LEBDA MEDICAL JOURNAL, VOL. 11, NO. 1 (30 APRIL 2026), PP. 99–107.

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

Evaluation of the antibacterial Properties of Olive leaf Extract on Selected Pathogenic Bacteria (*Staphylococcus haemolyticus and Klebsiella pneumoniae*)

Ayada Ali Abuhdema[a], Awatif Mohammed Bin Mahmoud[a], Najat Muammer Eawidan[a], Ryad Moftah Alati[a], Mohamed Ali Mamash[a]

[a] Faculty of Pharmacy, Alasmarya Islamic University, Zliten- Libya.

* Corresponding author email: ry.alati@asmarya.edu.ly

A B S T R A C T

Background: Olive (*Olea europaea L.*) products, including fruit, oil, and leaves, are rich in bioactive phenolic compounds with diverse biological activities. Olive leaf extract has gained attention as a natural antimicrobial and antioxidant agent, with reported benefits for cardiovascular and immune health. Key constituents such as oleuropein and hydroxytyrosol exhibit inhibitory effects against pathogens including *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*. These findings highlight the therapeutic potential of olive-derived phytochemicals in combating antimicrobial resistance. Aim: The present study aimed to evaluate the antibacterial activity of olive leaf extract against two pathogenic bacteria: *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*.

Materials and Methods: The antibacterial potential of olive leaf extract was assessed using the agar well diffusion method, whereas ciprofloxacin and co-trimoxazole (effective against –ve gram and +ve gram bacteria respectively) were applied in the form of antibiotic discs and served as standard reference antibiotics. Zones of inhibition were measured in millimeters (mm) at different extract concentrations (15, 25, 30, and 60 mg/ml).

Results: For *Klebsiella pneumoniae*, the olive leaf extract produced inhibition zones of 0, 14 ± 0.4, 15.1 ± 0.6, and 17 ± 0.8 mm at concentrations of 15, 25, 30, and 60 mg/ml, respectively. For *Staphylococcus haemolyticus*, the inhibition zones were 11.6 ± 1.10, 14 ± 0.75, 15 ± 0.8, and 15.5 ± 1.29 mm at the same concentrations. Notably, the 60 mg/ml formulation demonstrated the highest antibacterial activity against *Klebsiella pneumoniae* (17 mm), which was comparable to the standard co-trimoxazole (16 mm).

Conclusion: Olive leaf extract exhibits significant antibacterial activity against both *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*. The strongest effect was observed at 60 mg/ml, suggesting its potential as a natural antimicrobial agent comparable to conventional antibiotics.

Keywords: Olive leaf extract, Antibacterial activity, Klebsiella pneumoniae, Staphylococcus haemolyticus

LEBDA MEDICAL JOURNAL, VOL. 11, NO. 1 (30 APRIL 2026), PP. 99–107.

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

1. Introduction

Olive (*Olea europaea L.*) fruit, oil, and leaves have a long history of nutritional, medicinal, and traditional use [1]. The olive tree is one of the most economically important species worldwide and is native to the Mediterranean region [2]. In recent decades, medicinal plants have attracted considerable scientific attention for their therapeutic potential in both human and veterinary medicine. This interest stems from their abundance of bioactive constituents, particularly flavonoids and phenolic derivatives. These molecules, which are categorized as secondary metabolites, are known to exert a wide spectrum of biological functions, including antioxidant, anti-inflammatory, antimicrobial, hypoglycemic, antihypertensive, and antiviral activities [3].

Olive leaf extract is considered a promising natural antimicrobial agent, possessing both antimicrobial and antioxidant properties. In addition, it has been associated with several health benefits, such as enhancing energy levels, lowering blood pressure, and supporting cardiovascular and immune system function [4]. Many reports describing the antimicrobial properties of phenolic compounds in olive products highlight hydroxytyrosol and oleuropein, which are primarily derived from olive fruit [5,6]. Olive leaf extracts have also been tested for their antibacterial efficacy against pathogenic bacteria, including *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* [5,6].

