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Phytochemical Analysis and *in Vitro* Assessment of Antimicrobial Activity of *Olea Europaea* Leaf Extract

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Annotation: Background: *Olea europaea* leaves are rich in phenolic acids, flavonoids, steroids, terpenoids. These phytochemicals are known for their anti-inflammatory, antioxidant and antimicrobial properties. **Objectives:** this study is designed to explore the phytochemical compounds in *Olea europaea* leaves extract and to evaluate the antimicrobial activity of it against pathogenic bacteria. **Materials and methods:** Extracts of *Olea europaea* first examined for their phytochemical constituents using standard preliminary screening tests. In addition, the antimicrobial activity of different concentrations of olive tree extract (10%, 20%, 30%, and 40%) was evaluated against four bacterial isolates against- *Staphylococcus aureus*, *Enterococcus ssp.*, *Escherichia coli*, and Klebsiella ssp. *in vitro*. The assessment was carried out using the agar well diffusion method, with levofloxacin disc used as a reference control for comparison. **Results:** the results of phytochemical analysis for the hydroethanolic extract of *Olea europaea* detected the presence of terpenoids, alkaloids, polyphenols, and flavonoids, while saponins were absent. The hydroethanolic extract of *Olea europaea* showed dose-dependent antimicrobial activity against the tested microorganisms, with greater inhibitory effects observed at higher extract concentrations. **Conclusion:** the finding of this study showed that Olea europaea leaf

extract exhibited antimicrobial activity in a dose dependent manner.

Keywords: antimicrobial activity, Olea europaea, plant extract, phytochemical compounds.

1. Introduction

The shortage of antimicrobial agents is a growing global issue have serious consequences for patient care and public health. This problem has developed mainly from the overuse and misuse of antibiotics and other antimicrobials, limited production because they are less profitable compared to newer drugs and shortages of raw materials [1].The serious consequences include appropriate treatment delays or force doctor to use less effective alternatives, prolonged or repeated infections, increased costs and overuse of wide spectrum antibiotics that contributes to the emergence and spread of antimicrobial resistance [2]. Therefore, it is essential to investigate for new agents to combat antibiotic resistance [3]. Before the advent of antibiotics, herbal treatment recognized as a primary aspect of traditional remedies, and even today, many modern drugs originate from medicinal plants [4]. However, the widespread and often inappropriate use of antimicrobial agents against bacterial, viral, and fungal infections has resulted in growing antimicrobial resistance, making these infections highly difficult to manage. As the result of this health problem, researchers are now turning to plant-based therapies as promising alternatives for helping to tackle antibiotic resistance [5]. one of interest is *Olea europaea* plant.

The olive tree *(Olea europaea)* is a small evergreen tree which belongs to the Oleaceae family and is mainly cultivated in the Mediterranean region, which produces nearly 98% of the world's olive products [6]. It has a wide range of interesting nutritional and health-promoting benefits, as both its oil and fruits are widely used as essential components of the daily diets in many cultures worldwide [7]. For centuries, different parts of the olive tree have been utilized in traditional medicine. Particularly, olive leaves have been accounted for their antidiabetic and antihypertensive effects [8]. Moreover, previous studies have reported that Olea europaea leaves extracts exhibited antioxidant, antiatherogenic, anti-inflammatory, and cholesterol lowering properties. These therapeutic properties of *Olea europaea* are mainly attributed to oleuropein, a key bioactive phenolic compound that accounts approximately 6–9% of the leaf's dry weight. Oleuropein is a phenolic compound that is largely receiving attraction due to its well- established antioxidant, anti-inflammatory and antimicrobial properties. Besides oleuropein, olive leaves were identified a rich source of many phytochemicals such as, steroids, alkaloids, rutin, quercetin, triterpenes and hydroxytyrosol. Many scientific evidence reported that these phytochemical compounds collectively contributed to many beneficial health effects associated with *Olea europaea* leaf extract [9,10]. In recent years, several studies have validated the antibacterial and antiviral potential of Olea europaea [11]. Olive leaf extracts have been widely evaluated against numerous microorganisms, including *Staphylococcus aureus, Escherichia coli, Candida albicans, Pseudomonas aeruginosa, Salmonella Enteritidis, Cryptococcus neoformans, and Klebsiella pneumoniae* [12]. The present study conducted to investigate the phytochemical compounds of in *Olea europaea* extract and to assess its antimicrobial activity against some gram-positive and gram-negative bacteria in comparison to reference antibiotic control levofloxacin.

