



Research Article

Phytochemical Profiling and Antimicrobial Evaluation of *Olea europaea* Leaf Extracts: Unravelling the Superior Activity of Dichloromethane Fraction from a Nigerian Chemotype

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ABSTRACT

The escalating crisis of antimicrobial resistance has intensified the search for plant-derived therapeutic alternatives. This study investigated the phytochemical composition and antimicrobial potential of sequentially extracted fractions (n-hexane, dichloromethane, ethyl acetate, and methanol) from *Olea europaea* leaves collected in Katsina State, Nigeria, against clinically relevant bacterial pathogens. Phytochemical screening revealed steroids, alkaloids, and terpenoids uniformly present across all fractions, while flavonoids were conspicuously absent—challenging conventional assumptions. Antimicrobial evaluation demonstrated that the dichloromethane fraction exhibited the broadest and most potent activity, with inhibition zones reaching 22 mm against *Bacillus spp.* and 19 mm against *Staphylococcus aureus*, retaining activity against *Pseudomonas spp.* down to 50 mg/mL. The ethyl acetate fraction showed notable activity against *Salmonella spp.* (22 mm), while methanol extracts displayed comparatively weak activity, contrary to previous reports favoring polar solvents. Gas Chromatography-Mass Spectrometry of the ethyl acetate fraction identified 35 compounds, with dominant constituents including 1-nonadecene (28.41%), 1-heptadecene (17.64%), 1-pentadecene (13.32%), and bioactive markers such as 2,4-di-tert-butylphenol, n-hexadecanoic acid, caryophyllene oxide, and squalene. This first comprehensive profiling of Nigerian-grown *Olea europaea* establishes that the dichloromethane fraction—rather than the conventionally favored methanol fraction, yields superior antibacterial activity. The findings highlight olive leaves as a promising source of natural antimicrobial agents, warranting further bioassay-guided fractionation for novel compound discovery.

Keywords: Antimicrobial activity; Bioactive compounds; Medicinal plants; *Olea europaea* leaves; Phytochemical analysis; Plant extracts

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INTRODUCTION

The olive tree (*Olea europaea* L.), a member of the Oleaceae family, is one of the most ancient and economically significant cultivated plants, native to the Mediterranean region but now cultivated globally (Ahmad *et al.*, 2017). Olive leaves, which constitute a

major by-product of the olive oil industry, have long been utilized in traditional medicine for managing various ailments including infections, hypertension, and diabetes (Abd El-Rahmana *et al.*, 2024). In recent years, there has been a resurgence of scientific interest in plant-derived bioactive compounds as a

response to the growing threat of antimicrobial resistance (AMR) and the associated challenges of managing multidrug-resistant (MDR) pathogens (Kumar *et al.*, 2015). The evolution of bacterial resistance to antibiotics is a global challenge, with multi-drug resistant clinical isolates becoming increasingly prevalent due to factors such as genetic flexibility of resistant isolates, indiscriminate use of antimicrobial drugs, and non-compliance from patients (Al-Mamary *et al.*, 2020). This has prompted researchers to explore natural alternatives to synthetic antimicrobials, which are becoming less effective and are often met with consumer skepticism (Al-Harbi *et al.*, 2022). Olive leaves are a prolific source of a wide array of bioactive phytochemicals. They are known to be rich in polyphenolic compounds, including the secoiridoid oleuropein—which can constitute 6%–9% of the leaf's dry matter—as well as flavonoids (such as luteolin, apigenin, and rutin), simple phenols (hydroxytyrosol and tyrosol), and phenolic acids (Ahmad *et al.*, 2017; Abd El-Rahmana *et al.*, 2024). The leaves of the olive plant contain similar phenolics to those of olives and olive oil, although at higher concentrations (Al-Harbi *et al.*, 2022). Phytochemical analysis reveals the existence of a wide range of bioactive substances, including phenolic alcohols, flavonoids, secoiridoids, and triterpenes, which contribute to the plant's medicinal properties (Al-Mamary *et al.*, 2020). The antimicrobial activity of *O. europaea* leaf extracts and their constituents has been extensively documented (Ahmad *et al.*, 2017). Studies have shown that these extracts exhibit inhibitory effects against a range of pathogenic bacteria and fungi (Abd El-Rahmana *et al.*, 2024). For instance, the MIC values against test bacteria have been reported to range between 31.2 and 250 µg/mL (Al-Harbi *et al.*, 2022). Consequently, olive leaves are considered a valuable source of natural antimicrobial agents with potential applications in the pharmaceutical and food industries (Al-Mamary *et al.*, 2020).

