unique qualities and features from these phytoconstituents (Baht, 2021). Plant extracts generally include numerous types of bioactive metabolites with different polarities; thus, breaking them down into their constituent chemical components still presents a considerable challenge to identification and characterization procedures. A typical procedure involves isolating bioactive metabolites using different forms of separation methods, such as thin-layer chromatography (TLC) and column chromatography.

2. Literature review

Planarchromatography, mostly referred to as High-performance liquid chromatography (HPLC), is one method Anuradha (2017) recommends utilizing to acquire pure chemicals. It is a popular method used for the separation of chemical components in multicomponent mixtures, such as plant extracts (Hawry et al., 2016). Bioactive compounds from medicinal plants have been identified using various instrumentation techniques. These include Fourier-transform infrared (FTIR)spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, gas chromatography-mass spectrometry (GC-MS) research, etc. (Ojo et al., 2021). Over the years, studies on plant and animal bioactive compounds such as alcohols, acids, esters, long-chain hydrocarbons, steroids, amino and nitro compounds etc. have resulted in development of various analytical and extraction methods such as gas chromatography (GC) with flame ionization detection (FID), capillary electrophoresis, high-performance liquid chromatography (HPLC), spectrophotometry, gas chromatography (GC), mass spectroscopy (MS). Quest for a more reliable technique has led to the development of the gas chromatography-mass spectroscopy (GC-MS) technique: a combination of gas chromatography (GC) (a high precision separation method) and mass spectroscopy (MS) (a high precision identification technique).

GC-MS provides a highly precise qualitative and quantitative approach for both volatile and semi-volatile chemical compounds (Alexander et al., 2019). Recently, GC-MS has established itself as a leading technology for characterizing secondary metabolites from various plant species (Gomathi et al., 2017; Saikarthik et al., 2017). The consequences of studying chemical molecules derived from plants have drawn more attention in recent years. According to Al Talebi et al. (2023), biotechnology breakthroughs have made it possible to explore natural chemicals faster and more precisely than ever before, which has led to the isolation of bioactive molecules with health advantages. Implementing a suitable extraction process is also crucial (Parys et al., 2022). The herb *P. osun* has been utilized traditionally in the treatment of many diseases. In our previous study, the leaves and stem bark of *P. osun* have been reported to contain alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, cardiac glycosides, and phenols (Fadeyi et al., 2022). This study is aimed at identifying the phytochemical constituents, antibacterial properties, GC-MS, and FTIR analyses of the oily isolate from the ethyl acetate leaf extract of *P. osun*.

3. Tables and Figures

Table 1: Antibacterial activity of ethyl acetate oily fractions from the leaves of *P. osun*

Bacterial	Leaf oily fraction (mg/mL)			
	10	5	2.5	C
	ZONE OF INHIBITION in mm			
Echerichia coli	30	25	25	32
Salmonella Typhi	30	25	24	35
Staphylococcus aureus	30	27	26	40

C = Control (Chloramphenicol 10mg/mL)

Table 2: FTIR Analysis of the oily fraction from the leaf of *P. osun*

Absorption band (cm^{-1})	Functional group	Stretching vibration.
2914, 2847	Alkane	CH sp3
2359	Si-H	
1728	aldehyde	C=O
1175	sulphonyl	S=O
719	alkane	C-C, C-H rock

Table 3: GCMS data of *P. osun* leaf oil

No	Hit compound	Formula	M.wt	Therapeutic uses
1	Trans-. beta. -Ionone	$C_{13}H_{20}O$	192.30	fragrance, protect against UVB photoaging
2	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	antioxidant, antibacterial, antifungal, anticancer (Zhao, 2020)
3	2-(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180.24	analgesic, antibacterial, antidiabetic (Mujeeb *et al.*, 2014)
4	Tetradecanoicacid	$C_{14}H_{28}O_2$	228.37	headaches, skin diseases antimicrobial (Shobayo*et al.*, 2015)
5	Neophytadiene	$C_{20}H_{38}$	278.52	antimicrobial, anti-inflammatory (Bhardwaj *et al.*, 2020)
6	2-Pentadecanone,6,10, 14-trimethyl	$C_{18}H_{35}O$	268.48	antibacterial, antinociceptive, anti-inflammatory (Vats *et al.*, 2020)
7	1,13-Tetradecadiene	$C_{14}H_{26}$	194.36	
8	1,2-Benzenedicarboxylic acid	$C_{16}H_{22}O_4$	278.34	antimicrobial, antinematicidal