Staphylococcus haemolyticus is a facultative anaerobic, spherical, Gram-positive bacterium belonging to the Staphylococcaceae family [7]. It is frequently isolated from hospitalized patients and is notable for its resistance to multiple antimicrobial agents. *Klebsiella pneumoniae* is a Gram-negative bacterium [8] that, although part of the normal flora of the mouth, skin, and intestine, can cause severe pulmonary infections when aspirated into the lungs, particularly affecting the alveoli and leading to bloody sputum. Several studies have demonstrated that phenolic compounds such as oleuropein, rutin, and hydroxytyrosol exert significant inhibitory effects against *Klebsiella pneumoniae* [9].

Aim: This study was conducted to assess the antibacterial efficacy of ethanolic olive leaf extract against two clinically significant pathogens, *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*.

2. Materials and Methods

2.1 Plant Material Identification and Authentication

The leaves of *Olea europaea* were collected and subjected to botanical identification and authentication under the supervision of Dr. Adel Ahmed Mauafa, Department of Chemistry of Natural Products, Faculty of Medical Technology, Misurata University, Libya. The plant material was examined for morphological characteristics, including leaf shape, venation, and surface features, and compared with standard taxonomic references to confirm species identity. Authentication was performed to ensure purity of the sample and to exclude adulteration or contamination with other plant species. This process guaranteed that the plant material used in the present study was scientifically validated and suitable for subsequent experimental procedures.

2.2 Plant Material

Samples of *Olea europaea* leaves were collected during the winter season in January 2023 from the Zliten region in northern Libya. The leaves were air-dried at ambient room temperature (20–30 °C) for twenty days. Subsequently, they were ground into a fine powder using an electric mill (ABENCOR® Hammer Mill (MC2 Ingeniería y Sistemas, Spain, manufactured in 2012) and stored in closed containers at room temperature in the dark until required for extraction.

Corresponding author email: ry.alati@asmarya.edu.ly

LEBDA MEDICAL JOURNAL, VOL. 11, NO. 1 (30 APRIL 2026), PP. 99–107.

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

2.3 Bacterial Strains

The bacterial strains used in this study were clinical isolates of *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*, obtained from the Department of Microbiology at Zliten Medical Center-Libya.

2.4 Plant Sample Preparation

Fifty grams of dried leaves powder were macerated in 500 mL of absolute ethanol (96%) and continuously stirred for 48 hours using a magnetic stirrer equipped with a hot plate. The mixture was subsequently filtered through Whatman No. 1 filter paper, and the resulting filtrate was collected. The solvent was evaporated to dryness in a hot-air oven maintained at 40 °C for 24 hours to yield the crude extract. The dried residue was stored at 4 °C in a refrigerator until further use.

2.5 Antibacterial Activity

2.5.1 Preparation of Test Solutions

The dried extract was dissolved in distilled water to prepare test solutions at concentrations of 15, 25, 30, and 60 mg/mL [10].

2.5.2 Preparation of Culture Media

A total of 38 g of Mueller–Hinton agar powder was dissolved in 1 L of distilled water with thorough mixing until complete solubilization was achieved. The prepared medium was then sterilized by autoclaving at 121 °C for 15 minutes. After sterilization, it was aseptically dispensed into sterile Petri dishes and left to solidify under aseptic conditions before use.

The disk diffusion test, also known as the agar diffusion test or Kirby–Bauer method, was employed to evaluate the antibacterial activity. Mueller–Hinton agar plates were inoculated with the test organisms by streaking a sterile swab in a back-and-forth motion across the surface of the agar. The plate was then rotated by 60° and the streaking procedure repeated. This process was performed once more to ensure an even distribution of the inoculum, resulting in a confluent lawn of bacterial growth.