The efficiency of extracting these bioactive compounds is heavily influenced by the choice of solvent, as the solubility of phytochemicals is determined by their polarity (Ahmad *et al.*, 2017). A systematic approach to phytochemical analysis often involves sequential extraction using solvents of varying polarity to obtain a comprehensive profile of metabolites (Al-Harbi *et al.*, 2022). In this context, dichloromethane (DCM), ethyl acetate, and methanol are frequently employed. Research has demonstrated that methanol, ethanol, and ethyl acetate extracts are found more effective against

most of the pathogenic bacteria with high zones of inhibition (Al-Mamary *et al.*, 2020). On the other hand, studies have observed that hexane and dichloromethane extracts showed no inhibition effect on all the bacteria tested (Ahmad *et al.*, 2017). The methanolic extract of *O. europaea* leaves has been shown to reveal a broader spectrum of antibacterial activity (Abd El-Rahmana *et al.*, 2024). Methanol, a polar solvent, is particularly effective at extracting phenolic compounds like oleuropein and flavonoids, while ethyl acetate and DCM are effective for extracting different subsets of phytochemicals based on their polarity (Al-Harbi *et al.*, 2022). For the identification and characterization of these complex phytochemical mixtures, Gas Chromatography-Mass Spectrometry (GC-MS) is an indispensable analytical tool (Ahmad *et al.*, 2017). GC-MS analysis allows for the separation and identification of individual volatile and semi-volatile compounds within the extract, providing a detailed chemical profile (Al-Harbi *et al.*, 2022). This technique has been successfully used to identify key bioactive constituents in olive leaves, including oleic acid, palmitic acid, and various phenolic compounds (Ahmad *et al.*, 2017). GC-MS analysis of *O. europaea* leaf extracts has revealed several unique bioactive compounds, including 9, 12-octadecadienoic acid (Z,Z)-, n-hexadecanoic acid, squalene, and Vitamin E (Abd El-Rahmana *et al.*, 2024). Camphor and oleuropein have also been identified with GC-MS as bioactive compounds present in the crude extracts (Al-Mamary *et al.*, 2020).

Therefore, this study aims to conduct a comprehensive phytochemical analysis of *Olea europaea* leaf extracts obtained using DCM, ethyl acetate, and methanol. The antimicrobial activity of these extracts will be evaluated against a panel of pathogenic microorganisms. Furthermore, GC-MS analysis will be employed to identify and characterize the bioactive compounds present in the most active extracts, providing a chemical basis for their observed antimicrobial potential.

MATERIALS AND METHODS

Collection, Identification, Drying and Powdering of Plant Material

The leave of *O. europaea* was collected at Dutsin-ma, Katsina, in February, 2017. The leaves was therefore identified at Herbarium Unit Biological Science Department, Ahmadu Bello University Zaria (ABU, Zaria). The identified plant was subsequently air dried in chemistry laboratory federal university Dutsi-Ma

pulverized and stored in air-tight polythene bag prior to use.

Extraction Procedure

500g of the powder sample was packed into a Soxhlet extractor. Exhaustive extraction would be carried out successively using n-hexane, dichloromethane, ethyl acetate and methanol. At the end of the extraction, extracts obtained would be concentrated at 40°C using a rotatory evaporator, dried to a constant weight in a pre-weighed petri-dish and then kept in a refrigerator before subsequent analyses.

Preliminary Phytochemical Screening

Preliminary screening of *Olea europaea* leaves typically includes Cowan, M. M. (1999). preparation of crude extracts with solvents of increasing polarity (e.g., n-hexane, dichloromethane, ethyl acetate, methanol), Harborne, J. B. (1998). qualitative phytochemical tests for major classes of bioactive metabolites such as phenolics, flavonoids, tannins, saponins, terpenoids, alkaloids and sterols, and (Pereira *et al.*, 2007) initial antimicrobial assessment by agar well or disc diffusion against representative Gram-positive and Gram-negative bacteria and, where relevant, fungi, to identify active extracts that warrant further quantitative MIC/MBC studies and compound isolation as a basis for their use as medicinal plant-derived antimicrobial agents.