				(Sholkamy *et al.*, 2020)
9	Hexadecanoicacid, methyl ester	$C_{17}H_{34}O_2$	270.45	anti-inflammatory (Sonmezdag *et al.*, 2016)
10	Isophytol	$C_{20}H_{40}O$	296.54	fragrance (McGinty *et al.*, 2010)
11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	antioxidant, antibacterial (Johannes *et al.*, 2016)
12	Hexadecanoicacid,	$C_{18}H_{36}O_2$	284.48	antioxidants, hypocholesterolemic (Yu *et al.*, 2005)
13	Isopropylpalmitate	$C_{19}H_{38}O_2$	298.50	skin care, antistatic (Khan, *et al.*, 2023)
14	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	analgesic, ucerogenic anti- inflammatory (Hadi*et al.*, 2016)
15	9-Octadecenoic acid	$C_{19}H_{36}O$	296.48	antibacterial, antitumor (Palaniappan et al., 2022)
16.	Phytol	$C_{20}H_{40}O$	296.53	antioxidant,***antinociceptive*** (de Moraes *et al.*, 2014)
17	Methylstearate	$C_{19}H_{38}O_2$	298.50	antimicrobial, anti-inflammatory (Nakaziba *et al.*, 2022)
18	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.50	anti-acne, antibacterial, anti-inflammatory agent (Soliman & AbdelWahab 2024)
19	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	antioxidant, nematicide (Ahmad *et al.*, 2015)
20	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.53	anticancer, anti-inflammatory (Xie et al., 2022)
21	3,7,11,15-Tetramethylhexadec-2-en-1-ylacetate	$C_{22}H_{42}O_2$	338.57	antineoplastic, anti-inflammatory, antifungal (Kurashov *et al.*, 2016)
22	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667.39	antifungal, antioxidant (Lutfia *et al.*, 2022)
23	4,8,12,16-Tetramethylheptadecan-4- Olide	$C_{21}H_{40}O_2$	324.54	
24	Hexadecanoicacid,hexylester	$C_{22}H_{44}O_2$	340.58	hepatoprotective, anticancer (Krishnamoorthy and Subramaniam, 2014)

25	Undecane,2-cyclohexyl-	$C_{17}H_{34}$	238.45	
26	2,5-Dihydroxybenzoicacid,	$C_7H_6O_4$	154.12	antioxidant (Annapoorani *et al*., 2013)
27	2,4-Bis(dimethylbenzyl)-6-t-butylphenol	$C_{28}H_{34}O$	386.57	antioxidant (song *et al*., 2018)
28	Phytyl,2-methylbutanoate	$C_{25}H_{48}O_2$	380.65	
29	Imidosulfurousdifluoride, methyl-	CH_3F_2NS	99.10	
30	alpha.-TocospiroA	$C_{29}H_{50}O_4$	462.70	antioxidant, anti-inflammatory neuroprotective (Yuan *et al*., 2014)
31	alpha.-TocospiroB	$C_{29}H_{50}O_4$	462.70	antioxidant, anti-inflamatory (salih, *et al*., 2022)
32	Tetracosane	$C_{24}H_{50}$	338.39	insomnia, fatigue, (Kawahara & Okino, 2022)
33	Octacosane	$C_{28}H_{58}$	394.76	antibacterial, antitumor (Kakalis *et al*., 2023)
34	Heptacos-1-ene	$C_{27}H_{54}$	378.42	
35	2-Nonacosanone	$C_{29}H_{58}O$	422.77	antidepressant, anti-inflammatory (Lee & Hyun, 2018)
36	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.71	wound healing, anticancer, anti-inflammatory