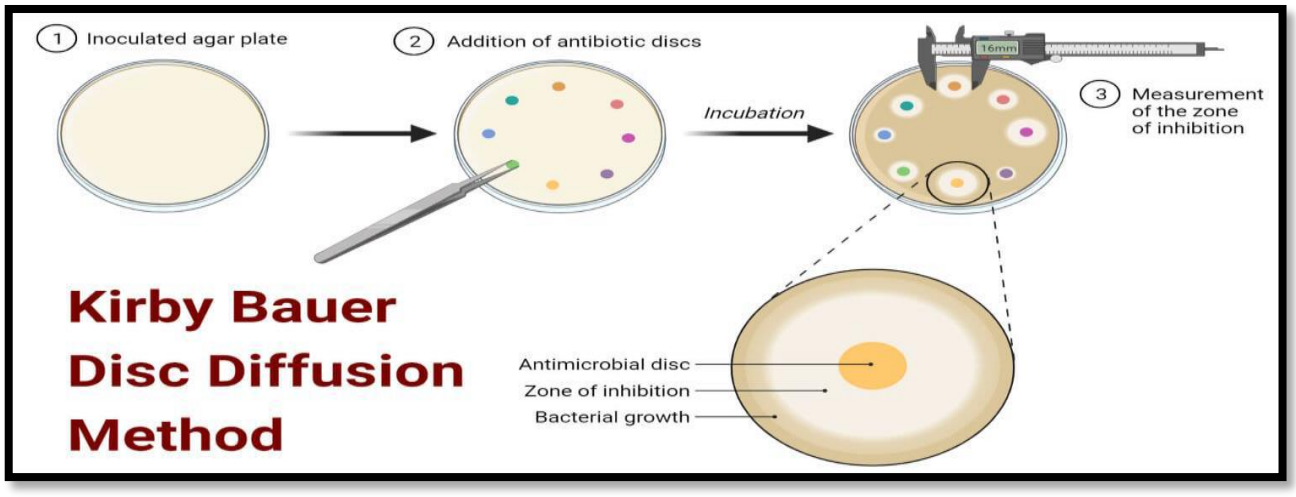

Figure 1: Kirby Bauer disc diffusion method [11]

Corresponding author email: ry.alati@asmarya.edu.ly

LEBDA MEDICAL JOURNAL, VOL. 11, NO. 1 (30 APRIL 2026), PP. 99–107.

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

Antibacterial-impregnated disks were subsequently placed on the surface of the inoculated agar. Ciprofloxacin (5µg) and co-trimoxazole (255µg) were used as standard reference antibiotics. The plates were incubated at 37 °C for 18-24hrs, within 15 minutes of applying the antibiotic disks (the antibiotics were applied in the form of antibiotic discs as standard reference antibiotics, whereas the olive leaf extract was tested using the agar well diffusion method). [11]

2.5.3 Diffusion Method / Agar Plate Method / Cup Plate Method

The cup plate method was employed to evaluate the antibacterial activity of the samples. In this technique, the test solution placed in a cavity (cup) diffuses through the agar layer in a Petri dish. The diffusion continues until the growth of the inoculated microorganisms is inhibited, resulting in a distinct circular zone of growth restriction surrounding the cavity containing the antimicrobial substance.

The antibacterial activity was quantified by measuring the diameter of the inhibition zone in millimeters using a calibrated scale

Experimental procedure:

Sterile borers were employed to prepare wells of 4 mm diameter in the agar medium of each Petri dish. Each well was filled with 200 µL of the test sample using a micropipette. Three wells were designated for the standard drug in each plate, and the corresponding zones of inhibition were measured for comparison with those of the test samples. In addition, a separate reference plate was prepared, in which discs of ciprofloxacin (5 µg), co-trimoxazole (25 µg). All plates were maintained at room temperature to allow effective diffusion of both the test samples and the standards, and subsequently incubated at 37 ± 1 °C for 24 hours. The appearance of distinct inhibition zones around the wells indicated antibacterial activity, and the diameters of these zones were measured and recorded. The procedure was adapted with minor modifications from established protocols [12].

3. Results and Discussion

The antibacterial activity of olive leaf ethanolic extracts were determined by measured zone of inhibition and compared with the zone of inhibition of standard co- trimoxazole and ciprofloxacin.