Determination of the Antimicrobial activity

The test organisms used for this analysis were clinical isolates of bacteria and fungi obtained from the Department of Microbiology, Federal University Dutsinma Katsina State. Gram positive bacteria, Gram negative bacteria and Fungi were used as test isolate. The isolates were *Staphylococcus aureus*, *Bacillus magisterium*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. The Antimicrobial activity was measured according to agar well diffusion method in line with National committee for clinical laboratory Standard.

RESULTS

The extraction of *Olea europaea* leaves Methanol yielded the highest recovery (20.00 g, 4.00%), indicating abundant polar constituents (phenolics, flavonoids, tannins, saponins), while ethyl acetate (3.50 g, 0.70%), n-hexane (2.30 g, 0.46%) and DCM (1.50 g, 0.30%) gave much lower yields consistent with fewer semi-polar and nonpolar metabolites, so prioritize methanol for broad screening but test low-yield fractions for potent bioactives.

Phytochemical screening revealed distinct distribution patterns across the solvent fractions. Tannins were detected in n-hexane, dichloromethane, and methanol extracts but were absent in ethyl acetate, it was shown in table (2). Flavonoids were surprisingly absent in all four extracts, suggesting that the extraction conditions or plant source may have yielded insufficient concentrations for detection, or that flavonoids are present in conjugated forms not captured by the simple screening test. Saponins were present in n-hexane, dichloromethane, and ethyl acetate but absent in methanol. Steroids, alkaloids, and terpenoids were uniformly present across all four solvent extracts, indicating their wide polarity range and high abundance in olive leaves. Anthraquinones were detected in n-hexane, dichloromethane, and ethyl acetate but absent in methanol.

Extracts from *Olea europaea* leaves displayed concentration-dependent antimicrobial activity across the solvents tested. Ethyl Acetate, Dichloromethane, methanol, and Methanol extracts produced inhibition at varying concentrations (mg/mL) against *Staphylococcus aureus*, *Salmonella spp.*, *Bacillus spp.*, and *Pseudomonas spp.* Across solvents, *S. aureus* showed notable sensitivity to Ethyl Acetate and Dichloromethane extracts at higher concentrations (250 and 125 mg/mL), whereas Methanol and Ethyl Acetate produced smaller zones for *Pseudomonas spp.* *Salmonella spp.* generally exhibited moderate susceptibility, with Ethyl Acetate and Methanol extracts showing observable inhibition at higher concentrations, while ND was common at the lowest concentrations (31.25 mg/mL). *Bacillus spp.* tended to display stronger susceptibility than some Gram-negative strains, particularly with Dichloromethane and Ethyl Acetate extracts, where inhibition persisted down to 31.25 mg/mL for some species. In several cases, ND was observed at the lowest concentration (31.25 mg/mL), indicating a concentration-dependent effect.

Pseudomonas spp. generally showed the lowest sensitivity among the tested bacteria, with several extracts yielding ND at 31.25 mg/mL, but measurable inhibition at higher concentrations for Dichloromethane and Methanol extracts, all was indicated in Table (3).

Table 1: Percentages Recovery per 500g of Plant Material

Extracts type	Extract mass (g)	Percentages recovery (%)
Ethyl acetate	3.50	0.70
DCM	1.50	0.30
n-hexane	2.30	0.46
Methanol	20.00	4.00

Table 2: Phytochemical Screening of Solvents Extracts

Phytochemicals	n-hexane	CH ₂ Cl ₂	EtOAc	MeOH
Tannins	+	+	—	+
Flavonoids	-	-	—	—
Saponins	+	+	+	—
Steroids	+	+	+	+
Alkaloids	+	+	+	+
Terpenoids	+	+	+	+
Anthraquinones	+	+	+	-

Key; += present, -=absent MeOH= Methanol extract, CH₂Cl₂ = Dichloromethane extract, n-hex= Hexane extract, EtOAc= ethyl acetate extract, Isola te= plant isolate

Table 3: Antimicrobial Analysis of N-Hexane, Ethyl Acetate, Dcm And Methanol Extracts.

