

The potential effect of olive leaf extracts in combating antibiotic-resistant bacteria

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Abstract

Employing olive leaves as a plant-derived antimicrobials sources against the resistant bacteria to antibiotics is emphasized by research. The bacterial growth as well as formation of biofilms may be inhibited via olive leaf extracts (OLE), which have been raised in bioactive polyphenols such as hydroxytyrosol along with oleuropein.

To evaluate the antibacterial efficacy of OLE against some multidrug resistant bacteria (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *S. pyogenes*, *B. cereus*). Distilled water had been utilized to prepare *O. europaea* leaf extracts. Agar diffusion well method had been utilized to assess the microbial attributes of olive leaf aqueous extract against clinically significant bacteria. The extract of olive leaf (OLE) had been significant versus *Bacillus cereus* with inhibitory, 10.2 mm. Our results confirm OLE's encouraging antibacterial activity against pathogens which are resistant to multiple antibiotics. But prior such extracts are applied in medical settings, more research on accurate dose optimization as well as safety over time assessments is necessary.

Key words: Antimicrobial resistance, Bacteria, Olive leaf extract

I. Introduction

With approximately one million and hundred forty thousand fatalities were due to resistant of bacteria regarding drug in 2021, antimicrobial resistance (AMR) has emerged as a major global root of mortality. This number is expected to increase dramatically in the lack of successful preventative measures, likely leading to around two million direct deaths per year by 2050 (Sullivan and coworkers). As direct inhibitors or by enhancing the effects of conventional antibacterial medications for getting around resistance mechanisms, some phytochemicals derived from plants have demonstrated encouraging activity versus MDR (multidrug resistant) pathogens (Jubair and coworkers).

Olea europaea L. which is named as olive tree, a well-known for producing virgin olive oil, is a defining Mediterranean feature region. The fusiform, coriaceous, grayish-green leaves of the tree are 5cm to 6cm long and 1–1.5 cm wide. The characteristics of the olive are influenced by a number of factors, including tree age, cultivation, and pruning techniques. The fruit is a drupe made up of an endocarp, mesocarp, and epicarp. Nutritionists and cardiologists are interested in the health benefits of olives because of their unique chemical composition and sensory qualities, despite their unremarkable morphology (Granados-Principal et al., 2010; Borjan et al., 2020).



Oleuropein range is (1 to 14%) in olive leaves as well as (0.005-0.12 %) in olive oil (Vogel et al. and coworkers). Furthermore, contrast with olive fruit 110mg per 100g and 23mg per 100 ml of EVOO, phenolic compounds are 145mg per 100g of fresh leaf (Lockyer et al., 2017). Oleuropein usually contributes to various indole alkaloids synthesis by forming glycosidic bonds along with iridoids during terpenes secondary metabolism (Soler-Rivas et al., 2020). Oleuropein also has anti-clastogenic qualities, scavenges free radicals, as well as prevents tumors growth. Additionally, Oleuropein reduced blood glucose and cholesterol levels; improve lipid metabolism for obesity management as well as preventing low-density lipoprotein oxidation (Mohammadi et al., 2016). Flavonoids are remarkably prevalent as well as extensively spread class of olive leaves polyphenols (Borjan et al., 2020).

Olive trees have long been utilized extensively in medicine (Hashmi et al., 2015). OLE have several applications as a hypotensive (Ismail et al., 2021), antioxidant (Lins and coworkers), and hypoglycemic (Wainstein and coworkers). OLE polyphenols were linked to reduction in the growth of pancreatic cancer cells as well as the induction of apoptosis (Goldsmith et al., 2018). The European Medicines Agency (EMA) has actually authorized the utilization of OLE as an oral supplement (Clusa and coworkers). OLE is generally safe at low doses, according to multiple *in vitro* and *in vivo* investigations. Even at doses as raised as 1,000 mg/kg, oleuropein, the key phenolic component of olive leaves, showed no negative or minimal effects in animal models (Gonzalez-Pastor and coworkers).

