ISSN: 03875547
Volume 45, Issue 01, February, 2022

Activity of *Olea europaea* and *Punica granatum* leaves extract on some bacterial samples isolated from different clinical sources

Suhad khalid sgheer[1*], Sumaya Ayad abdulrazzaq[2], Shrook Gany Yassin[1], Saade Abdalkareem Jasim[2]

Educational Directorate of Karbala, Iraq[1]
Medical Laboratory Techniques Department, Al-maarif University College, Iraq[2]

Corresponding Author: 1*

Keywords:
Olea europaea, Punica granatum, Antibacterial activity, Medical plants.

ABSTRACT

Antibiotic resistance in pathogenic microorganisms is on the rise, prompting the development of novel antimicrobial options. Many plant components have received a lot of interest in Iraq as alternatives to traditional treatment. Two Iraqi medicinal plants, *Olea europaea* and *Punica granatum*, were examined in this study. One sort of solvent, hot boiled water, was used to extract the plant sample. The plant extract was tested as an antibacterial agent against *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli*, and *Pseudomonas aeruginosa* in three concentrations (100, 150, and 200 mg/ml) using the agar-well diffusion method. Antibacterial activity was found in all of the tested plant extracts at various concentrations, and this activity varied based on the bacteria species, plant type, solvent type, and extract concentration. Plant extracts are more sensitive to Gram-positive bacteria than they are to Gram-negative bacteria, depending on the kind of bacterium. Increases in extract concentration, on the other hand, have been linked to an increase in the diameter of the inhibitory zone. *Olea europaea* extract generated the highest diameter inhibitory zone.

1. INTRODUCTION

Due to the increase in antibiotic resistance around the world, drug manufacturers have turned to medicinal plants as the only way to inhibit the bacterial resistance [1]. *Punica granatum* L, also known as pomegranate, is a member of the Punicaceae family, which belongs to the order Myrtals, subclass rosidae, and class mangudiopsida. Pomegranate has been used as a culinary medicine and herbal medicine for over 3000 years [2]. Olive tree (*Olea europaea*, Oleaceae) leaves are widely used in traditional herbal medicine to prevent and treat a number of diseases, particularly in the Mediterranean region [3], [4]. *Klebsiella pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa* are more bacteria that resistance for many antibiotics [5]. *Punica granatum* L." Iraqi Roman" has been used to treat dysentery, diarrhea, Alzheimer's illness, male infertility, and arthritis. It has antiviral, antioxidant, anticancer, and anti-proliferative properties [6]. *P. granatum* constituents have been linked to a variety of biological and pharmacological functions. The key contributors to the health benefits of pomegranate leaves were discovered to be phenolics. These phenolics

have a strong ability to connect to a variety of molecular structures, including proteins and glycoproteins, and hence can help to combat bacterial resistance [7], [8]. The olive tree is usually recognized as having the highest antioxidant activity among natural antioxidants [9], mainly to its oil, fruits, and leaves. The study's main goal is to see if pomegranate and olive leaves can suppress bacteria that are resistant to antibiotics.

2. Methods

2.1 Plant leaves preparation

The leaves of the *Punica granatum* and *Olea europaea* plants were taken from the city of Ramadi - Anbar Governorate, and the plants were confirmed by the key for the classification of the plant.

2.2 Bacterial isolates preparation

Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and ultimately *Klebsiella pneumoniae* were all isolated and molecularly identified using the *16 srRNA* gene.

2.3 DNA extraction

They extracted, genomic DNA from isolates using Geneaid Genomic DNA Purification Kit (UK) and produced as directed by the company.