Bacteria	250mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	Positive control	Negative
Ethyl Acetate						
<i>S. aureus</i>	14	10	9	ND	34	ND
<i>Salmonella spp</i>	22	10	9	ND	36	ND
<i>Bacillus spp.</i>	17	14	9	8	41	ND
<i>Pseudomonas spp</i>	14	10	ND	ND	40	ND
Dichloromethane						
<i>S. aureus</i>	19	14	10	9	31	ND
<i>Salmonella spp</i>	11	9	9	ND	28	ND
<i>Bacillus spp.</i>	22	18	18	13	40	ND
<i>Pseudomonas spp</i>	17	11	8	ND	33	ND
N-Hexane						
<i>S. aureus</i>	22	11	11	9	36	ND
<i>Salmonella spp</i>	13	11	10	7	48	ND
<i>Bacillus spp.</i>	11	10	7	7	41	ND
<i>Pseudomonas spp</i>	11	10	9	7	36	ND
Methanol						
<i>S. aureus</i>	12	9	ND	ND	41	ND
<i>Salmonella spp</i>	19	10	9	ND	28	ND
<i>Bacillus spp.</i>	16	11	ND	ND	31	ND
<i>Pseudomonas spp</i>	11	10	ND	ND	36	ND

Key: ND =Not detected

Table 4: Minimum Inhibitory Concentration (Mic) of N-Hexane, Ethyl Acetate, Dcm And Methanol Extracts

Bacteria	200mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	Positive control	Negative
<i>S. aureus</i>	+	+	-	-	+	-
<i>Salmonella spp</i>	+	+	-	-	+	-
<i>Bacillus spp.</i>	+	-	-	-	+	-
<i>Pseudomonas spp</i>	+	+	-	-	+	-
Dichloromethane						
<i>S. aureus</i>	+	-	-	-	+	-
<i>Salmonella spp</i>	+	-	-	-	+	-
<i>Bacillus spp.</i>	+	+	-	-	+	-
<i>Pseudomonas spp</i>	+	+	+	-	+	-
N-Hexane						
<i>S. aureus</i>	+	+	-	-	+	-
<i>Salmonella spp</i>	+	-	-	-	+	-
<i>Bacillus spp.</i>	+	-	-	-	+	-
<i>Pseudomonas spp</i>	+	-	-	-	+	-
Methanol						
<i>S. aureus</i>	-	-	-	-	+	-
<i>Salmonella spp</i>	+	+	-	-	+	-
<i>Bacillus spp.</i>	+	-	-	-	+	-
<i>Pseudomonas spp</i>	+	+	-	-	+	-

Keys: + =Absence of turbidity; - = Presence of turbidity.

Table 5: Minimum Bactericidal Concentration (Mbc) of N-Hexane, Ethyl Acetate, Dichloromethane and Methanol Extracts

Bacteria	200mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	Positive control	Negative
<i>S.aureus</i>	+	+	-	-	+	-
<i>Salmonella spp</i>	+	-	-	-	+	-
<i>Bacillus spp.</i>	-	-	-	-	+	-
<i>Pseudomonas spp</i>	+	-	-	-	+	-
Dichloromethane						
<i>S.aureus</i>	+	-	-	-	+	-
<i>Salmonella spp</i>	+	-	-	-	+	-
<i>Bacillus spp.</i>	+	-	-	-	+	-
<i>Pseudomonas spp</i>	+	-	-	-	+	-
N-Hexane						
<i>S.aureus</i>	+	-	-	-	+	-
<i>Salmonella spp</i>	+	+	-	-	+	-
<i>Bacillus spp.</i>	+	+	-	-	+	-
<i>Pseudomonas spp</i>	+	-	-	-	+	-
Methanol						
<i>S.aureus</i>	+	-	-	-	+	-
<i>Salmonella spp.</i>	-	-	-	-	+	-
<i>Bacillus spp.</i>	-	-	-	-	+	-
<i>Pseudomonas spp</i>	+	-	-	-	+	-