OLE demonstrated *in vitro* anti-microbial action due to phenol derivatives, in particular hydroxy-tyrosol and oleuropeins (Borjan et al., 2020) against a variety of pathogens, including *C. albicans*, *E. coli*, dermatophytes, *H. pylori*, methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), as well as anaerobic periodontal pathogens (Sudjana et al., 2014; Karygianni et al., 2014; Zorić et al., 2016). It has been effectively used in the food industry to prevent foodborne pathogens (*Listeria monocytogenes*, *E. coli*, and *Salmonella enteritidis*) from forming biofilms (Liu et al., 2017), control *S. aureus* growth throughout Kasar cheese preservation (Ayana and Turhan, 2009), as well as conserve seafood (Ahmed et al., 2014).

II. Materials and Method

Olive leaves extract (OLE) preparation

After thoroughly cleaning the plant materials with running tap water to get rid of any superficial debris, they had been allowed to air dry for six days at room temperature in a well-ventilated, shaded area. The leaves had been crushed into a finely ground powder in a commercial blender after dryness in a dehydrator. After precisely weighing twenty grammes of powdered olive leaves, each one was submerged in 200 millilitres of distilled water. To guarantee reproducibility and avoid contamination, the extraction was carried under carefully monitored laboratory circumstances. To aid in the extraction of bioactive compounds, the mixtures had been left at room temperature for twenty-four hours with sporadic light stirring. Following maceration, the extracts were filtered through several layers of gauze and then 180 millilitres of filter paper to get rid of coarse particles. A rotary evaporator operating at 100°C was used to concentrate the resulting filtrates under reduced pressure. Soxhlet extraction was then used to further concentrate the distilled water extract until a sufficiently dense residue was obtained. At a solvent-to-powder ratio of 1:5, an approximate yield of 4 grammas was obtained from the original leaf powder. The extract's dried fine powder was reconstituted in DMSO to create a stock solution with a concentration of 100 mg/ml. Stock samples were kept at room temperature until needed.



Bacterial culture

The samples utilized in this investigation had been obtained from patients with firm infections. G⁺ as well as G⁻ bacteria had been taken from a variety of the clinical samples, such as stool, blood, urine, and swab cultures, from patients of various ages as well as genders. To guarantee adherence to ethical research standards, all isolates have been obtained with informed patient consent as well as approved by the committee on institutional ethics.

E. coli, *P. aeruginosa*, as well as *K. pneumoniae* had been between the G⁻ isolates, whereas *S. aureus*, *S. pyogenes*, along with *B. cereus* had been among the G⁺ isolates. To maintain viability and purity throughout the investigation, all bacterial isolates had been regularly kept on Mueller-Hinton agar (MHA) (Oxoid, Sigma) and subcultured onto new nutrient agar plates as needed.

Antimicrobial assay

OLE antibacterial features had been assessed through Agar well diffusion technique (Azoro, 2002). Twenty millilitres of (MHA) (Oxoid, Sigma) were added to the plates. The pathogenic strains were spread on the surface of MHA after being diluted with sterile water to a density of 10⁹ CFU/ml. These agar plates had been separated to 7 mm-diameter wells, and every well had been filled with dried extract that had been dissolved in DMSO at concentrations of 10, 15, 25, 30, and 50 mg/ml. After incubating culture plates for twenty four hours at 37°C, the inhibition zone diameter had been measured and reported in millimeters to ascertain the antimicrobial efficacy.

Results

The pathogenic bacteria under test were effectively inhibited by OLE. According to **Table 1**, the extract that worked best against *Bacillus cereus* had an inhibitory effect of 10.2 mm.