2. Materials and Methods

2.1 Collection and extraction of *Olea europaea* leaves

Olea europaea leaves was brought from olive trees in home garden in Hilla city. The plant material was dried in shadow and grounded into fine particles. A 500 gm of dried plant powder

was filled into a thimble and then placed in a Soxhlet extractor. The extraction procedure was performed using (80:20) (ethanol: water) solvent. The extract was evaporated using rotary evaporator (Buchi / Germany) at a temperature not exceeding 40 °C and then the exudate was kept at 4 °C until further use [13].

2.2 Preliminary qualitative phytochemical analysis

These qualitative phytochemical tests were carried out on the hydroethanolic extract of *Olea europaea* using standard methods to detect the active phytochemicals. The Lead acetate test was used to detect phenolic compounds by adding one ml of 10% lead acetate solution to five ml of alcoholic extract. If a bulky white precipitate is formed, indicating the presence of polyphenols [14]. The Wagner's test was employed to identify alkaloids by mixing 10 ml of the alcoholic extract with dilute HCl and then the mixture was treated with one or two drops of Wagner's reagents (1.27 g of I_2 and 2 gm of KI in 100ml of H_2O). When a reddish-brown precipitate is appeared that is confirmed the presence of alkaloids [15]. For the steroid's detection, the Liebermann Burchard test was carried out by putting 2 mL of the plant extract with 2 ml chloroform and then treated with concentrated acetic acid and sulphuric acid. If the green color is observed that is reflected the presence of steroids [16]. Additionally, the Ferric Chloride test was performed for detection of tannins by mixing 2 mL of 5% (w/v) $FeCl_3$ solution with 2 mL of the plant extract. If tannins are present, a dark green or blue-black coloration is appeared [17]. For identification of saponins, a foam test was performed. For detection of terpenes, mixing five ml of olive leaf extract with two ml of chloroform and then addition of 3 ml of concentrated H_2SO_4 to mixture to form separate layer. The observation of a reddish-brown color in interface indicated the presence of terpenes [14].

2.3 Antimicrobial activity test

Olea europaea leaves hydroethanolic extract was investigated at various concentrations (20%, 40%, 60%, 80%, 100%) against *Enterococcus ssp.*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella ssp.* by agar diffusion method. Levofloxacin antibiotic disc (5µg) (Sigma, USA) was utilized as reference control for antibacterial activity, A loopful of growth from each isolate was transferred into nutrient broth and incubated at 37 °C for 18–20 hours. The cultures were then diluted in normal saline to match the 0.5 McFarland standard, producing a uniform suspension with a cell density of 1.5×10^8 CFU/ml. Petri dishes containing Brain Heart Infusion Agar (BHA) were inoculated with the bacterial suspension by streaking the surface evenly using a sterile swab. In each agar plate, five wells of 6 mm diameter were made by using a sterile cork borer, and then each well was filled with 100 µl of the test extracts at the respective concentrations using a sterile micropipette, while the central well filled with reference control. Then, agars were incubated at 37°C for 24 hours.

Levofloxacin (5 µg) was utilized as the reference control, while distilled water served as the negative control. After 24 hours incubation, antibacterial activity was determined by measuring the diameter of the inhibition zones (in millimeters) surrounding each well. All experiments were performed in triplicate, and the mean inhibition zone diameter was calculated [18].

3. Results And Discussion

The preliminary phytochemical screening results of *Olea europaea* leaves extract are shown in table -1-.