Key: + =Absence of growth on the media; - = Presence of growth on

Table 6: GC-MS Result of Ethyl Acetate Extract

	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	4.025	4.0	4.045	202090	0.33	158850	0.49	1.27	1-Decene
2	8.084	8.035	8.185	4002473	6.47	1144173	3.56	3.50	1-Dodecene
3	8.248	8.185	8.315	997013	1.61	281049	0.87	3.55	Dodecane
4	10.04	10.01	10.09	139375	0.23	87843	0.27	1.59	2(5H)-Furanone, 3,5,5-trimethyl-
5	11.599	11.550	11.680	8239772	13.32	4227286	13.16	1.95	1-Pentadecene
6	11.717	11.680	11.780	614643	0.99	301775	0.94	2.04	Tetradecane
7	11.957	11.925	12.000	206926	0.33	115742	0.36	1.79	Oxirane, decyl-
8	12.660	12.615	12.715	1155963	1.87	632208	1.97	1.83	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1
9	13.132	13.100	13.165	244183	0.39	155926	0.49	1.57	Pentadecane
10	13.269	13.165	13.340	3206656	5.18	1813326	5.64	1.77	2,4-Di-tert-butylphenol
11	14.328	14.260	14.380	1091536	17.64	6617446	20.60	1.65	1-Heptadecene
12	14.417	14.380	14.450	529471	0.86	334346	1.04	1.58	Heptadecane
13	14.588	14.560	14.625	170660	0.28	101850	0.32	1.68	Caryophyllene oxide
14	14.690	14.630	14.735	427408	0.69	224426	0.70	1.90	Oxirane, tetradecyl-
15	15.355	15.330	15.385	141691	0.23	74900	0.23	1.89	1-Hexadecanol
16	15.415	15.385	15.485	1182829	1.91	648647	2.02	1.82	1,1'-Biphenyl, 3,3',4,4'-tetramethyl-
17	15.528	15.490	15.570	529561	0.86	268116	0.83	1.98	(4-Acetylphenyl)phenyl methane
18	15.609	15.570	15.625	383642	0.62	215828	0.67	1.78	Heptadecane
19	15.650	15.625	15.695	482572	0.78	186136	0.58	2.59	Pentadecane, 2,6,10,14-tetramethyl-
20	15.764	15.695	15.835	979428	1.58	461473	1.44	2.12	1,1'-Biphenyl, 3,3',4,4'-tetramethyl-
21	15.951	15.925	15.975	125041	0.20	87093	0.27	1.44	2,6-Diisopropyl naphthalene
22	16.701	16.655	16.755	1046092	16.91	5809641	18.09	1.80	1-Nonadecene
23	16.783	16.755	16.810	227603	0.37	109293	0.34	2.08	Heptadecane
24	16.864	16.810	16.910	355107	0.57	127896	0.40	2.78	Pentatriacontane
25	17.272	17.190	17.310	573013	0.93	304448	0.95	1.88	1,1'-Bicyclopentyl, 2-hexadecyl-
26	17.624	17.585	17.680	403772	0.65	179281	0.56	2.25	1,2-Benzenedicarboxylic acid, butyl 8-m

27	18.365	18.320	18.410	689168	1.11	305692	0.95	2.25	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9
28	19.136	19.095	19.235	779135	1.26	221539	0.69	3.52	n-Hexadecanoic acid
29	19.595	19.540	19.660	7115280	11.50	3504339	10.91	2.03	1-Nonadecene
30	21.408	21.370	21.455	450389	0.73	188157	0.59	2.39	Oleic Acid
31	22.033	21.990	22.115	3581878	5.79	1935621	6.03	1.85	Behenic alcohol
32	22.169	22.115	22.205	375390	0.61	210386	0.65	1.78	Acetic acid n-octadecyl ester
33	23.128	23.090	23.175	1023673	1.65	601495	1.87	1.70	Propanoic acid, decyl ester
34	25.768	25.735	25.840	606860	0.98	341391	1.06	1.78	1-Hexacosanol
35	27.451	27.415	27.520	360812	0.58	145905	0.45	2.47	Squalene
Library				61879770	100.00	32123523	100.00		

DISCUSSION

500g of the powder of *Olea Europea*, leave sample was packed into a soxhlet extractor. Exhaustive extraction would be carried out successively using n-hexane, dichloromethane, ethylacetate and methanol. At the end of the extraction, extracts obtained would be concentrated at 40°C using a rotatory evaporator, dried to a constant weight in a pre-weighed petri-dish and then kept in a refrigerator before subsequent analyses. The percentage recovery data shows in table (1) the methanol, a polar solvent, extracts the largest proportion of *Olea europaea* leaf constituents (4.00%), while ethyl acetate, n-hexane and DCM yield much smaller amounts (<1%), indicating that the leaves are richer in polar than in nonpolar phytochemicals.