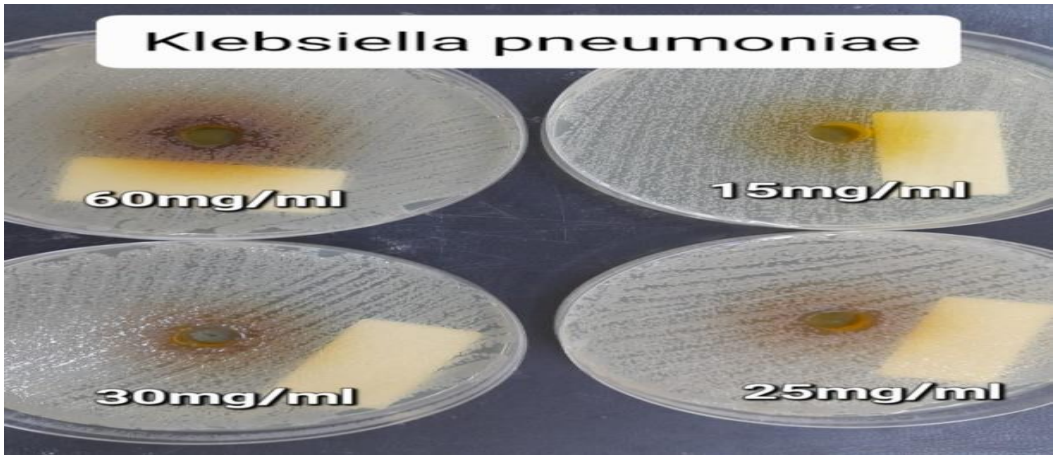

Figure 2. Antibacterial activity of *Olea europaea* (olive leaf) extract against *Klebsiella pneumoniae* using the agar well diffusion

Corresponding author email: ry.alati@asmarya.edu.ly

LEBDA MEDICAL JOURNAL, VOL. 11, NO. 1 (30 APRIL 2026), PP. 99–107.

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

method Different concentrations of the extract (15, 25, 30, and 60 mg/ml) were tested. The inhibition zones around the wells indicate that the extract exhibits dose-dependent antibacterial activity, with larger zones observed at higher concentrations (notably at 60 mg/ml).

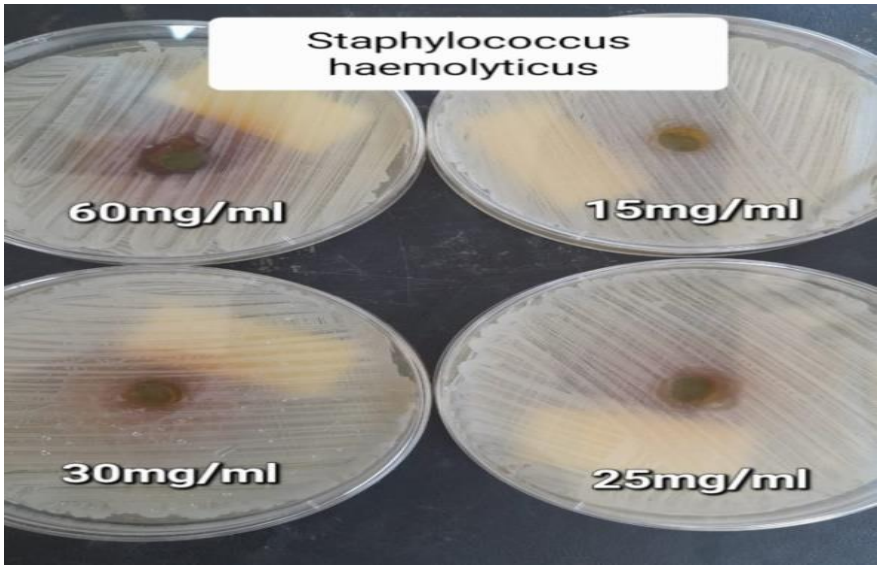

Figure 3. Antibacterial activity of *Olea europaea* (olive leaf) extract against *Staphylococcus haemolyticus*

Figure 3. displays antibacterial activity of *Olea europaea* (olive leaf) extract against *Staphylococcus haemolyticus* using the agar well diffusion method. Different concentrations of the extract (15, 25, 30, and 60 mg/ml) were tested, showing clear zones of inhibition around the wells. The diameter of the inhibition zones increased proportionally with extract concentration, with the highest activity observed at 60 mg/ml, indicating a dose-dependent antibacterial effect.