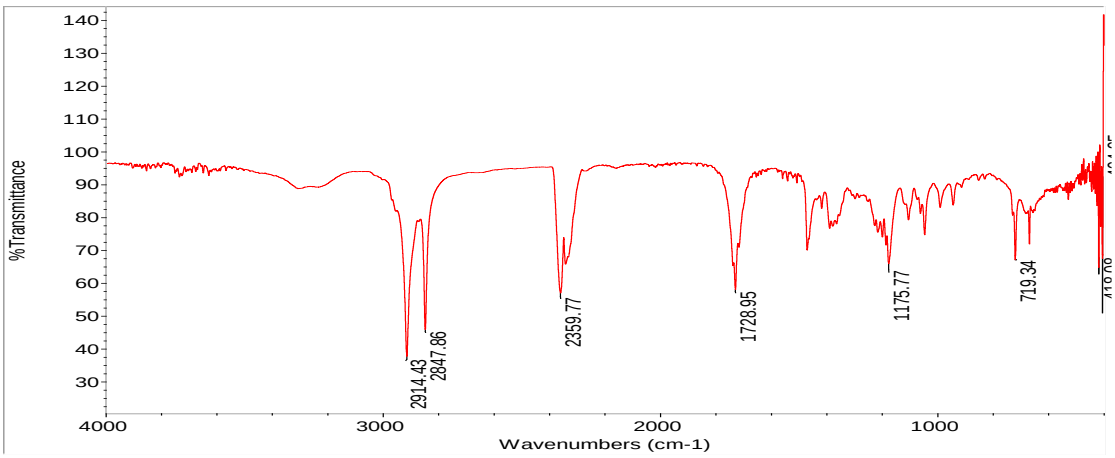


Figure 1: Spectrum from FTIR analysis of oil fraction of leaf of *P. osun*

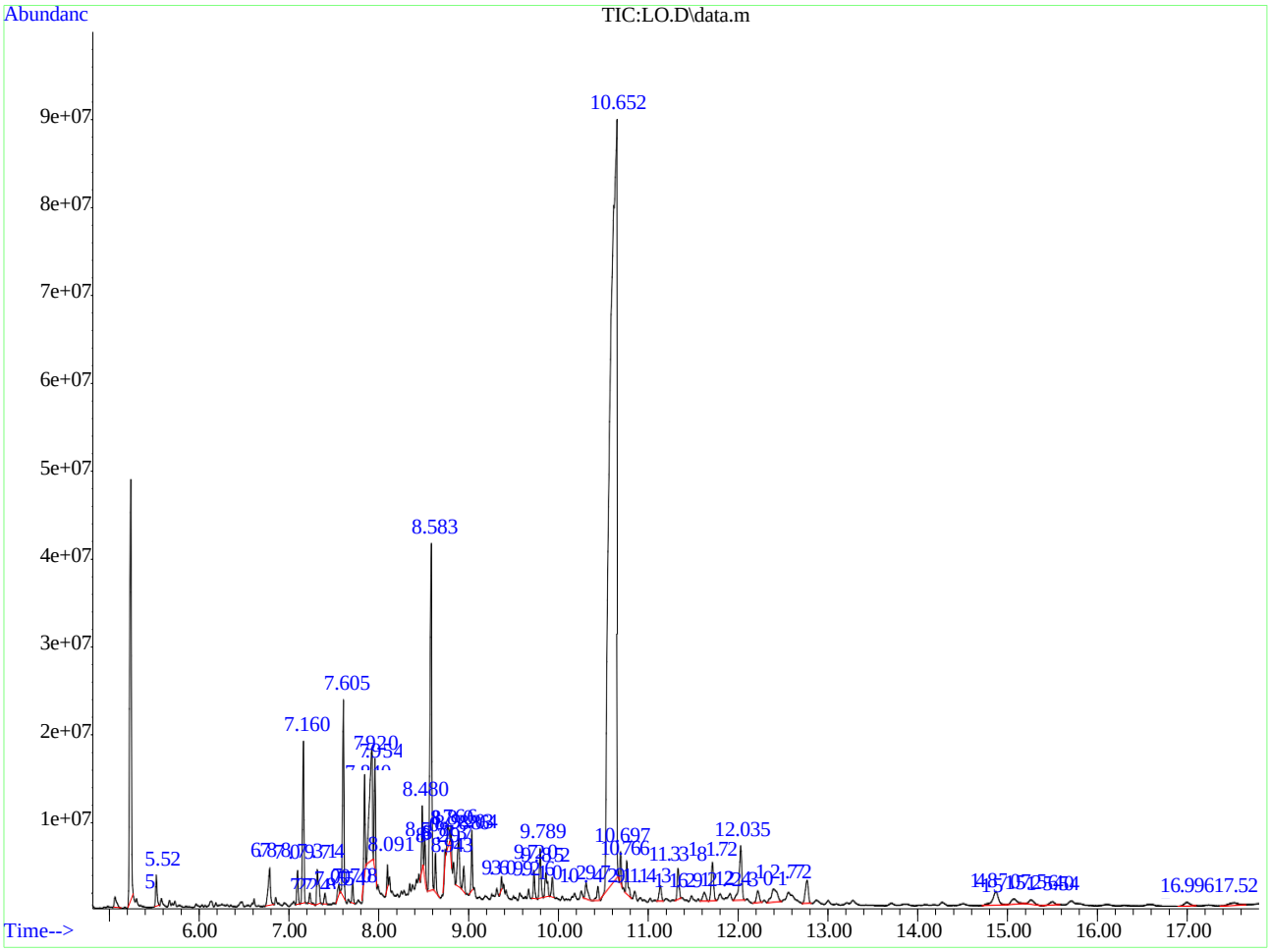
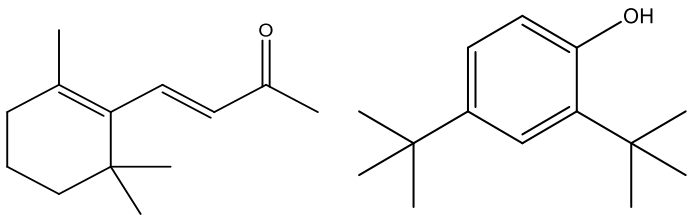
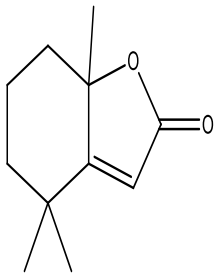


