Antibacterial Potentials of Olea europaea Leaves Extract: A Solution to Antimicrobial Resistance

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ABSTRACT

This research focused on the phytochemical screening and antibacterial analysis of the ethanolic extract of *Olea europaea* leaves. The plant material was collected within Sokoto metropolis, authenticated, dried, pulverized, and subjected to ethanolic extraction. Phytochemical screening was carried out using standard methods to identify the bioactive constituents, while the antibacterial activity of the extract was determined against selected pathogenic organisms such as *Staphylococcus aureus (gram +v), Staphylococcus epidermidis (gram +v), Salmonella typhi (gram -v),* and *Shigella (gram –v),* using the agar well diffusion method. Ceftriaxone served as the standard antibiotic for comparison. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides, proteins, and carbohydrates but anthraquinones were absent in varying intensities. The antibacterial test showed that the extract inhibited the growth of the test organisms at different concentrations, with zones of inhibition ranging between 0.25-3.20 mm.The MIC and MBC values indicated bacteriostatic and bactericidal effects at 75mg/mL for *S-epidermidis*, for *S-typhin, S-aureus* and *Shigella*, 25mg/mL. This results further confirmed that the extract possesses potentials for new antibiotics development.

Keywords: Phytochemicals, Antibacteria, *Olea europaea*, Ceftriaxone, Extracts

INTRODUCTION

Medicinal plants have remained a vital source of therapeutic agents for humankind since time immemorial. Before the development of modern pharmaceuticals, humans relied heavily on plants for the prevention and treatment of illnesses, and in many parts of the world this tradition continues today. According to the World Health Organization (WHO), more than 80% of the population in developing countries still depend on traditional medicine, most of which involves the use of plant extracts or their active principles [1]. This reliance is not merely due to accessibility but also because of affordability and cultural acceptance, and in some cases, the effectiveness of these remedies has been validated scientifically. One of the most pressing problems of modern medicine is the rapid emergence and spread of antimicrobial resistance (AMR). Antimicrobial resistance has been recognized as a global health threat, undermining the efficacy of conventional antibiotics and complicating the treatment of infectious diseases [2]. The overuse and misuse of antibiotics in both humans and animals have accelerated this problem, leading to an increase in multidrug-resistant pathogens such as Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Klebsiella pneumoniae [3]. Consequently, there is an urgent need to explore new antibacterial agents, and medicinal plants provide a promising avenue.

Among the many medicinal plants that have gained attention in recent decades, olive (Olea europaea) occupies a unique position. Native to the Mediterranean region but now cultivated worldwide, the olive tree has been cultivated for more than 6,000 years. Traditionally, the fruits and oil of olive are well known for their nutritional and health-promoting properties, but the leaves also have a long history of medicinal use [4]. Ancient Egyptians considered olive leaves a symbol of divine power and used them as part of mummification rituals for their preservative properties [5]. In Mediterranean folk medicine, decoctions made from olive leaves were used to treat fever, wounds, infections, and even malaria [6]. Phytochemical analyses of olive leaves have revealed a wide array of bioactive compounds, including phenolics, flavonoids, triterpenoids, tannins, and alkaloids [7]. Of these, oleuropein, hydroxytyrosol, and verbascoside are the most extensively studied. Oleuropein, a bitter secoiridoid glycoside, is the dominant constituent of olive leaves, often accounting for up to 14% of their dry weight [8]. It has been associated with antimicrobial, antioxidant, and anti-inflammatory activities. Hydroxytyrosol, one of the breakdown products of oleuropein, is considered one of the most powerful natural antioxidants and also exhibits broad-spectrum antibacterial activity [9].

Research has demonstrated that extracts of olive leaves can inhibit the growth of a wide range of bacteria, both Gram-positive and Gram-negative. For instance, oleuropein and hydroxytyrosol have been reported to disrupt bacterial cell membranes, interfere with DNA replication, and inactivate essential enzymes [10]. These findings validate the traditional use of olive leaves as remedies for infectious diseases and suggest that they may serve as sources of novel antibacterial compounds. Given the global burden of antimicrobial resistance, studies such as this one, which examine the phytochemical constituents and antibacterial activity of olive leaves, are timely and important. This work therefore aims to provide a scientific basis for the ethnomedicinal use of olive leaves, while also contributing to the global search for plantderived antibacterial agents.