The zones of inhibition produced by the ethanolic extract of olive leaf at concentrations of 15, 25, 30, and 60 mg/mL against *Klebsiella pneumoniae* were 0 (no zone of inhibition), 14 ± 0.4, 15.1 ± 0.6, and 17 ± 0.8 mm, respectively. For *Staphylococcus haemolyticus*, the inhibition zones at the same concentrations were 11.6 ± 1.10, 14 ± 0.75, 15 ± 0.8, and 15.5 ± 1.29 mm, respectively, as presented in Table 2. The inhibition zones observed for the standard antibiotics co-trimoxazole and ciprofloxacin against *Klebsiella pneumoniae* were 16 ± 0.8 mm and 22 ± 0.8 mm, respectively. Against *Staphylococcus haemolyticus*, the inhibition zones were 16 ± 0.8 mm and 26 ± 0.8 mm, respectively (Figure 2 and 3).

The antibacterial activity of the negative control (distilled water) was also assessed, and no inhibition zones were observed (figure 4).

Corresponding author email: ry.alati@asmarya.edu.ly

LEBDA MEDICAL JOURNAL, VOL. 11, NO. 1 (30 APRIL 2026), PP. 99–107.

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

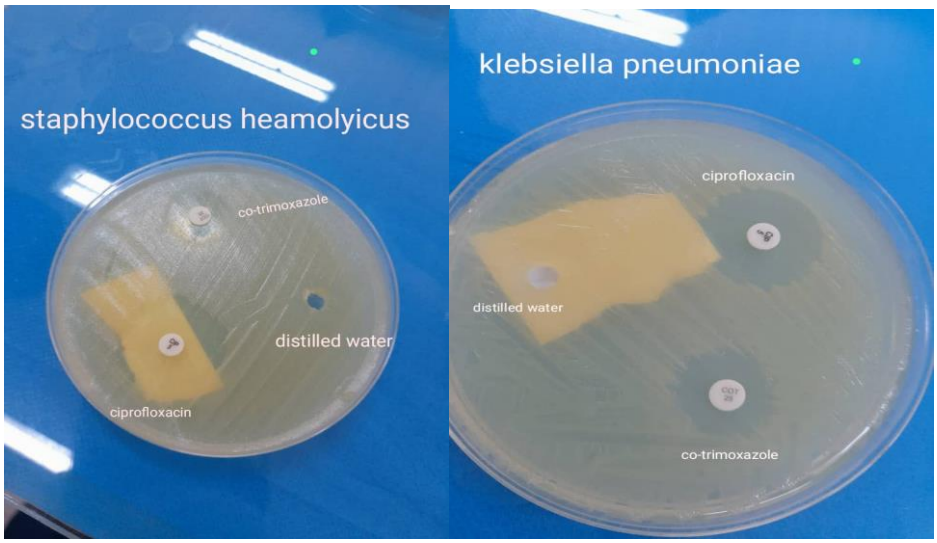

Figure 4. Inhibition zones of standard antibiotics (ciprofloxacin 5 µg and co-trimoxazole 25 µg) compared with distilled water control against *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*

As it can be seen in Figure 4, clear zones of inhibition were observed around ciprofloxacin and co-trimoxazole discs, indicating effective antibacterial activity, while distilled water showed no inhibitory effect.