Figure 2: GCMS chromatogram of oily fraction from leaf extract of *P. osun*

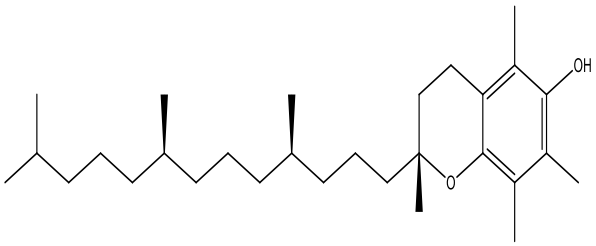


Trans-. beta. -Ionone

2,4-Di-tert-butylphenol



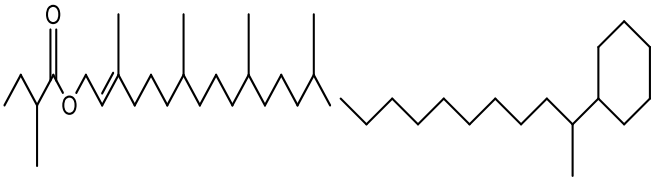
2(4h)-benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-



dl-.alpha.-Tocopherol
Caution: Stereochemical terms discarded: dl

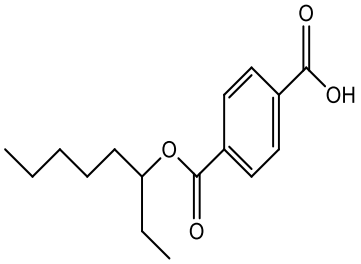
2-Nonacosanone

Tetracosane

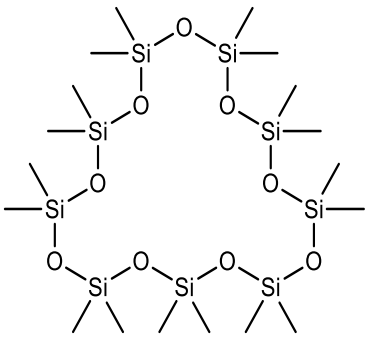


Phytyl, 2-methylbutanoate

Undecane, 2-cyclohexyl-



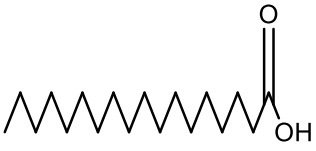
1,4-Benzenedicarboxylic acid, ethylhexyl ester
This name appears to be ambiguous



