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Phytochemical Screening, Antimicrobial, and Antioxidant Activities of *Olea europaea* Leaf Extracts

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Olea europaea L. (family Oleaceae) is a medicinal plant traditionally used to manage infections, hypertension, diabetes, and inflammatory conditions. This study evaluated the phytochemical composition, antimicrobial, and antioxidant activities of O. europaea leaves. Dried leaves were sequentially extracted using n-hexane, methanol, and water. Phytochemical screening revealed that methanolic and aqueous extracts contained phenolics, flavonoids, tannins, saponins, alkaloids, and cardiac glycosides, whereas n-hexane lacked cardiac glycosides and steroids. Antimicrobial activity was assessed against Salmonella spp., Escherichia coli, Aspergillus niger, and Candida albicans using the agar well diffusion method, with Ceftriaxone (10–30 mg/mL) and fluconazole (10–30 mg/mL) as controls. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined by broth dilution. All extracts exhibited antimicrobial activity, with inhibition zones of 0–2.9 mm (bacteria) and 0–2.8 mm (fungi). Methanolic extract demonstrated the highest antibacterial activity against Salmonella spp. (2.20 mm at 30 mg/mL), while the aqueous extract showed the strongest antifungal activity against C. albicans (2.80 mm at 30 mg/mL). MICs ranged from 0.05 to 8.56 mg/mL and MFCs from 0.56 to 14.32 mg/mL. Antioxidant activity, measured by DPPH free radical scavenging (5–640 µg/mL), was concentration-dependent, with methanolic extract achieving 87.36% inhibition at 640 µg/mL. These findings indicate that O. europaea leaves are a rich source of bioactive compounds with moderate antimicrobial and potent antioxidant properties, highlighting their potential as natural therapeutic agents for managing infections and oxidative stress-related conditions.

Keywords: *Olea europaea*, phytochemicals, antimicrobial activity, antioxidant, DPPH, MIC.

1. Introduction

The increasing prevalence of infectious diseases and the rise of antimicrobial resistance have intensified the search for new therapeutic agents, particularly from natural sources [1-2]. Medicinal plants have long been recognised as reservoirs of bioactive compounds with antimicrobial, antioxidant, anti-inflammatory, and other pharmacological properties [3–6]. Among these plants, *Olea europaea* L. (family Oleaceae), commonly known as the olive tree, is widely cultivated in Mediterranean regions and valued for both its nutritional and medicinal benefits [7,8].

While olive oil is well known for its cardioprotective and anti-inflammatory effects [9-10], other parts of the plant—especially the leaves—contain high levels of phenolic compounds such as oleuropein and hydroxytyrosol, which have demonstrated antimicrobial and antioxidant activities [11-13]. Previous studies have reported the ability of olive leaf extracts to inhibit bacterial and fungal growth [14,15], as well as to scavenge free radicals, thereby preventing oxidative stress-related disorders [16-18]. However, variations in solvent polarity, extraction methods, and plant source can significantly influence the yield and activity of these phytochemicals, and comparative studies using different solvents remain limited [19-20].

Figure 1.1: *Olea europaea* Leaves

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Given the growing problem of drug-resistant pathogens and the need for safe, plant-based antioxidants, further investigation into *O. europaea* leaves is warranted. This study aims to (i) qualitatively screen the phytochemical constituents of *O. europaea* leaves extracted with solvents of varying polarity (n-hexane, methanol, and water), (ii) evaluate their antimicrobial activity against selected bacterial (*Salmonella spp.*, *Escherichia coli*) and fungal (*Aspergillus niger*, *Candida albicans*) pathogens, and (iii) assess their antioxidant potential using the DPPH assay. The findings are expected to contribute to the understanding of *O. europaea*'s pharmacological properties and support its potential application in natural product-based therapeutics.

2. Materials and Methods

2.1 Materials

All chemicals and reagents used in conducting the tests were analytical grade such as, methanol, ethanol (Sigma, UK), DPPH (Sigma, UK), Sulphuric acid (Fisher scientific), n-hexane (Sigma, UK), ferric chloride (Sigma, UK), NaOH (Sigma, UK), Acetic anhydride (Sigma, UK), potassium iodide (Sigma, UK), Ammonia (Sigma, UK). Ciprofloxacin and Ceftrazone were purchased from Zumunci Pharmaceuticals Ltd., Sokoto, Nigeria. All the chemicals used have a purity of ≥ 99 %. The materials were stored appropriately to avoid degradation, and de-ionised water was used throughout the experiments.