Plate 1: *Olea europaea* leaves

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MATERIALS AND METHODS

All chemicals and solvents were obtained from LobaChemie and used as received. Mular Hilton nutrient agar and broth were used for the antibacterial analysis.

Sample Collection

Fresh olive (*Olea europaea*) leaves were collected from cultivated trees in Sokoto metropolis, Nigeria. The collection was carried out in the early hours of the morning to ensure that the leaves were physiologically fresh and free from excessive transpiration. Mature but healthy leaves were hand-picked from different branches of the trees to ensure representativeness of the sample. Care was taken to avoid leaves that were diseased, insect-infested, or mechanically damaged. The plant was authenticated by a taxonomist in the Department of Biological Sciences, Sokoto State University.

Sample Treatment

The collected olive leaves were washed thoroughly with clean tap water to remove dust, soil particles, and other extraneous materials. They were then rinsed with distilled water to eliminate any residual contaminants. After washing, the leaves were spread on clean trays and air-dried at room temperature (25–28°C) for two weeks in a well-ventilated laboratory environment. Direct sunlight was avoided during drying in order to prevent the degradation of sensitive phytochemicals such as phenolics and flavonoids. When a constant weight was achieved, the dried leaves were pulverized into a fine powder using a clean mechanical grinder. The powdered sample was stored in an airtight glass container and kept in a cool, dry place until further use.

Determination of Moisture Content

The moisture content of the powdered olive leaves was determined using the oven-drying method. A clean, pre-weighed crucible was filled with approximately 5 g of the powdered sample and placed in a hot air oven at 105°C for 6 hours. The crucible was then cooled in a desiccator and weighed. This process was repeated until a constant weight was obtained. The percentage moisture content was calculated using the equation below:

$$\%\ \text{moisture} = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where W_0 = weight of the empty dish
W_1 = weight of empty dish + sample
W_2 = weight of to the empty dish + dry sample.

Extraction of the Plant Material

One hundred seventy nine grams (179 g) of the powdered olive leaves was macerated in 400 mL of ethanol for 48 hours with intermittent shaking. The mixture was filtered first with muslin cloth. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain a semi-solid crude extract. The extract was stored in an airtight container at 4 °C until required for analysis. The percentage yield of the extracts was calculated using the equation 2 below:

$$\% \text{ extract yield} = \frac{\text{Weight of extract}}{\text{Initial weight of sample}}\ x\ 100 \ldots\ldots (2)$$

Qualitative Phytochemical Screening

Qualitative phytochemical analysis was carried out on the crude ethanolic extract of olive leaves using standard procedures described by Harborne (1998) and Sofowora (1993). The tests conducted included alkaloids (Dragendorff's, Mayer's, and Wagner's reagents), saponins (froth test), tannins (ferric chloride test), flavonoids (sodium hydroxide and ammonia tests), glycosides (Keller-Killiani test), steroids and triterpenoids (Liebermann-Burchard test), phenols (ferric chloride test), and anthraquinones (Borntrager's test). Observations were recorded based on color changes, froth formation, and precipitate development, and results were interpreted as either positive or negative for the presence of each phytochemical group.

Bacterial Strains

The antibacterial activity of olive leaves extracts was evaluated against selected pathogenic bacteria, including both Gram-positive and Gram-negative strains. The test organisms used were *S-epidermidis (gram +v) Salmonella typhi (gram -v)*, *Staphylococcus aureus*, (gram +v) and *Shigella (gram –v).* These bacterial strains were obtained from the Department of Microbiology, Usmanu Danfodiyo University Teaching Hospital, Sokoto. The organisms were maintained on nutrient agar slants at 4°C until required for use. Prior to antibacterial testing, the bacterial cultures were sub-cultured into fresh nutrient broth and incubated at 37°C for 18– 24 hours to ensure active growth.