2.4 Detection of 16srRNA genes

All samples are prepared for (PCR) after the measurement of the concentration and purification of the DNA. Where it is prepared the forward (5'- AGAGTTTGATCCTGGCTCA- 3') and reverse (5' - GGTTACCTTGTTACGCTT - 3') primer for *16srRNA* gene [10]. The *16srRNA* gene specific interaction mixture included 12.5 µl of master mix, 1 µl of each primer, 1.5 µl of DNA, and 9 µl of distil water. After that, the *16srRNA* gene conditions were as follows: One cycle of initial denaturation at 94°C for five minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, and the final extension at 72°C for 10 minutes. On a 1% agarose gel, the PCR products were separated. With a current of 70 volts and 65 amps, the gel is left to run for 75 minutes. Following PCR amplification, agarose gel electrophoresis was used to establish the presence of amplification, followed by electrophoresis for the PCR product after staining with red safe and visualizing with UV light [11].

2.5 Sequencing

Using their ABI 3730xl genomic analyzer, Macrogen Inc. sequenced the 16srRNA gene. The Bio Edit program and the BLAST software were used to do the homology search, which may be found at the National Center Biotechnology Information (NCBI) online at http://www.ncbi.nlm.nih.gov.

2.6 Preparation of the aqueous extract

The aqueous extract of the leaves of the *Punica granatum* and *Olea europaea* plants was prepared by taking 5 g of the leaves of the two plants after crushing them well through a blender with the addition of 100 ml of distilled water, then stirred the mixture with a magnetic stirrer (40 C°) for one hour, then filtered the mixture by gauze. The mixture was filtered, then the extract was dried and stored for use [12].

2.7 Inhibitory efficacy of extracts

Using a sterile swab stick, each isolate's standardized inoculate was swabbed onto the surface of Mueller Hinton Agar in separate Petri dishes. Extract discs and standard Tetracycline (TET 30g) were applied to the infected media's surface. The plates were inverted and incubated aerobically at 37°C for 18 hours after allowing 30 minutes for the extract to diffuse into the agar. The test organisms formed a zone of inhibition

around each extract and standard antibiotic disc, which was subsequently quantified [13].

Agar well diffusion and dilution tests were used to assess the antibacterial properties of aquatic extract. 500 ml of microbial suspension was placed onto Mueller Hinton Agar (MHA) medium and cultivated in three directions using a sterile swab in the agar well diffusion method. On the surface of the agar plates were fixed paper discs made from filter paper and saturated with varied quantities of the aqueous extract to be evaluated. After a 24-hour incubation period at 37°C, microbial cultures were measured in millimeters for the presence or absence of a growth zone.

3. Results and Discussion

DNA was extracted from different isolates extraction kit equipped from Geniad Company, after the completion of the extraction process should measure the concentration and purity of samples, shown in fig.1.

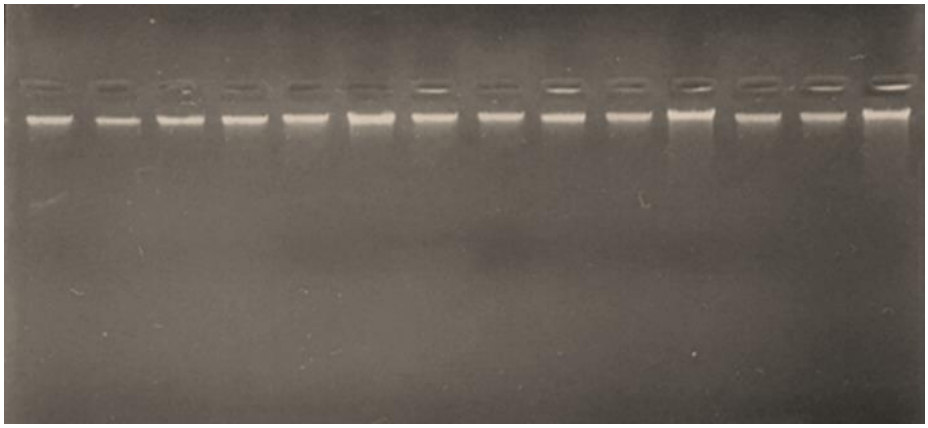

Figure 1: Genomic DNA extraction gel electrophoresis For 1:00 hours, run a 1% agarose gel at 6 vol/cm.