2.2 Methods

2.2.1 Sample Collection and Identification

Mature green leaves of *Olea europaea* were collected from a market in Yauri local government, Kebbi state, Nigeria. The leaves were collected in a dry black nylon bag, and the sample was identified with ID number LHT145 at the herbarium of the Department of Biological Sciences, Usman Danfodio University, Sokoto.

2.2.2 Sample Preparation

The sample of *Olea europaea* was dried in the shade for 72 hours and ground into a fine powder using a pestle and mortar. The powdered sample was transferred and stored in a screw-capped glass in a desiccator until required.

2.3 Extraction of the Leaves

2.3.1 n-Hexane Extraction

Powdered *Olea europaea* leaves (115 g) were macerated in 750 mL of n-hexane for 72 hours at room temperature with intermittent agitation to facilitate the extraction of non-polar phytochemicals. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated by evaporating the solvent under reduced pressure using a rotary evaporator at 40 °C. The resulting crude extract was weighed, recorded for yield calculation, and stored in an airtight container until further analysis.

2.3.2 Methanolic Extraction

Powdered *Olea europaea* leaves (125 g) were macerated in 680 mL of methanol for 72 hours at room temperature with intermittent agitation to enhance the dissolution of phytochemicals. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C. The resulting crude methanolic extract was weighed, recorded for percentage yield determination, and stored in an airtight container until further analysis [21].

2.3.3 Aqueous Extraction

Powdered *Olea europaea* leaves (100 g) were soaked in 1000 mL of distilled water and maintained at 28 °C for 72 hours with intermittent stirring to enhance extraction efficiency. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated by evaporating the water under reduced pressure at 40 °C. The crude aqueous extract was weighed, recorded for percentage yield determination, and stored in an airtight container until further analysis [22].

2.4 Qualitative Phytochemical Screening of the Extracts

The tests for flavonoids, tannins, saponins, steroids/triterpenoids, alkaloids, cardiac glycosides and anthraquinones were carried out according to the methods described by [23].

2.5 Antibacterial Studies

2.5.1 Preparation of test organisms and inoculum standardisation

Pure cultures of *Salmonella* spp. and *Escherichia coli* were obtained from the departmental culture collection. Each organism was subcultured onto nutrient agar (NA) and incubated at 37 °C for 18–24 h. Colonies from an overnight plate were transferred into sterile normal saline, and the turbidity was adjusted to 0.5 McFarland standard (≈1.5 × 10^8 CFU/mL) by visual comparison. The standardised suspension was then diluted 1:100 in sterile broth to obtain a working inoculum of ≈1.5 × 10^6 CFU/mL for agar inoculation [24].

2.5.2 Agar-well diffusion assay and controls

Mueller–Hinton agar (MHA) plates were prepared, allowed to solidify, and the surface dried. Using a sterile cotton swab, the working bacterial inoculum was evenly streaked over the entire agar surface to

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produce a lawn of growth. Wells (2 mm diameter) were bored in the agar using a sterile corn borer (2 mm). Test solutions of each extract were prepared at the desired concentrations (10, 20, 30 mg/mL) in an appropriate solvent (aqueous extracts in sterile distilled water; methanolic and n-hexane extracts reconstituted in a small volume of solvent, then diluted in sterile distilled water or 5% DMSO such that the final DMSO concentration ≤5% v/v). Into each well, 50 µL of test solution was dispensed.

2.5.3 Determination of Minimum Inhibitory Concentration (MIC)

The MICs of the *Olea europaea* leaf extracts against the test bacterial isolates were determined using the broth microdilution method in sterile 96-well microplates as described by Andrews [36], with slight modifications. Stock solutions of each extract (100 mg/mL) were prepared in an appropriate solvent (aqueous extract in sterile distilled water; methanolic and n-hexane extracts pre-dissolved in a small volume of DMSO and diluted with Mueller Hinton broth (MHB) to a final DMSO concentration ≤5% v/v). Two-fold serial dilutions of each stock were made in MHB to give final test concentrations ranging from 0.019 to 10 mg/mL.

2.6 Antifungal studies

2.6.1 Sabouraud Dextrose Agar (SDA) preparation

SDA was prepared by dissolving peptone (10 g), dextrose (40 g) and agar (15 g) in 1 L of distilled water. The mixture was heated to dissolve components, adjusted to pH ~5.6, and sterilised by autoclaving at 121 °C for 15–20 min. After cooling to ~50–55 °C, the medium was poured into sterile Petri dishes (approx. 20 mL per plate) and allowed to solidify under aseptic conditions. Plates were stored at 4 °C and brought to room temperature before use.