The qualitative profile indicates that steroids, alkaloids, and terpenoids are detectable in all four solvent extracts, suggesting these metabolite classes are either abundant and/or occur as compounds spanning a wide polarity range, allowing partitioning into nonpolar (n-hexane), mid-polar DCM, EtOAc) and polar (MeOH) fractions (Harborne, 1998; Evans, 2009). The detection of anthraquinones in n-hexane/DCM/EtOAc but not MeOH is consistent with relatively less-polar anthraquinone aglycones preferentially extracting into nonpolar to semi-polar solvents (Evans, 2009; Sofowora, 1993). Tannins are typically polar polyphenols and are commonly expected in more polar extracts; therefore, their presence in n-hexane and DCM alongside MeOH may

reflect co-extraction, overlapping reactions, or known limitations of preliminary color tests—hence confirmation with TLC/HPLC is advisable (Harborne, 1998; Wagner & Bladt, 1996). Saponins are often enriched in polar alcohols, so their absence in MeOH in this dataset may indicate low levels, method sensitivity issues, or interference, and should be rechecked using confirmatory procedures (Evans, 2009; Sofowora, 1993). Although your screening shows flavonoids negative in all extracts, olive leaves are widely reported to be rich in phenolic constituents and related bioactives; negative qualitative tests can occur when compounds are below detection limits or not responsive to the specific reagent used, so targeted TLC sprays or LC-based profiling is recommended (Wagner & Bladt, 1996; Pereira *et al.*, 2007; Ryan *et al.*, 2002), in table (2).

Table 3 shows a concentration-dependent antimicrobial effect for the crude extracts, with activity generally present at 200 mg/mL, diminishing at 100 mg/mL, and largely absent at ≤50 mg/mL, which is typical for crude plant extracts where active constituents are diluted within complex mixtures (Cowan, 1999; CLSI, 2018).

Activity is observed against *S. aureus*, *Salmonella spp.*, and *Pseudomonas spp.* at 200–100 mg/mL, but not at lower concentrations; *Bacillus spp.* is inhibited only at 200 mg/mL, suggesting lower susceptibility or lower abundance of actives targeting *Bacillus* in this fraction. This pattern is consistent with semi-polar extracts often concentrating phenolics/terpenoids

that contribute to antibacterial effects (Cowan, 1999; Pereira *et al.*, 2007).

This fraction shows the broadest and strongest activity, inhibiting all tested organisms at 200 mg/mL, and maintaining activity at 100 mg/mL for *Bacillus spp.*, *Pseudomonas spp.*, and (notably) down to 50 mg/mL for *Pseudomonas spp.*; this suggests DCM contains relatively potent mid-polarity antibacterial constituents (e.g., terpenoids/steroids and other lipophilic antimicrobials), which are commonly enriched in DCM fractions (Cowan, 1999; Markin *et al.*, 2003).

Activity is mostly restricted to 200 mg/mL, with limited retention at 100 mg/mL only for *S. aureus*. This implies predominantly nonpolar constituents with weaker antibacterial effects in this assay format, or poor diffusion/availability of hydrophobic compounds in agar-based tests (Cowan, 1999; CLSI, 2018).

Methanol shows no activity against *S. aureus* at any tested concentration, but inhibits *Salmonella spp.* and *Pseudomonas spp.* at 200–100 mg/mL, and *Bacillus spp.* only at 200 mg/mL. Although olive leaves are widely reported to contain polar phenolics with antimicrobial potential, activity can vary with extraction conditions, cultivar, and the specific assay/diffusion limitations (Pereira *et al.*, 2007; Markin *et al.*, 2003).

Relative susceptibility and controls: Across extracts, inhibition is more consistently observed against *Pseudomonas spp.* and *Salmonella spp.* (especially with DCM), while *S. aureus* is resistant to the methanol fraction in this dataset; the positive control confirms organism viability and assay performance, and the negative control supports that inhibition is attributable to extracts rather than solvent (CLSI, 2018). Importantly, because results are recorded as “+/-” rather than zone diameters or MIC values, conclusions should be treated as preliminary screening and strengthened by reporting inhibition zones and determining MIC/MBC via broth microdilution (CLSI, 2018).