Table 1. the phytochemicals analysis results of *Olea europaea* hydroethanolic extract

Phytochemical compounds	*Olea europaea* extract
flavonoids	+
alkaloids	+
steroids	+
tannins	+

saponins	-
terpenoids	+

The preliminary phytochemical analysis test for *Olea europaea* leaves hydroethanolic extract detected the presence of various bioactive compounds that include polyphenols, tannins, steroids, alkaloids, and terpenoids, whereas saponins were absent. These findings are lined with those documented by tawfeeq (2023) and ibrahim *et al.* (2025) [20] who identified various phytochemical metabolites, such as flavonoids, tannins, terpenoids, saponins, alkaloids and steroids in olea europaea leaves extracts. These phytochemicals support the pharmacological effects of olive leaves extract. Moreover, Ghanem et al. (2019) performed a comprehensive chemical and biological analysis olive leaves extract, indicating their phenolic and flavonoid composition, as well as their antioxidant and antimicrobial activities. The (80:20) methanolic leaves extract exhibited the highest total polyphenol content and showed strong antioxidant activity *in vitro*. Notably, oleuropein was demonstrated as the key polyphenolic compound that contributed markedly to the potent antioxidant and antimicrobial properties of olive leaves extract [21]. Also, with respect to the terpenoid fraction in *Olea europaea* leaves, the finding of this study showed the positive results that are consistent with previous studies [22,23]. Terpenoid compounds in *Olea europaea* leaves have a key role in many of medicinal properties including antioxidant, anti-inflammatory and antibacterial, antifungal, and antiviral activities. Their antimicrobial activities are believed to be result from the disruption of microbial cell wall disruption or inhibition of microbial enzymes [24]. In regard to steroid compounds, the present findings are consistent with those documented by Nora, *et al*., (2012) [25] who demonstrated the presence of steroidal metabolites in *Olea europaea* leaf ethanolic extract. These compounds are thought to play a significant role in the biological activities of olive leaves extracts, including antioxidant, anti-inflammatory, antimicrobial, cardioprotective, antidiabetic, and anti-arthritic effects. In addition, Steroids present in Olea europaea may act synergistically with other phytochemicals, such as phenols and flavonoids, thereby enhancing the overall pharmacological effects of the extract [26]. With regard to saponin constituents, Marwa et al. (2023) [27] reported the presence of saponins in their analysis of *Olea europaea* leaf extracts, in contrast to the findings of the present study.

These variation in results among different studies may be attributed to several factors, including extraction parameters (such as solvent type, temperature, extraction time, and technique), analytical sensitivity (related to the reagents used and detection technique employed), and sample preparation methods, including drying, grounding, and storage of plant prior to analysis. Additionally, geographical and environmental conditions may influence on extraction results because the chemical composition of plants can vary depending on growing region, soil characteristics, and the season during which leaf collecting and drying [25].

The antibacterial activity of Olea europaea leaf extract at a range of concentrations (20%, 40%,60%,80% and 100%) were evaluated in vitro against *S. aureus, Enterococcus ssp., E. coli and Klebsiella* ssp. and compared to standard levofloxacin (5µg) (table2, figure1).

Table 2: The inhibitory effect (inhibition zone mm) of different olive leaf extract concentrations against bacterial isolates

Bacterial types	Olive leaf extract concentration %					levofloxacin (5µg)
	20%	40%	60%	80%	100%	
Staphylococcus aureus	**0**	**10**	**15**	**18**	**25**	**13**
Enterococcus ssp.	**0**	**9**	**13**	**15**	**20**	**11**
Escherichia coli	**0**	**0**	**10**	**13**	**30**	**16**
***Klebsiella* ssp.**	**0**	**0**	**0**	**10**	**27**	**15**

The results showing no antibacterial inhibition activity of olive leaf extract at 20% concentration

for all tested bacteria and the antibacterial activity increased with increase extract concentration with inhibition zone range 8-30mm. The extract has maximum inhibition at concentration (100%). S. aureus inhibition zone increased from 10mm (40%) to 25mm (100%), showing good sensitivity, while the inhibition zone of Enterococcus ssp. ranged from 9mm at (40%) to 20 mm at (100%). The Olea europaea leaves extract shows inhibitory effect on *E. coli* and *Klebsiella* only at concentration 60%,80%,100%. In this study, the inhibitory effect of olive leaf extract varied according to the type of Bacteria, with highest antimicrobial activity on both *E. coli* and *Klebsiella ssp*. than others bacteria at concentration 100%.