2.6.2 Potato Dextrose Agar (PDA) preparation

PDA was prepared by boiling peeled, sliced potatoes (approx. 200 g) in 1 L of distilled water for 20–25 min, decanting and straining the potato infusion through sterile muslin to obtain 1 L filtrate. Dextrose (20 g) and agar (15 g) were added to the filtrate, dissolved by heating, and the medium sterilised by autoclaving at 121 °C for 15–20 min. After cooling to 50–55 °C, the medium was poured into sterile Petri dishes and allowed to solidify. Plates were stored at 4 °C until use.

2.6.3 Antifungal susceptibility testing (agar-well diffusion and controls)

For agar-well diffusion, SDA (for yeasts) or PDA (for filamentous fungi) plates were inoculated by evenly swabbing the surface with the standardised fungal suspension to produce a uniform lawn. Wells (2 mm diameter) were bored using a sterile cork borer and 50 µL of each extract concentration (10, 20 and 30 $mg \cdot mL^{-1}$) was dispensed into designated wells. Positive (fluconazole), negative (solvent) and sterility controls were included on each plate. Plates were allowed to stand at room temperature for 30–60 min to permit diffusion, then incubated at 28–30 °C for 48–72 h (filamentous fungi) or 24–48 h (yeasts). Zones of inhibition were measured in millimetres (mm). All assays were performed in triplicate, and the mean ± SD is reported.

2.6.4 Determination of Minimum Fungicidal Concentration (MFC)

MFCs were determined following broth microdilution MIC assays. After incubation, wells showing no visible fungal growth were identified. From each such well, 10 µL was aseptically plated onto fresh SDA or PDA plates and incubated at 28–30 °C for 48–72 h. The MFC was recorded as the lowest extract concentration that produced no visible colony growth on the recovery plates (indicating ≥99.9% killing). Appropriate growth, sterility and solvent controls were included. MFC determinations were performed in duplicate and reported as mean ± SD.

2.7 Preparation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Determination of Antioxidant Activity

DPPH solution was prepared by dissolving 0.04 grams of DPPH in 100 ml of methanol to obtain a concentration of 0.1 mM. The solution is stored in a dark bottle and kept at 4°C to protect it from light and maintain its stability. For the assay, various concentrations of the plant extract (ranging from 20 to 200 µg/ml) are prepared in methanol. To each concentration of the extract, an equal volume of DPPH solution is added, and the mixture is incubated in the dark at room temperature for 30 minutes. The absorbance of the resulting solution is measured at 517 nm using a UV-Vis spectrophotometer. The decrease in absorbance indicates the scavenging activity of the extract against DPPH free radicals, which is calculated as a percentage of antioxidant activity [25].

3. Results and Discussion

3.1 Results

The percentage yields of 4.25 %, 9.80 % and 7.39 % (Table 1) were obtained for the methanolic, aqueous extract and hexane extracts, respectively.

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3.1.1 Extraction of *Olea europaea*

The mass of the fraction obtained and the percentage yield are presented in Table 3.1

Table 1: Mass of extract (g) and the percentage yield of the *Olea europaea* extracts (%)

Extract	Original mass (g)	Mass (g) of extract	Percentage yield (%)
Methanol	125	5.31	4.25
Aqueous	100	9.80	9.80
n-hexane	115	8.50	7.39

3.1.2 Qualitative Phytochemical Screening of *Olea europaea* Leaves Extract

The qualitative phytochemical screening of the extracts obtained showed the presence of phenolic compounds, saponin, tannin, flavonoids, cardiac glycosides, steroids and alkaloids (Table 2).

Table 2: Qualitative Phytochemical Screening of *Olea europaea* Leaf Extracts

Phytochemical Constituents	Test	n-hexane extract	Methanolic extract	Aqueous extract
Phenolic	$FeCl_3$	+	+	+
Saponin	Frothing	+	+	+
Tanin	$FeCl_3$	+	+	+
Flavonoids	Alkaline	+	+	+
Cardiac glycosides	Keller-Killiani's	-	+	+
Steroids	Salkowski's	-	-	+
Alkaloid	Mayer's	+	+	+

Key: (+) detected, (-) not detected

3.1.3 Antibacterial Activity of *Olea europaea* Leaf Extract

The details of the results obtained from the antibacterial activity test of *Olea europaea* leaves extract are shown in Tables 3(a), 3(b), and 3(c). The result deduced that the extract had activity on the isolates with a zone of inhibition ranging between 4-16 mm.