The electrophoresis findings revealed that the size band was 1500 bp after staining with red safe, as shown in fig. 2.

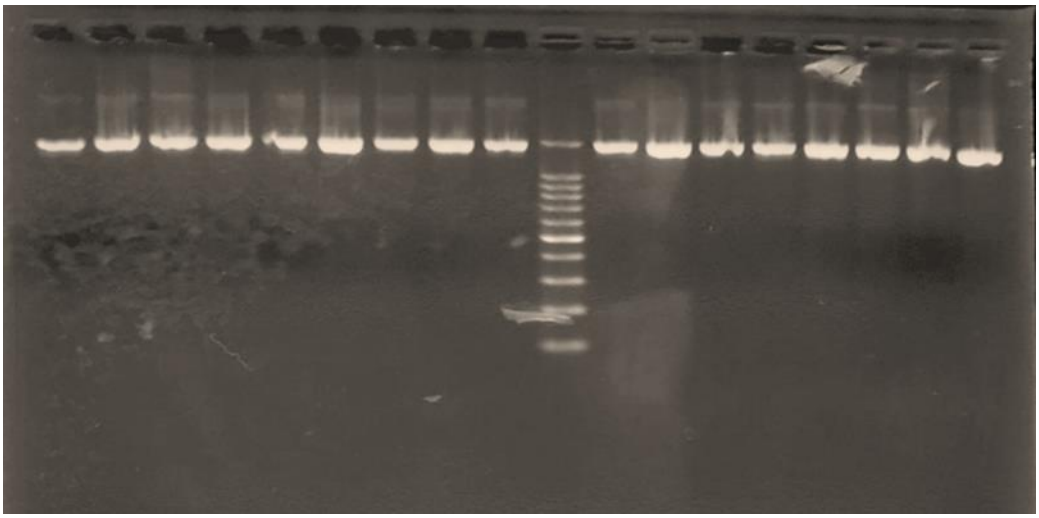

Figure 2: The band size produced by PCR is 1500bp. Electrophoresis on 1.5 % agarose at 5 volt/cm2 was used to separate the products. 75 minutes of 1x TBE buffer M: DNA ladder (100-1500 bp), lane (1-17) PCR result with 1500 bp band size, visible under UV light.

After sending the samples for sequencing, the results of the analysis showed 5 isolates of *Staphylococcus*

aureus, 6 isolates of *Escherichia coli*, 3 of *Pseudomonas aeruginosa* and 3 isolates of *Klebsiella pneumoniae*.

In recent decades, many natural compounds have been identified as sources of effective antibacterial agents, with research focused on plants as antimicrobial agents. Alkaloids, flavonoids, tannins, and terpenoids, which are secondary metabolites found in plants, have been proven to have antibacterial capabilities in the control of pathogenic microbes. As shown in table 1 and fig 3, all analyzed plant extracts had antibacterial activity against various bacterial isolates at varying doses in this study.

Table 1: Inhibition zones of two plant extracts at 200 mg/ml against several bacterial strains, measured in millimeters.

Type of Extract	Plant	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Aqueous	*Olea europaea*	41	33	27	25
	Punica granatum	24	19	12	9