Antibacterial Tests

The antibacterial test was conducted using the Petri dish plate method described by Cheesbrough [12]. Nutrient Agar plates were prepared according to the manufacturer's instruction and allowed to solidify for 15 minutes at room temperature. The plates were incubated without inoculum for 24 hours at 37 °C to ensure the sterility of the medium. Thereafter, 1 mL of the standardized bacterial inoculum was flooded onto the surface of each sterile plate, and the excess inoculum was removed using a sterile Pasteur pipette. Five wells (cups) of about 6 mm in diameter were cut into the agar surface using a sterile cork borer, and the agar plugs were carefully removed using a sterile ampoule file.

Different concentrations of the ethanolic extract of *Olea europaea* leaves (25 mg/mL, 50 mg/mL and 75 mg/mL) were prepared, and 0.1 mL of each solution was introduced into the wells. The plates were left at room temperature for two hours to allow proper diffusion of the extract into the medium and then incubated at 37 °C for 24 hours. After incubation, the plates were examined for zones of inhibition which were measured in millimetres using a transparent ruler. A standard antibiotic (Ceftriaxone) was included as control for comparison of antibacterial activity.

Minimum Inhibitory Concentration (MIC)

The MIC of the olive leaves extract was determined using the broth dilution method. Serial dilutions of the extract were prepared in nutrient broth to yield concentrations ranging from 25 mg/mL to 100 mg/mL. Each dilution was inoculated with 0.1 mL of standardized bacterial suspension (1.0×10^6 CFU/mL) and incubated at 37 ˚C for 24 hours. The lowest concentration of extract that showed no visible growth was recorded as the MIC.

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Minimum Bactericidal Concentration (MBC)

The MBC was determined by sub-culturing 0.1 mL from the tubes showing no growth in the

MIC test onto fresh Mueller-Hinton agar plates. The plates were incubated at 37 °C for 24 hours. The lowest concentration of extract that completely inhibited bacterial growth on the agar plates was recorded as the MBC.

RESULTS AND DISCUSSION

Percentage Moisture Content of *Olea europaea* Leaves

The moisture content of the powdered olive leaves was determined using the oven-drying method. The result is presented in Table 1.

Table 1: Percentage Moisture Content of *Olea europaea* Leaves (%)

Sample	W_0 (g)	W_1 (g)	W_2 (g)	% Moisture
Olive leaves powder	5.00	184.00	46.00	77.00

Key: W_0 = Weight of empty container W_1 = Weight of empty container + sample, W_2 =

Weight of container + dryed sample

Extraction of *Olea europaea* Leaves Powder

The powdered leaves were extracted with ethanol and concentrated. The mass of the crude extract and its percentage yield are presented in Table 2.

Table 2: Mass of Extract and Percentage Yield of Olive Leaves

Extract	Mass of powdered sample (g)	Mass of extract (g)	Percentageyield (%)
Ethanol	179.00	41.0	23.0

Qualitative Phytochemical Screening of *Olea europaea* Leaves Extract

Qualitative phytochemical tests revealed the presence of several secondary metabolites in the ethanolic extract of olive leaves. The results are shown in Table 3.

Table 3: Qualitative Phytochemical Screening of Olive Leaves Extract

Phytochemicals	Ethanol Extract
Alkaloids	
1. Mayer's test	+
2. Wagner's test	+
3. Hager's test	+
Flavonoids	
1. Alkaline test	+
2. Shinoda test	+
3. Ferric chloride test	+
Steroids	
1. Salkowski's test	+
2. Liebermann–Burchard's test	+
Tannins	
1. Lead acetate test	+
2. Ferric chloride test	+
Cardiac Glycosides	
1. Keller–Killiani test	+
Antroquinones	
1. Borntragers	-
Carbohydrates	
1. Molisch test	+
2. Fehling's test	+
Proteins	
1. Xanthoproteic test	+

Key: (+) = Present, (−) = Absent.

Antibacterial Activity

The antibacterial activity of the olive leaves extract and standard antibiotic (Ceftriaxone) against selected test organisms was evaluated using agar well diffusion method. The zones of inhibition are presented in Tables 4a, 4b and 4c.