Table 3 (a): Antibacterial Activity of *Olea europaea* leaves Extract

Test plant (*Olea europaea*)	Test organism	Conc. (mg/mL)	Zone of inhibition (mm/cm)
Aqueous extract	*Salmonella spp*	10	1.80
		20	1.95
		30	2.05
Methanolic extract	*Salmonella spp*	10	2.20
		20	2.20
		30	2.20
n-hexane extract	*Salmonella spp*	10	1.80
		20	1.73
		30	2.90
Aqueous extract	*Escherichia coli*	10	0.00
		20	0.00
		30	2.00
Methanolic extract	*Escherichia coli*	10	0.00
		20	1.60
		30	2.70
n-hexane extract	*Escherichia coli*	10	0.78
		20	2.70
		30	1.80
Positive control (Ceftriaxone)	*Salmonella spp*	10	7.35
		20	7.35
		30	7.35
	Escherichia coli	10	7.60
		20	7.60
		30	7.60
Negative control (Distilled water)	*Salmonella spp*	10	0.00

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		20	0.00
		30	0.00
	Escherichia coli	10	0.00
		20	0.00
		30	0.00

Table 3(b): Minimum Inhibitory Concentration (MIC) of *Olea europaea* Leaves Extract for *Salmonella spp (Sal. Spp) and Escherichia coli (E.Coli)*

Extract	**Concentration (mg/mL) *Sal. Spp***			**Concentration (mg/mL) *E. Coli***		
	10	20	30	10	20	30
Methanolic extract	8.56	3.12	1.56	1.0	1.08	2.80
Aqueous extract	0.19	0.17	0.05	1.65	1.68	1.65
n-hexane extract	1.78	1.56	1.38	0.88	1.86	2.35

3.1.4 Antifungal Activity of *Olea europaea* Leaves Extract

The details of the results obtained from the antifungal activity test of *Olea europaea* leaves extract are shown in Tables 3(a), and 3(b). The result deduces that the extract has activity on the isolate with a zone of inhibition ranging between 4-16 mm.

Table 4(a): Antifungal Activity of *Olea europaea* Leaves Extract

Test plant (*Olea europaea*)	**Test organism**	**Conc. (mg/mL)**	**Zone of inhibition (mm/cm)**
Aqueous extract	*Aspergilus niger*	10	0.00
		20	0.80
		30	2.00
Methanolic extract	*Aspergilus niger*	10	0.00
		20	1.80
		30	2.20
n-hexane extract	*Aspergilus niger*	10	0.00
		20	0.00
		30	0.00
Aqueous extract	*Candida albican*	10	1.90
		20	2.10
		30	2.80
Methanolic extract	*Candida albican*	10	0.00
		20	0.00
		30	1.80
n-hexane extract	*Candida albican*	10	0.00
		20	0.00
		30	2.20
Positive control (Fluconazole)	*Aspergilus niger*	10	2.10
		20	2.10
		30	2.10
	Candida albican	10	1.80
		20	1.80
		30	1.80
Negative control (Distilled water)	*Aspergilus niger*	10	0.00
		20	0.00
		30	0.00
	Candida albican	10	0.00
		20	0.00
		30	0.00

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Table 4(b): Minimum Fungicidal Concentration (MFC) of *Olea europaea* Leaves Extract for *Aspergilus niger and Candida albican*

Extract	Concentration (mg/mL) As. niger			Concentration (mg/mL) Can. al.		
	10	20	30	10	20	30
Methanolic extract	10.50	9.98	9.00	2.56	0.58	0.56
Aqueous extract	12.80	12.80	12.80	3.39	1.08	1.00
n-hexane extract	8.66	5.00	5.00	10.35	14.32	10.00

3.1.5 Antioxidant Activity of *Olea europaea* Leaves Extract

The details of the results obtained from the antioxidant activity test of *Olea europaea* leaves extract are shown in Table 4(a), and 4(b). Table 4 (a) shows the detailed result of the methanolic extract, including the absorption wavelength of the 1st, 2nd and 3rd and also the percentage inhibition. While Table 3.5 (b) shows the result of aqueous and n-hexane extract for percentage inhibition only.