Cyclononasiloxane, octadecamethyl-

2-Pentadecanone, 6,10, 14-trimethyl

Octadecanoic acid, ethyl ester



Octadecanoic acid

Linoleic acid ethyl ester

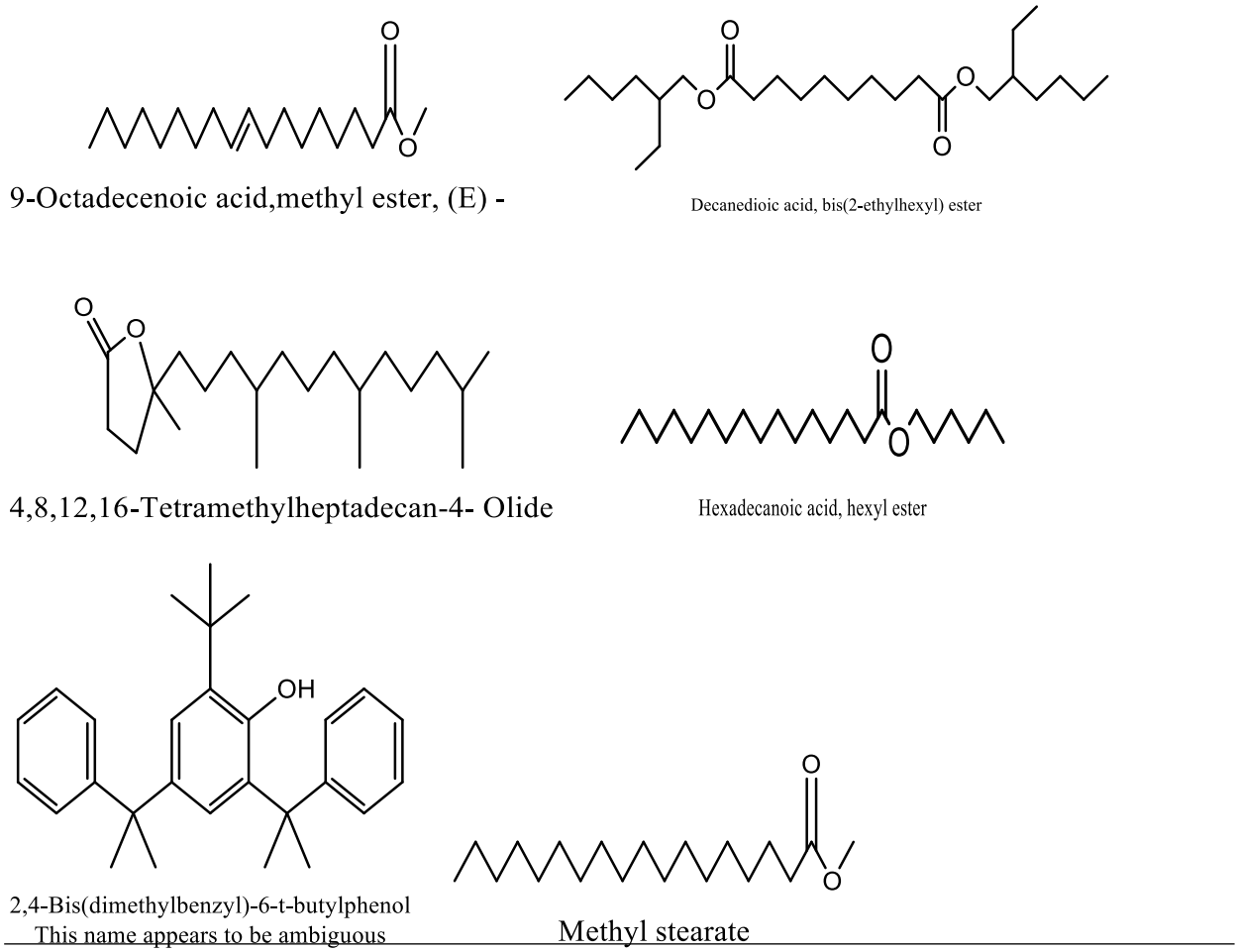


Figure 3: Chemical structures of some compounds from the GCMS spectrum

4. Research methodology

4.1. Sample preparation, extraction, and column chromatography separation technique

The plant's identification was carried out at National institute for pharmaceutical research and development (NIPRD), Nigeria (herbarium number-NIPRD/H/7251). The collected leaves of *P. osun* was air-dried and ground into a powder. The powdered sample was macerated using ethyl acetate. The column chromatographic method was used to chromatograph the crude extract obtained using n-hexane and ethyl acetate in ratio 8:2 solvent system. As a portion, a thick, golden-yellow, oily material that dissolves in chloroform was produced.

4.2. Analysis of extract using gas chromatography-mass spectroscopy

Phytochemical constituents of the oily isolate obtained was analysed using GC-MS. To identify the compounds using the peaks obtained, the unknown peaks were compared with known peaks in the mass spectral electronic databases from Wiley and NIST (National Institute of Standards and Technology) (Stein, 1990; Mahier et al., 2010).

4.3. Analysis of extract using Fourier transform infrared

The FTIR spectroscopic analysis of the sample was carried out by scanning the oily samples using Nicolet IS 5 Thermo Fisher Scientific, USA FTIR spectrophotometer between 4000 and 400 cm^{-1} wavelengths. Information regarding the distinctive functional groups found in the samples is provided in the FTIR spectrum Figure 1 and Table 2.