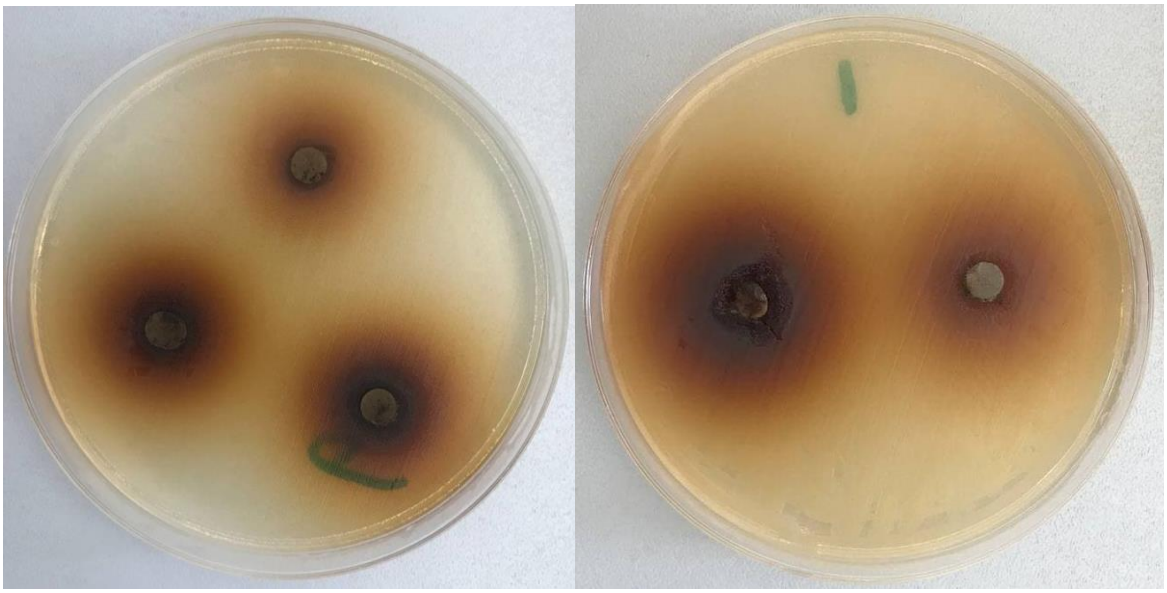

Figure 3: The Mueller Hinton Agar was used to test the antibacterial activity of plant extracts using the agar well diffusion method.

According to several studies [14], [15], these medicinal herbs could be used as alternative therapeutic agents for a variety of diseases. There are often no established procedures for test parameters such as inoculum size, inoculum preparation process, bacterial growth conditions, or standardized sizes for inhibitory zones or endpoints, making comparisons between results in different research difficult [16].

According to the findings of this study, the antibacterial activity of the plant extracts examined differed based on a number of criteria, including the bacteria species, plant type, solvent type, and extract concentration. This is in line with [17], who claims that the solvent type, extract concentration, and extraction technique all play a role in defining plant extract antibacterial activity.

Gram +ve bacteria, regardless of solvent type, are more sensitive to most plant extracts than Gram-ve bacteria, depending on the bacteria species. In crude extract, the diameters of inhibition zones for *S. aureus, E. coli, and K. pneumoniae* were (24-41) mm, (19-33) mm, and (12-27) mm, respectively, whereas the diameters of

inhibition zones for *P. aeruginosa* were (9-25) mm, as shown in Table 1. Plant extracts having high antibacterial activity against Gram-negative bacteria may not usually have considerable antibacterial activity against Gram-positive bacteria, according to [18], [19].

4. Conclusions

The results show that the two medicinal plants studied have antibacterial activity against both Gram negative and Gram positive pathogenic bacteria, supporting their historic use as an alternative treatment. Several factors, such as the bacteria species and plant type, influenced this antibacterial action.

5. References

[1] da Silva, L. E., Confortin, C., & Swamy, M. K. (2021). Antibacterial and Antifungal Plant Metabolites from the Tropical Medicinal Plants. In Bioactive Natural Products for Pharmaceutical Applications (pp. 263-285). Springer, Cham.

[2] Bown. D. Encyclopaedia of Herbs and their Uses. Dorling Kindersley , London .1995 ISBN 0-7513-020-31.

[3] Boss A, Bishop KS, Marlow G, Barnett MP, Ferguson LR. Evidence to Support the Anti-Cancer Effect of Olive Leaf Extract and Future Directions. Nutrients. 2016 Aug;8(8):E513.