Table (1): Olive leaf aqueous extracts' antibacterial activity at various concentrations (inhibition zones in millimeters)

Extract mg/ml	<i>E-coli</i>	<i>P-aeruginosa</i>	<i>Klebsiella</i>	<i>S-aureus</i>	<i>S- pyogenes</i>	<i>B-cereus</i>
10	-	-	-	-	1.0	-
15	1.1	-	1.0	1.2	2.3	1.0
25	4.5	2.3	4.6	3.3	4.5	2.2
35	7	5	6.2	5.8	6.9	8.1
50	9.3	6.6	8.3	7.4	8.1	10.2

Discussion

Similar to other plant-derived biomolecules like green tea, oregano, and thyme, which usually work by causing oxidative stress or compromising the integrity of microbial membranes, olive leaf extracts have an antimicrobial mechanism against both Gram-positive and Gram-negative pathogens (Magyari-Pavel et al., 2024).

The current study findings revealed that OLE successfully inhibited tested pathogenic bacteria. The extract that worked best against *Bacillus cereus* had an inhibitory effect of 10.2 mm.

Extracts from olive (*O. europaea*) leaves have broad-spectrum antimicrobial qualities and had shown notable in vitro efficacy against pathogens frequently linked to respiratory and gastrointestinal tract infections (**de Oliveira et al., 2024**). The antimicrobial effectiveness of olive leaf extract is ascribed to the synergistic interaction between its phenolic constituents, including oleuropein and hydroxytyrosol, along with additional elements, like as fatty acids (**de Oliveira et al., 2024**). Olive leaf extract has strong antimicrobial properties; at sub-inhibitory concentrations, it inhibits MDR *Pseudomonas aeruginosa* strains' ability to form biofilms by 82% (**Esfandiary et al., 2024**). Additionally, olive leaf extracts possessed strong bactericidal efficacy against tetracycline and ciprofloxacin-resistant *Campylobacter* species (**Silvan et al., 2022**).

It was found that OLE possesses antibacterial activity in opposition to pathogens transmitted by food, such as *L. monocytogenes*, *Salmonella spp.*, *E. coli*, as well as *Staphylococcus aureus* (**Techathuvanan et al., 2014**). For instance, OLE has been used to lower bacterial levels in organic leafy greens and prawns (**Moore et al., 2011; Ahmed et al., 2014**). In a different investigation, **Korukluoglu et al., (2010)** examined how the solvent used for extraction affected the antibacterial activity of *S. aureus*, *E. coli*, *Salmonella Enteritidis*, and *Salmonella Typhimurium*. They also noted that ethanol olive extracts had the strongest antibacterial activity against *Salmonella Enteritidis* as well as *E. coli*.

A commercial OLE had been shown by **Sudjana et al., (2009)** to be efficient against *Staphylococcus aureus*, *Helicobacter pylori*, as well as *Campylobacter jejuni* at low MIC concentrations (0.31–0.78% v/v). With minimum bactericidal concentration (MBC) values ranging from 5.5 to 22.5 mg mL⁻¹, it was discovered that all cultivars of OLE were efficient against *S. aureus* and methicillin-resistant *S. aureus* (**Martín-García et al., 2022**).

Bacillus subtilis had been fewer vulnerable than *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *S. aureus*, according to another study (**Markin et al., 2003**). In seventy eight percent of the outbreaks, *S. aureus* as well as its enterotoxin A had been rendered inactive by the commercial olive powder as well as 4-hydroxytyrosol (**Friedman et al., 2011**).

Furthermore, a study by **Najee et al., (2018)** demonstrated that *Olea europaea* fatty oil inhibited the development of biofilm on drug-resistant bacterial and fungal pathogens by effectively interacting with microbial surface structures required for growth, such as membrane, in addition to suggested that it could decrease the pathogen's motility. According to a different study, olive leaf methanolic extract exhibited crucial antioxidant as well as antibiofilm properties (**Edziri et al., 2019**). Furthermore, it has been reported that olive leaf can be a cheap, sustainable, and abundant source of biophenols (**Martín-Vertedor et al., 2016**).

Conclusion: These results confirm OLE's encouraging antimicrobial activity against pathogens that are resistant to multiple drugs. However, before these extracts are used in clinical practice, more research on accurate dose optimization as well as safety over time assessments is necessary.

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