Table 1- Antibacterial activity of olive leaf ethanolic extracts at different concentration

Tests	*Strain: Staphylococcus haemolyticus*				*Strain: Klebsilla pneumonia*			
	repetitions of tests				repetitions of tests			
	Rep1	Rep2	Rep3	Rep4	Rep1	Rep2	Rep3	Rep4
Control- distilled water	0	0	0	0	0	0	0	0
Ciprofloxacin-Standard	26	27	26	25	22	21	23	22
Co-trimoxazol-Standard	16	16	15	17	16	17	16	15
Olive leaf extract (15mg\ml)	12	11	10.5	13	0	0	0	0
Olive leaf extract (25mg\ml)	14	15.5	14	15	14	13.5	14	14.5
Olive leaf extract (30mg\ml)	15	14	16	15	15	16	14.5	15
Olive leaf extract (60mg\ml)	16	14	17	15	17	16	18	17

Corresponding author email: ry.alati@asmarya.edu.ly

LEBDA MEDICAL JOURNAL, VOL. 11, NO. 1 (30 APRIL 2026), PP. 99–107.

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

Table 2: Means and SD of Standard Drugs and Olive leaf Extract

Drug (controlled disk and extracts concentrations)	*Staphylococcus haemolyticus*		*Klebsilla pneumonia*	
	Mean	**STD**	**Mean**	**STD**
Control – distilled water	0	0	0	0
Ciprofloxacin- Standard	26	0.81650	22	0.81650
Co-trimoxazole- Standard	16	0.81650	16	0.81650
Olive leaf extract (15mg/ml)	11.625	1.10868	0	0
Olive leaf extract (25mg/ml)	14	0.7500	14	0.40825
Olive leaf extract (30mg/ml)	15	0.81650	15.1250	0.62915
Olive leaf extract (60mg/ml)	15.5	1.29099	17	0.81650

Note: The mean and SD were calculated from table1)