4.4. Antibacterial analysis

Three pathogenic bacteria- *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*, were obtained from Biotechnology Advanced Research laboratory of Sheda Science and Technology Complex, Abuja, Nigeria. The organisms obtained were validated using suitable growth media and subsequently utilized for the study. Diffusion techniques in Agar Wells were used to measure antibacterial activity. On tryptic soy agar, all the bacteria were cultivated for a full day. After four hours of incubation in Muller-Hinton broth, the turbidity of three colonies was measured using 0.5 MacFarland and corrected as necessary. Every inoculum was applied to the Muller Hinton Agar plates using a sterile swab. Four wells (6 mm) were bored in the inoculated media using a sterilized cork-borer of 6 mm. A 100 µL aliquot of varying concentrations of the extracts was introduced into three of the four wells and left for 30 minutes. As the Control, 100 µL chloramphenicol (10 mg/mL) was placed in the fourth well. The zone of inhibition was measured in millimeters after 18 to 24 hours of incubation (CLSI, 2020).

5. Results and discussions

The antimicrobial screening of the viscous oily substance obtained from the column chromatography separation of the ethyl acetate extract of *P. osun* leaf at various concentrations (Table 1**)** showed positive activities comparable to the standard drug, chloramphenicol which was used as a controlled experiment. *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* were shown to be vulnerable to the oily sample, according to the results. *Staphylococcus aureus*, a gram-positive bacterium, has been reported to cause inflammatory diseases (Rasquel-Oliveira *et al*., 2025). *Escherichia coli*, a gram-negative organism, is responsible for ill health conditions such as intestinal and extraintestinal infections, gastrointestinal infections, pneumonia, bacteremia, urinary tract infections, abdominal and pelvic infections, also meningitis. *Salmonella typhi,* a gram-negative pathogen that is responsible for typhoid fever.

At the highest concentration of test for instance, 10 mg/mL, all the test organisms recorded zones of inhibitions compared favourably with the chloramphenicol, used as the standard control drug (Table 1). Figure 1 shows the FTIR analysis while the absorption bands, and the functional groups present are displayed in Table 2. From the spectrum and the table, the functional groups noticeably present include C-H alkane stretch at 2914, 2847 cm^{-1}, C=O stretch of aldehyde functional group (1728 cm^{-1}). At 2359 cm^{-1}, Si-H group and sulphonyl were observed at peak 1175 cm^{-1}while alkane C-H rock at 719 cm^{-1}. Figure 1 shows the FTIR analysis while the absorption bands, and the functional groups present are displayed in Table 2. From the spectrum and the table, the functional groups noticeably present include C-H alkane stretch at 2914, 2847 cm^{-1}, C=O stretch of aldehyde functional group (1728 cm^{-1}). At 2359 cm^{-1}, Si-H group and sulphonyl were observed at peak 1175 cm^{-1}while alkane C-H rock at 719 cm^{-1}.

6. Implications of the study

This study has demonstrated the potential of the leaf extract of *P. osun* to exhibit antioxidant, anti-inflammatory, antifungal, antibacterial, antitumor, and analgesic properties. Thus, careful analysis of this plant may lead to the development of novel drugs in order to complement the existing antimicrobial drugs and combat multidrug-resistant (MDR) microorganisms.

7. Recommendations and suggestions

Further work is recommended, particularly in clinical trials and synthetic pathways for commercial purposes.

8. Conclusion

This research revealed some of the chemical compounds that nature has embedded in the leaf of *P. osun,* which are therapeutic ingredients that are responsible for the traditional medicinal use of the plant for the treatment of ailments and diseases. This gives credence to nature as a reservoir of bioactive compounds of medicinal value.

ORCID

Adewale Fadeyi https://orcid.org/0000-0001-7152-7131
Saheed Akiode https://orcid.org/0000-0002-2629-046X
Fatokun Ayodeji Olakunle https://orcid.org/0000-0001-6581-9699
Monday Fenu https://orcid.org/0009-0002-2826-7308
Oluremi Adeniran https://orcid.org/0000-0001-7235-6549