Table 5(a): Antioxidant Activity of *Olea europaea* for methanolic extract

T.T	Assay	DH_2O(ml)	DPPH(ml)	% inhibition
1	5	955	2	8.74
2	10	990	2	10.35
3	20	980	2	32.22
4	40	960	2	65.70
5	80	920	2	79.81
6	160	840	2	84.82
7	320	680	2	86.58
8	640	360	2	87.36

Table 5(b): Antioxidant Activity of *Olea europaea* for Aqueous and n-hexane percentage inhibition

Assay	DH_2O	n-hexane extract (%)	Aqueous extract (%)
5	955	3.54	20.85
10	990	8.88	35.00
20	980	10.35	38.50
40	960	15.83	55.86
80	920	20.58	54.80
160	840	22.80	80.00
320	680	25.00	96.00
640	360	48.90	97.77

3.2 Discussion

The percentage yields of *Olea europaea* leaf extracts obtained with n-hexane, methanol, and water were 7.39%, 4.25%, and 9.80%, respectively. The higher yield in the aqueous extract may be attributed to the greater solubility of polar phytochemicals, such as phenolics, tannins, and glycosides, in water. Solvent polarity plays a key role in the extraction efficiency of plant constituents; polar solvents such as water and methanol generally extract hydrophilic compounds, while non-polar solvents like n-hexane favour lipophilic compounds such as fatty acids, waxes, and terpenoids. Similar trends in yield variation with solvent polarity have been reported in earlier studies on *O. europaea* and other medicinal plants [26]. Qualitative phytochemical screening revealed the presence of phenolic compounds, flavonoids, tannins, saponins, alkaloids, and cardiac glycosides in both methanolic and aqueous extracts, while the n-hexane extract lacked cardiac glycosides and steroids. The absence of cardiac glycosides in the n-hexane extract is consistent with their polar nature, making them insoluble in non-polar solvents. Conversely, steroids were detected only in the aqueous extract, which may be due to differences in extraction efficiency or possible contamination in other solvents. The phytochemical profile obtained in this study is in agreement with previous reports on *O.*

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europaea leaves [26]. The antibacterial activity assessment showed that all extracts exhibited varying inhibitory effects against *Salmonella spp.* and *Escherichia coli*, with zones of inhibition ranging from 0.00 to 2.90 mm. The methanolic extract demonstrated the highest activity against *Salmonella spp.* (2.20 mm at 30 mg/mL), while activity against *E. coli* was comparatively lower. MIC values ranged from 0.05 to 8.56 mg/mL, indicating that some extracts were active at relatively low concentrations. These differences in sensitivity may be due to variations in the cell wall structures of Gram-negative bacteria, which can influence permeability to phytochemicals. In the antifungal assays, the aqueous extract showed the strongest inhibition against *Candida albicans* (2.80 mm at 30 mg/mL), while activity against *Aspergillus niger* was generally lower, with no inhibition observed for the n-hexane extract. MIC and MFC values indicated fungicidal effects at concentrations between 0.56 and 14.32 mg/mL. The observed antifungal activity may be linked to phenolic compounds and flavonoids, which have been reported to disrupt fungal cell walls and membranes. The antioxidant activity, assessed by the DPPH free radical scavenging assay, showed a concentration-dependent increase for all extracts. The methanolic extract exhibited the highest activity, reaching 87.36% inhibition at 640 µg/mL, followed by the aqueous extract, while the n-hexane extract showed the lowest scavenging potential. These differences reflect the varying solubility of antioxidant compounds, such as polyphenols, in solvents of different polarities. The results confirm that *O. europaea* leaves are a source of bioactive compounds capable of neutralizing free radicals, supporting their potential role in reducing oxidative stress-related damage.

4. Conclusion

This study demonstrated that aqueous extraction of *Olea europaea* leaves produced the highest yield of bioactive compounds compared to methanolic and n-hexane extractions. Phytochemical screening revealed the presence of alkaloids, phenolics, flavonoids, tannins, saponins, steroids, and cardiac glycosides in varying proportions depending on solvent polarity. The methanolic extract showed the strongest antibacterial activity against *Salmonella spp.*, while the aqueous extract exhibited the highest antifungal activity against *Candida albicans*. Antioxidant assays confirmed strong, concentration-dependent free radical scavenging, with the methanolic extract showing the highest activity. These findings support the potential use of *O. europaea* leaves as a natural source of antimicrobial and antioxidant agents.

Conflict of Interest

The authors declare no conflict of interest.

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