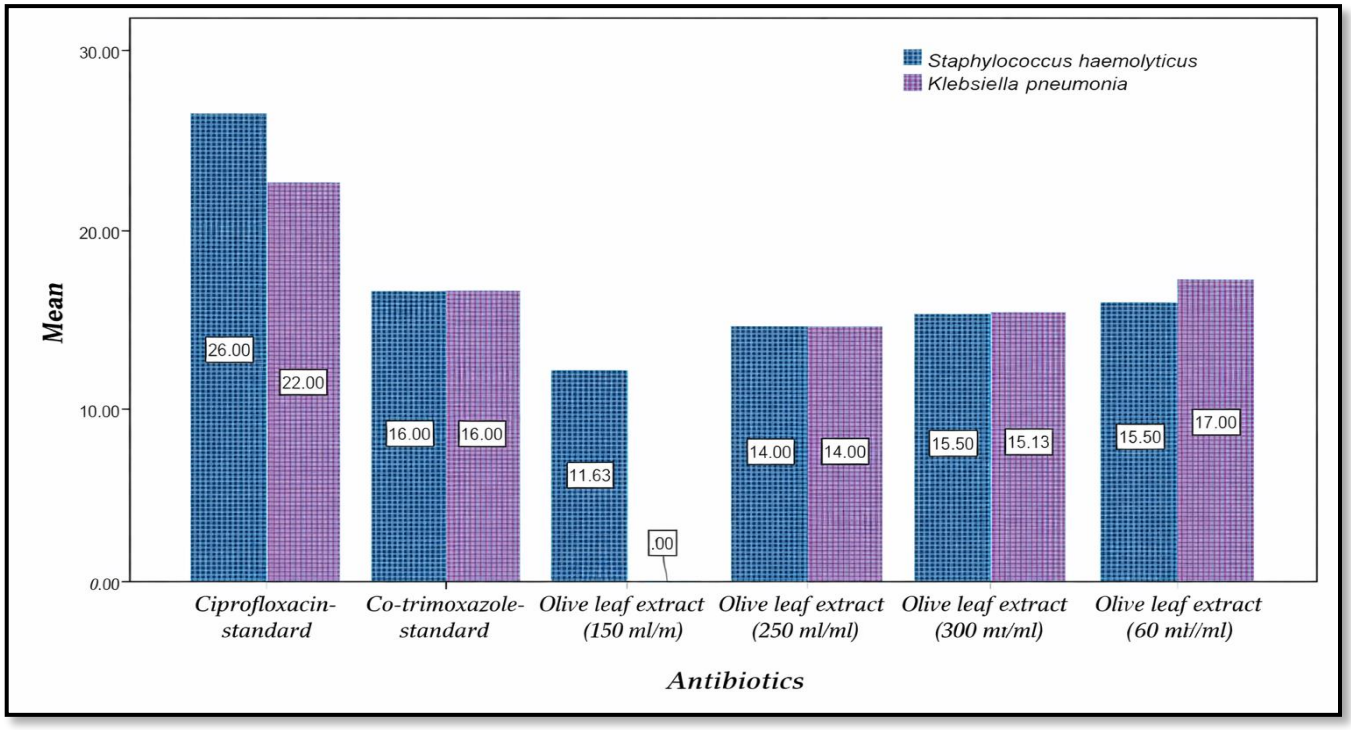

Figure 5. Comparison of antibacterial activity of olive leaf ethanolic extract with standard and control drugs

The ethanolic extract of olive leaf examined in this study demonstrated notable antibacterial activity against the tested microorganisms, specifically *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*. As shown in Table 1, the extract at a concentration of 60 mg/mL

Corresponding author email: ry.alati@asmarya.edu.ly

LEBDA MEDICAL JOURNAL, VOL. 11, NO. 1 (30 APRIL 2026), PP. 99–107.

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

exhibited the highest inhibitory effect, producing zones of inhibition of 17 mm against *K. pneumoniae* and 15.5 mm against *S. haemolyticus*.

Comparable findings have been reported in previous studies Keskın et al., 2012.[11] observed that the aqueous extract of olive leaves inhibited *K. pneumoniae* with a zone of inhibition measuring 11 mm. Similarly, Malik, 2015 [13] reported that olive leaf extracts were effective against Gram-positive strains (*Staphylococcus aureus* and *Bacillus cereus*), while lower activity was noted against Gram-negative strains. The highest inhibition was detected against *S. aureus*, a common Gram-positive bacterium associated with food poisoning. However, the activity of olive leaves and arugula seed extracts was lower than that of gallic acid. In the case of *B. cereus*, gallic acid produced a zone of inhibition of 8.76 mm, compared to 4.86 mm and 5.33 mm for olive leaf and arugula seed extracts, respectively.

Further confirmation was reported by Aicha Debib et al. [10], who examined leaf extracts of *Olea europaea* (Chemlali cultivar). Their findings indicated antimicrobial properties against a broad range of microorganisms. The petroleum ether fraction displayed pronounced inhibitory activity against *Escherichia coli* (13 mm) and *Salmonella enterica* (13 mm), whereas only moderate inhibition was noted for other bacterial species. Conversely, the methanolic fraction exhibited limited activity against most of the tested organisms, except for *S. enterica*, which showed a marked inhibition zone (15 ± 1.1 mm). Importantly, *Klebsiella pneumoniae* was identified as the most resistant strain in that investigation [14].

The formulation containing 60 mg/mL of olive leaf ethanolic extract exhibited the highest antibacterial activity against *Klebsiella pneumoniae*, producing a zone of inhibition of 17 mm. This effect was comparable to that of the standard antibiotic co-trimoxazole, which demonstrated a zone of inhibition of 16 mm.

4. Conclusion

Olive leaf extract showed notable antibacterial activity against both *Staphylococcus haemolyticus* and *Klebsiella pneumoniae,* with the most pronounced inhibition observed at 60 mg/ml. These findings suggest its potential as a natural antimicrobial agent with efficacy comparable to conventional antibiotics.

ETHICAL APPROVAL

Not Applicable.

CONFLICT OF INTEREST

The authors declare that they have no financial, professional, or personal relationships that could influence or appear to influence the work reported in this study.

AUTHORS' CONTRIBUTIONS

A.A.A. and M. A. M: Conceived and designed the study, supervised experimental work, and contributed to manuscript drafting.

A.M.B**:** Performed laboratory experiments, collected data, and assisted in data analysis.

N.M. E**:** Conducted statistical analysis, prepared tables and figures, and contributed to interpretation of results.

R.A: Provided pharmacological expertise, reviewed the manuscript critically for intellectual content, and finalized the draft for submission.