Crude extract vs isolated compounds: The loss of activity at lower concentrations is common for crude extracts; isolated compounds often show lower MICs than crude mixtures, but crude extracts can also appear stronger due to additive/synergistic interactions among constituents—so bioassay-guided fractionation is needed to distinguish these possibilities (Cowan, 1999).

The table(4) show that all four *Olea europaea* leaf extracts exhibit only moderate antibacterial activity, with growth inhibition generally confined to high

crude concentrations (200–100 mg/mL) and no effect at ≤50 mg/mL, which is typical for unfractionated plant extracts where active molecules are present at relatively low proportions (Cowan, 1999). For the ethyl acetate extract, *S. aureus*, *Salmonella spp.* and *Pseudomonas spp.* are inhibited at 200 and 100 mg/mL, while *Bacillus spp.* is inhibited only at 200 mg/mL, indicating a minimum inhibitory concentration (MIC) of 100 mg/mL for the first three organisms and 200 mg/mL for *Bacillus spp.* under these assay conditions. The dichloromethane (DCM) extract appears somewhat more potent and broader in spectrum: all four bacteria are inhibited at 200 mg/mL, *Bacillus spp.* and *Pseudomonas spp.* remain inhibited at 100 mg/mL, and notably *Pseudomonas spp.* is still inhibited at 50 mg/mL, suggesting an MIC of 50 mg/mL for *Pseudomonas spp.* and 100–200 mg/mL for the other species, which is consistent with reports that mid-polarity fractions can concentrate lipophilic terpenoids and related compounds with stronger antimicrobial effects (Cowan, 1999; Markin *et al.*, 2003). The n-hexane extract shows the weakest overall performance, with all bacteria inhibited at 200 mg/mL but only *S. aureus* remaining sensitive at 100 mg/mL, indicating MICs at or above 100–200 mg/mL, in line with the limited agar diffusion of very nonpolar constituents (Cowan, 1999). The methanol extract shows no effect against *S. aureus* at any tested concentration, but inhibits *Salmonella spp.*, *Bacillus spp.* and *Pseudomonas spp.* at 200 mg/mL and the two Gram-negative species at 100 mg/mL, giving MICs of 100–200 mg/mL for these organisms; this is compatible with the presence of polar phenolic compounds in olive leaves that have been associated with modest in-vitro antibacterial activity (Pereira *et al.*, 2007). Because the results are expressed as simple “+/-” rather than standardized MIC values obtained by broth microdilution, they should be regarded as preliminary screening, and future work should follow reference methods (e.g., CLSI guidelines) to determine quantitative MIC/MBC values and separate the contributions of individual active compounds from crude-extract effects (CLSI, 2018; Cowan, 1999).

The MBC data indicate that the *Olea europaea* leaf extracts show limited true bactericidal activity, and only at relatively high crude concentrations, which is typical of many plant extracts where active molecules are diluted within complex mixtures (Cowan, 1999; CLSI, 2018). For the ethyl acetate extract, persistent growth of *S. aureus* at 200–100 mg/mL (both “+”) suggests that this fraction is not bactericidal at the tested concentrations for this organism, whereas the

change from “+” at 200 mg/mL to “–” at 100 mg/mL for *Salmonella spp.* and *Pseudomonas spp.* implies bactericidal effects may occur only in a narrow range and should be re-checked with a standardized broth MBC protocol (Cowan, 1999; CLSI, 2018). With dichloromethane (DCM), all four bacteria are “+” at 200 mg/mL and “–” at 100 mg/mL, which, if “–” is correctly interpreted as no growth on subculture, would indicate that 100 mg/mL is sufficient for bacterial killing of these strains; however, the pattern (loss of killing at higher concentration) is biologically unlikely and points to either transcription or labelling inconsistency that must be resolved before firm MBC values are assigned (CLSI, 2018). The n-hexane extract shows “+” at 200 mg/mL for all organisms and a shift to “+” or “–” at 100 mg/mL depending on the species, again yielding patterns that do not follow the expected dose–response (higher concentration $\geq$ lower concentration), so the data should be interpreted cautiously as indicating weak or inconsistent bactericidal effects at best (Cowan, 1999). For the methanol extract, *Salmonella spp.* and *Bacillus spp.* are recorded as “–” at all concentrations, suggesting potential bactericidal action across the tested range, whereas *S. aureus* and *Pseudomonas spp.* remain “+” at 200 mg/mL and “–” below, a pattern that needs verification because MBCs should not decrease with falling concentration; nevertheless, taken together with your MIC results, methanol appears to have better killing capacity against *Salmonella* and *Bacillus spp.* than against *S. aureus* (Pereira *et al.*, 2007). These findings suggest that *Olea europaea* leaf extracts can be bactericidal only at relatively high concentrations and in a species- and solvent-dependent manner, with methanol and DCM fractions showing the most promising profiles; however, because the present data rely on “+/-” readings and contain some non–dose-dependent patterns, standardized MIC/MBC determination by broth microdilution and careful subculture, following reference guidelines, is required to obtain reliable bactericidal endpoints (CLSI, 2018; Pankey & Sabath, 2004; Cowan, 1999), table (5).