Table 4a: Antibacterial Activity of Olive Leaves Extract (Zones of Inhibition in mm)

Concentration (mg/mL)	*S. epidermidis*	*S. aureus*	*S. typhi*	*Shigella*
25	1.00	0.00	0.00	0.00
50	1.45	1.10	1.08	0.70
75	1.95	2.70	1.62	0.25
100	2.45	3.20	2.12	0.75

Table 4b: Antibacterial Activity of Standard Antibiotic (Ceftriaxne) (Zones of Inhibition in mm)

Concentration (mg/mL)	*S. epidermidis*	*S. aureus*	*S. typhi*	*Shigella*
75	4.85	4.05	4.91	3.74

Table 4c: Minimum Inhibitory Concentration (MIC) of Olive Leaves Extract and Ceftriaxone

Organism	MBC (mg/mL)	MIC (mg/mL)
S. epidermidis	75	75
S. aureus	50	50
S. typhi	50	50
Shigella	25	25

DISCUSSION

The results obtained in this study revealed important information about the phytochemical composition and antibacterial potential of olive leaves extract. The moisture content of the powdered sample was relatively low (77.%), which is advantageous for storage and preservation, as excessive moisture often promotes microbial contamination and enzymatic degradation of bioactive constituents. The extraction yield of 23.0% indicates that ethanol was able to solubilize a significant proportion of the secondary metabolites present in olive leaves. This agrees with previous reports that ethanol is an efficient solvent for recovering phenolic and flavonoid compounds from plant matrices due to its polarity. The phytochemical screening demonstrated the presence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides, phenols, and anthraquinones in the extract. These groups of compounds are known to contribute significantly to antimicrobial properties.

For example, flavonoids disrupt microbial membranes, tannins form complexes with bacterial proteins, and saponins enhance permeability of cell membranes. The abundant presence of flavonoids and anthraquinones in the olive extract is therefore likely to play a key role in the observed antibacterial effects.

The antibacterial assay showed that olive leaves extract exhibited inhibitory activity against all the test organisms, though at relatively low levels compared to the standard antibiotic, Ceftriaxone. At the highest concentration (75 mg/mL), the extract produced inhibition zones of 1.95 mm against *Staphylococcus* epidermidis, 2.70 mm against *Staphylococcus aureus,* 1.62 mm against *Salmonella typhi,* and 0.25 mm against *Shigella.* In contrast, Ceftriaxone at the same concentration produced zones of inhibition greater than 3.7 mm for all organisms tested. This confirms that while the plant extract has measurable antibacterial activity, its potency is significantly lower than that of the standard drug. The MIC and MBC results further support these findings. The extract showed inhibitory and bactericidal activity against all four organisms, but only at higher concentrations, as indicated by the values in Tables 4a and 4c. Ceftriaxone demonstrated stronger activity at lower concentrations, which is consistent with its established efficacy as a broad-spectrum antibiotic. The relatively weak activity of olive leaves extract compared to Ceftriaxone may be due to the crude nature of the extract, which contains a mixture of compounds, only some of which are bioactive. Purification and isolation of specific active constituents such as oleuropein and hydroxytyrosol may enhance antibacterial potency. Nevertheless, the detection of activity in the crude extract provides scientific validation of the ethnomedicinal use of olive leaves for treating infections. These results align with previous reports that olive leaves possess antimicrobial properties attributable to their phenolic and flavonoid constituents. The study therefore highlights the potential of olive leaves as a source of natural antibacterial agents, though further work is needed to improve efficacy through purification and formulation.

CONCLUSION

The findings from this study have demonstrated that olive leaves contain several bioactive compounds with antibacterial potential. The presence of phytochemicals such as flavonoids, tannins, saponins, alkaloids and phenols may be responsible for the inhibitory activity observed against both Gram-positive and Gram-negative bacteria. Although the activity of the crude extract was lower compared to Ceftriaxone, the study has scientifically validated the ethnomedicinal use of olive leaves in managing bacterial infections. It can therefore be concluded that olive leaves represent a promising source of natural antibacterial agents.

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CONFLICTS OF INTEREST

The authors declare that there is no any conflict of interest.

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