All authors read and approved the final manuscript.

Corresponding author email: ry.alati@asmarya.edu.ly

Lebda Medical Journal; |LMJ|; {Online ISSN:2520-095X}

Lebda Medical Journal Homepage: https://lebmedj.elmergib.edu.ly/index.php/LMJ/en

Received:11.04.2026; Revised:22.04.2026; Accepted:27.04.2026; Published:30.04.2026.

5. References

1. A. M. Ahmed, N. S. Rabii, A. M. Garbaj, and S. K. Abolghait, "Antibacterial effect of olive (Olea europaea L.) leaves extract in raw peeled undeveined shrimp (Penaeus semisulcatus)," *International Journal of Veterinary Science and Medicine,* vol. 2, no ,1 .pp. 53-56, 2014.
2. B. Fazeli-Nasab, M. Valizadeh, M. A. Hassanzadeh, and M. Beigomi, "Evaluation of the Antimicrobial Activity of Olive and Rosemary Leave Extracts Prepared with Different Solvents Against Antibiotic-Resistant Escherichia coli," *International Journal of Infection,* vol. 8, no. 3, 2021.
3. O. Ghomari *et al.*, "Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity," *Biotechnology Reports,* vol. 23, p. e00347, 2019.
4. Y. Liu, L. C. McKeever, and N. S. Malik, "Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens," *Frontiers in microbiology,* vol. 8, p. 113, 2017.
5. M. A. Aliabadi, R. K. Darsanaki, M. L. Rokhi, M. Nourbakhsh ,and G. Raeisi, "Antimicrobial activity of olive leaf aqueous extract," *Annals of Biological Research,* vol. 3, no. 8, pp. 4189-4191, 2012.
6. O.-H. Lee and B.-Y. Lee, "Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract," *Bioresource technology,* vol. 101, no. 10, pp. 3751-3754, 2010.
7. A. Shittu, J. Lin, D. Morrison, and D. Kolawole, "Isolation and molecular characterization of multiresistant Staphylococcus sciuri and Staphylococcus haemolyticus associated with skin and soft-tissue infections," *Journal of medical microbiology,* vol. 53, no. 1, pp. 51-55, 2004.
8. P. H. AG. "(Staphylococcus haemolyticus bacterium)." HARTMANN SCIENECE CENTER. https://www.hartmann-science-center.com/en/hygiene-knowledge/pathogens-a-z/pathogens-19/staphylococcus-haemolyticus (accessed 9 June 2023).
9. D. Borjan, M. Leitgeb, Ž. Knez, and M. K. Hrnčič, "Microbiological and antioxidant activity of phenolic compounds in olive leaf extract," *Molecules,* vol. 25, no. 24, p.2020 ,5946 .
10. A. Debib and M. N. Boukhatem, "Phenolic content, antioxidant and antimicrobial activities of "Chemlali" olive leaf (Olea europaea L.) extracts," *International Journal of Pharmacology, Phytochemistry and Ethnomedicine,* vol. 6, pp. 38-46, 2017.
11. J. J. Biemer, "Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method," *Annals of Clinical & Laboratory Science,* vol. 3, no. 2, pp. 135-140, 1973.
12. Z. Erdohan and K. Turhan, "Olive leaf extract and usage for development of antimicrobial food packaging," *Science against microbial pathogens: communicating current research and technological advances. Microbiol Book Ser,* vol. 3, pp. 1094.2011 ,1101-
13. S. N. Malik, "Antibacterial activity of olive (Olea europaea) leaves and arugula (Eruca sativa) seeds extract," *International Journal of Pharmacognosy and Phytochemical Research,* vol. 7, no. 2, pp. 307-310, 2015.
14. D. Keskın ,N. Ceyhan, A. Uğur, and A. D. Dbeys, "Antimicrobial activity and chemical constitutions of West Anatolian olive (Olea europaea L.) leaves," *Journal of Food, Agriculture & Environment,* vol. 10, no. 2, pp. 99-102, 2012.

Corresponding author email: ry.alati@asmarya.edu.ly