[4] Hassen I, Casabianca H, Hosni K. Biological activities of the natural antioxidant oleuropein: exceeding the expectation – A mini-review. J Funct Foods. 2015;18:926–40.

[5] L. Abdulazeem, S. Abdalkareem Jasim and W.J. Rajab, Anti-bacterial activity of gold nanoparticles against two type of antibiotic resistance pathogenic bacteria in Al-Hilla city, Materials Today: Proceedings, https://doi.org/10.1016/j.matpr.2021. 05.234.

[6] Bassiri-Jahromi S (2018) Punica granatum (Pomegranate) activity in health promotion and cancer prevention. Oncol Rev 12(1): 345. https://doi.org/10.4081/oncol.2018.345

[7] Servili M, Baldioli M, Selvaggini R, Macchioni A, Montedoro G. Phenolic compounds of olive fruit: one- and two-dimensional nuclear magnetic resonance characterization of Nüzhenide and its distribution in the constitutive parts of fruit. J Agric Food Chem. 1999 Jan; 47(1): 12–8.

[8] H. Wagner and G. Ulrich-Merzenich, “Synergy research: approaching a new generation of phytopharmaceuticals,” Phytomedicine, vol. 16, no. 2-3, pp. 97–110, 2009.

[9] Trabelsi, A., El Kaibi, M. A., Abbassi, A., Horchani, A., Chekir-Ghedira, L., & Ghedira, K. (2020). Phytochemical study and antibacterial and antibiotic modulation activity of Punica granatum (Pomegranate) leaves. Scientifica, 2020.

[10] Alamgir, A. N. M. (2017). Herbal drugs: their collection, preservation, and preparation; evaluation, quality control, and standardization of herbal drugs. In Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1 (pp. 453-495). Springer, Cham.

[11] Yusha’u, M., Hamza, M. M., & Abdullahi, N. (2021). Antibacterial activity of Adansonia digitata stem bark extracts on some clinical bacterial isolates. International Journal of Biomedical and Health Sciences,

6(3).

[12] ALI, Y. H., KHALAF, A. A., QANBAR, F. J., & ABDALKAREEM, S. (2020). Phylogenetic Tree of Some Nosocomial Bacteria Isolated from Ramadi Teaching Hospital. International Journal of Pharmaceutical Research, 12(4).

[13] Jasim, S. A., & Farhan, S. M. (2020). Variation of 16SrRNA gene for Some Nosocomial Bacteria Isolated from Ramadi Teaching Hospital for Women and Children. Indian Journal of Forensic Medicine & Toxicology, 14(4).

[14] Al-Sa'ady, A. T. (2020). Antibacterial screening for five local medicinal plants against nosocomial pathogens: Klebsiella pneumoniae and Staphylococcus epidermidis. Eurasian Journal of Biosciences, 14(1), 553-559.

[15] Salehi, B., Ata, A., V Anil Kumar, N., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., ... & Sharifi-Rad, J. (2019). Antidiabetic potential of medicinal plants and their active components. Biomolecules, 9(10), 551.

[16] Balouiri M, Sadiki M, Ibnsouda SK (2015) Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal 6(2): 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

[17] Kyung KH (2012) Antimicrobial properties of Allium species. Curr Opin Biotechnol 23(2): 142-147.

[18] Elisha IL, Francien SB, Lyndy JM, Jacobus NE (2017) The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts. BMC Complement Altern Med 17: 133. https://doi.org/10.1186/s12906-017-1645-z https://doi.org/10.1016/j.sjbs.2017.02.004

[19] Jasim, S. A., Feisal, H. T., & Abdulazeem, L. (2020). A Review: Antimicrobial Agent for Pseudomonas aeruginosa Isolated From Iraqi Patients. Systematic Reviews in Pharmacy, 11(12), 1117-1125.