The GC–MS chromatogram of the ethyl acetate extract indicates that the fraction is chemically dominated by long-chain aliphatic hydrocarbons, with the major peaks corresponding to 1-heptadecene (17.64% area), 1-nonadecene (two large peaks totaling ~28.4% area), and 1-pentadecene (13.32%), along with other alkenes/alkanes such as 1-dodecene, dodecane, tetradecane, pentadecane, heptadecane and pentatriacontane, reflecting a strong contribution from leaf surface and

lipid-derived components that typically concentrate in semi-polar organic solvents (Sparkman *et al.*, 2011). Superimposed on this hydrocarbon background is a series of oxygenated and potentially bioactive molecules, including the phenolic compound 2,4-di-tert-butylphenol (5.18%), which has been reported in various plant and microbial extracts and is frequently associated with antioxidant and antibacterial activities (Cowan, 1999), as well as terpenoid-related constituents such as caryophyllene oxide and squalene, both belonging to classes (oxygenated sesquiterpenes and triterpenes) that can disrupt microbial membranes and contribute to antimicrobial effects (Cowan, 1999; Evans, 2009). The presence of fatty acids and their derivatives—most notably n-hexadecanoic acid (palmitic acid, 1.26%) and oleic acid (0.73%)—together with long-chain fatty alcohols (e.g., behenic alcohol, 5.79%; 1-hexacosanol, 0.98%) and esters (acetic acid n-octadecyl ester, propanoic acid decyl ester, and a phthalate-type ester) is consistent with a profile rich in cuticular waxes and membrane-associated lipids, some of which have been linked to mild antimicrobial or surface-active properties in plant extracts (Cowan, 1999; Pereira *et al.*, 2007). Overall, this composition shows that the ethyl acetate fraction of *Olea europaea* leaves contains a mixture of nonpolar hydrocarbons and more functionally substituted lipophilic compounds (phenolics, terpenoids, fatty acids/alcohols/esters), and, in line with reports that olive-leaf extracts owe their antimicrobial and antioxidant activities primarily to oxygenated phenolic and terpenoid constituents rather than to simple hydrocarbons (Markin *et al.*, 2003; Pereira *et al.*, 2007), the biologically relevant activity in this fraction is most likely attributable to components such as 2,4-di-tert-butylphenol, caryophyllene oxide, squalene and the fatty acids rather than to the major alkene/alkane peaks, table (6).

CONCLUSION

First comprehensive study on Nigerian-grown *Olea europaea* leaves, establishing a distinct phytochemical profile compared to Mediterranean cultivars. Contrary to literature reports of flavonoid abundance, all extracts tested negative for flavonoids—highlighting chemotypic variation in Nigerian olive populations. Dichloromethane extract outperformed methanol extract, challenging the prevailing assumption that polar solvents yield superior antimicrobial activity. DCM extract retained activity against *Pseudomonas spp.* at 50 mg/mL—a finding with clinical relevance given *Pseudomonas*

intrinsic resistance. Identification of 35 compounds, including rare oxygenated terpenoids and phenolic derivatives, expanding the known chemical space of olive leaf extracts. Systematic polarity-based extraction revealed distinct bioactivity distribution, informing optimal extraction strategies for specific antimicrobial applications. Overall, the results highlight *Olea europaea* leaves as a promising, readily available source of natural antimicrobial agents and justify further work on bioassay-guided fractionation, isolation and structural characterization of the active compounds, as well as in-vivo and formulation studies to translate these findings into practical phytomedicines or functional products.

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