References

1. Ahmad Razi Othman, A. R. O., Norhani Abdullah, N. A., Syahida Ahmad, S. A., Intan Safinar Ismail, I. S. I., & Mohamad Pauzi Zakaria, M. P. Z. (2015). Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from Jatropha curcas L. plant root.Al Talebi, Z., Karhib, M. M., Taki, M. M., Abood, E., Mubarak, H. A., & Ahmed Ibrahim, C. S. (2023). Chemical Analysis, Antioxidant, and Antibacterial Potential of Aqueous Extract of Broccoli (Brassica oleracea) Using GC-ms. *Institut Razi. Archives*, *78*(2).
2. Alexander, P., Antony, J., & Rodgers, B. (2019). Lean Six Sigma for small-and medium-sized manufacturing enterprises: a systematic review. *International Journal of Quality & Reliability Management*, *36*(3), 378-397.
3. Annapoorani, C. A., & Manimegalai, K. (2013). Evaluation of biological activity and qualitative analysis of 2, 5-dihydroxybenzoic acid from Momordica charantia fruit. *Int J Pharm Sci Rev Res*, *22*(2), 89-95.
4. Anuradha, R. N. R. (2017). Isolation and HPLC quantitative analysis of flavonoids from flower extract of Punica granatum L. *Asian Journal of Pharmacy and Pharmacology*, *3*(4), 139-144.
5. Bhat, S. G. (2021). Medicinal plants and its pharmacological values. *Natural Medicinal Plants*.
6. Clinical and Laboratory Standards Institute (CLSI). (2020). Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100 (ISBN 978-1-68440-104-8 [Print]; ISBN 978-1-68440-105-5 [Electronic]). Clinical and Laboratory Standards Institute, USA

7. de Moraes, J., de Oliveira, R. N., Costa, J. P., Junior, A. L., de Sousa, D. P., Freitas, R. M., ... & Pinto, P. L. (2014). Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease Schistosomiasis mansoni. *PLoS neglected tropical diseases*, *8*(1), e2617.

8. Fadeyi A.E., Adeniran O. I., & Akiode O.S. (2022). Nutrients, Phytochemical, Antioxidant and Antimicrobial Analysis of *Pterocarpus osun* stem bark and leaf for their nutritional, medicinal capacity. Indo. *J. Chem. Res*., 10(1), 58-67, 2022. http://ojs3.unpatti.ac.id/index.php/ijcr

9. Gomathi Priyadarshini, A. A. E., Anthony, J., Rao, M. R. K., Prabhu, K., Ramesh, A., & Krishna, V. (2017). The GC MS analysis of one medicinal plant, Premna tomentosa. *Journal of Pharmaceutical Sciences and Research*, *9*(9), 1595-1597.

10. Hawrył, A., Świeboda, R., Hawrył, M., & Ziobro, A. (2016). Two-dimensional thin layer chromatography fingerprint profiles of ten Cirsium species by chemometric processing. *JPC-Journal of Planar Chromatography-Modern TLC*, *29*(6), 405-409.

11. Kakalis, A., Tsekouras, V., Mavrikou, S., Moschopoulou, G., Kintzios, S., Evergetis, E., ... & Haroutounian, S. A. (2023). Farm or Lab? A Comparative Study of Oregano's Leaf and Callus Volatile Isolates Chemistry and Cytotoxicity. *Plants*, *12*(7), 1472.

12. Kawahara, T., & Okino, T. (2012). Chlorosulfolipids. *Studies in Natural Products Chemistry*, *36*, 219-247.

13. Krishnamoorthy, K., & Subramaniam, P. (2014). Phytochemical profiling of leaf, stem, and tuber parts of Solena amplexicaulis (Lam.) Gandhi using GC-MS. *International scholarly research notices*, *2014*.

14. Kurashov, E. A., Fedorova, E. V., Krylova, J. V., & Mitrukova, G. G. (2016). Assessment of the potential biological activity of low molecular weight metabolites of freshwater macrophytes with QSAR. *Scientifica*, *2016*.

15. Lutfia, A., Munir, E., Yurnaliza, Y., & Basyuni, M. (2021). Chemical analysis and anticancer activity of sesterterpenoid from an endophytic fungus Hypomontagnella monticulosa Zg15SU and its host Zingiber griffithii Baker. *Heliyon*, *7*(2).

16. Mahier, T. J., Al-Doush, I. I., Al-Sheikh, A. K., Al-Tufail, M., & Bogusz, M. J. (2010). Standardized GC–MS (EI) procedure for monitoring the detection and identification performance applied to herbal remedies. *Accreditation and quality assurance*, *15*, 659-664.

17. Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *BioMed research international*, *2014*, 497606. https://doi.org/10.1155/2014/497606

18. Nakaziba, R., Amanya, S. B., Sesaazi, C. D., Byarugaba, F., Ogwal-Okeng, J., & Alele, P. E. (2022). Antimicrobial bioactivity and GC-MS analysis of different extracts of Corchorus olitorius L leaves. *The Scientific World Journal*, *2022*.

19. Ojo, O. A., Ojo, A.B., Okolie, C., Nwakama, M. A.C., Iyobhebhe, M., Evbuomwan, I.O., & Batiha, G. E. S. (2021). Deciphering the interactions of bioactive compounds in selected traditional medicinal plants against Alzheimer's diseases via pharmacophore modeling, auto-QSAR, and molecular docking approaches. *Molecules*, *26*(7), 1996.

20. Palaniappan, N., Balasubramanian, B., Arunkumar, M., Pushparaj, K., Rengasamy, K. R., Maluventhen, V., & Maruthupandian, A. (2022). Anticancer, antioxidant, and antimicrobial

properties of solvent extract of Lobophora variegata through in vitro and in silico studies with major phytoconstituents. *Food Bioscience*, *48*, 101822.

21. Parys, W., Dołowy, M., & Pyka-Pająk, A. (2022). Significance of chromatographic techniques in pharmaceutical analysis. *Processes*, *10*(1), 172.

22. Rasquel-Oliveira, F. S., Ribeiro, J. M., Martelossi-Cebinelli, G., Costa, F. B., Nakazato, G., Casagrande, R., & Verri, W. A. (2025). *Staphylococcus aureus* in Inflammation and Pain: Update on Pathologic Mechanisms. *Pathogens*, *14*(2), 185. https://doi.org/10.3390/pathogens14020185

23. Saikarthik, J., Ilango, S., Vijayakumar, J., & Vijayaraghavan, R. (2017). Phytochemical analysis of methanolic extract of seeds of Mucuna pruriens by gas chromatography mass spectrometry. *Int J Pharm Sci Res*, *8*(7), 2916-2921.

24. Salih, L., Eid, F., Elhaw, M., & Hamed, A. (2021). In vitro cytotoxic, antioxidant, antimicrobial activity and volatile constituents of Coccoloba peltata Schott cultivated in Egypt. *Egyptian Journal of Chemistry*, *64*(12), 7157-7163.

25. Shobayo, B. R., Ojo, D. A., & Agboola, D.A. (2015). Antibacterial Activity of Pterocarpus osun L. on Multi-Drug Resistant (MDR) *Escherichia coli* from Wound Infections in Abeokuta, South-West Nigeria. *Open Access Library Journal*. 2, e1434 DOI:10.4236/oalib.1101434

26. Soliman, H. M., & Abdel-Wahhab, M. A. (2024). Synthesis of Antibacterial Bioactive Compounds Using Linoleic Acid Extracted from Melon Seeds Oil and Evaluation of Its Waste Meal Ash for Fried Oil Regeneration. *Waste and Biomass Valorization*, *15*(1), 487-499.

27. Song, Y. W., Lim, Y., & Cho, S. K. (2018). 2, 4-Di-tert-butylphenol, a potential HDAC6 inhibitor, induces senescence and mitotic catastrophe in human gastric adenocarcinoma AGS cells. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1865*(5), 675-683.

28. Stein, S. E. (1990). National Institute of Standards and Technology (NIST) mass spectral database and software.

29. Vats, T., Unda, S. R., & Osborn, I. (2020). Heart rate variability and antinociception monitoring: A prospective tool to manage and assess pain. *Topics in pain management*, *35*(9), 1-10.

30. Xie, C., Wang, S., Cao, M., Xiong, W., & Wu, L. (2022). (E)-9-Octadecenoic Acid Ethyl Ester Derived from Lotus Seedpod Ameliorates Inflammatory Responses by Regulating MAPKs and NF-κB Signalling Pathways in LPS-Induced RAW264. 7 Macrophages. *Evidence-Based Complementary and Alternative Medicine*, *2022*.

31. Yuan, Z., Duan, H., Xu, Y., Wang, A., Gan, L., Li, J., ... & Shang, X. (2014). α-Tocospiro C, a novel cytotoxic α-tocopheroid from Cirsium setosum. *Phytochemistry Letters*, *8*, 116-120.

32. Zuvela, P., Skoczylas, M., Jay Liu, J., Bączek, T., Kaliszan, R., Wong, M. W., & Buszewski, B. (2019). Column characterization and selection systems in reversed-phase high-performance liquid chromatography. *Chemical reviews*, *119*(6), 3674-3729.