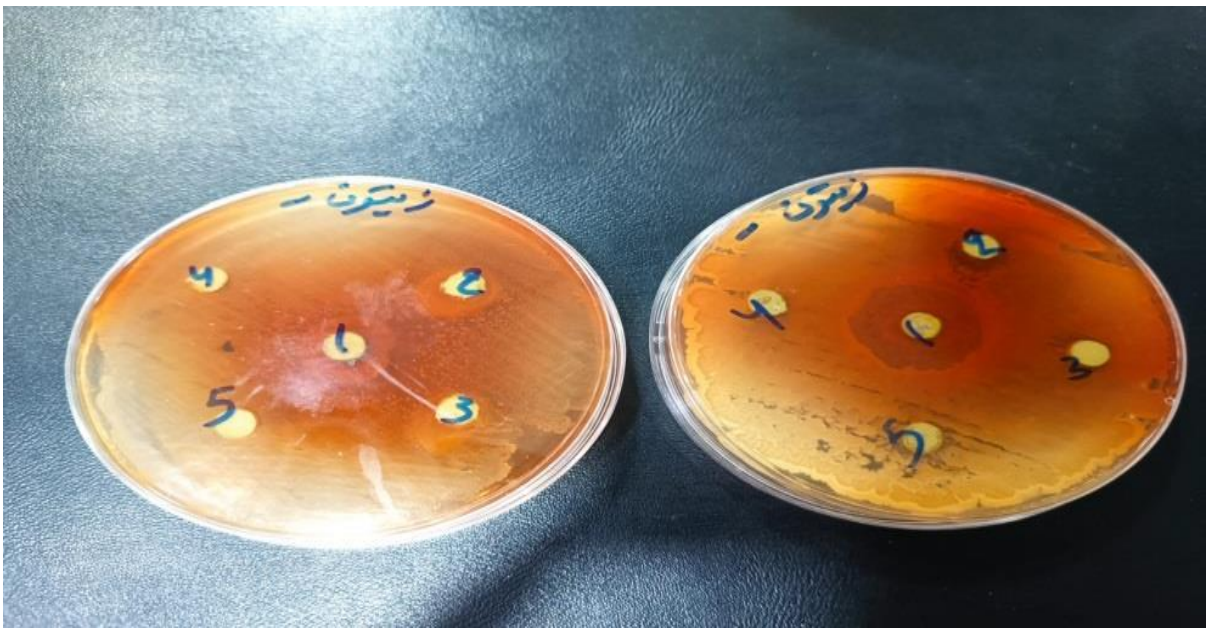

Figure 1. The impact of *Olea europaea* (olive leaf) extract against different bacteria

The current findings are in agreement with other studies like (Ben-Amor *et al.* (2021), who confirmed that olive leaf extract possesses biological efficacy against *Staphylococcus aureus, E. coli* and *Klebsiella spp.* [22]. A comparable result has been reported by Alsulaymani, *et al.*, (2021) that olive leaf extract possess activity on Enterococcus ssp. and *Escherichia coli* at similar concentrations [28]. The present results demonstrated a dose-proportional antibacterial activity, with higher extract concentrations leading to stronger bacterial inhibition. Gram-positive bacteria (*Staphylococcus* aureus and Enterococcus) exhibited high sensitivity than gram-negative bacteria at certain concentrations (*Escherichia coli* and *Klebsiella*), likely due to differences in microbial cell wall structure, as gram-negative bacteria have an outer membrane which can reduce the penetration of antimicrobial compounds [27, 28]. In the study done by Liu *et al.* (2017) who reported that *Olea europaea* leaves extract at a concentration of 62.5 mg/mL almost greatly inhibited the growth of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella spp.* This finding underscore that the antimicrobial effect is concentration-dependent, and lower concentrations might not yield significant inhibition, potentially explaining discrepancies between studies [29]. The variation in antibacterial activity of olive extract between this study and others studies could be due to differences in bacterial strains used, variability in extraction methods (solvent type, concentration, temperature, duration), differences in experimental conditions such as incubation time and medium used [25].

Based on these findings, we conclude that *Olea europaea* leaves extract appears to exhibit antimicrobial activity. The antimicrobial properties of this plant may be related to the phytochemical constituents, including steroids, polyphenols, alkaloids and tannins.

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