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A study of the Synergistic Effect of Olive Leaf Extract with some Antibiotics on Salmonella causing Food Poisoning

Aliaa Saadoon Al-Faraji[1], Adil Turki Al-Musawi[2], Sudad Jasim Mohammed[3]*

[1]Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq
Email: alia.sadon@yahoo.com ORCID: 0000-0003-4315-5611
[2] Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq
Email: adilalmusawi@mracpc.uobaghdad.edu.iq ORCID: 0000-0002-1309-4654
[3] Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq
Email: sudad@mracpc.uobaghdad.edu.iq ORCID: 0000-0002-1750-0576.
* Corresponding information's : Sudad Jasim Mohammed, Email: sudad@mracpc.uobaghdad.edu.iq

ABSTRACT

Olive oil contains up to 81% oleic acid, an omega-9 fatty acid and an unsaturated fat. Its benefits are numerous, including lowering LDL, cholesterol and raising HDL cholesterol, thus promoting cardiovascular health. The antioxidants in olive oil combat diseases and inflammation, including chronic conditions affecting the nervous and cardiovascular systems, as well as physical inflammations like arthritis and rheumatism. It is also a major source of vitamin E (an antioxidant that protects cells from damage) and vitamin K, helping to combat oxidative stress, which is linked to cancer and aging. This study was conducted to test the effectiveness of extracts from green olive leaves and their effect as antibacterial agents against Salmonella and E. coli bacteria, which are classified as common causes of some diseases such as food poisoning that humans and animals are exposed to. In addition, they are considered contaminants that cause spoilage of some foods. The synergistic effect of these leaves with antibiotics was also studied. The results of the study showed a variation in the inhibitory effect of olive leaf extracts against Salmonella and E. coli bacteria, and the Alcoholic extract was superior to the experimental bacteria that showed sensitivity to some antibiotics, while the synergistic effect was higher of (Gentamicin with Alcoholic extract of olive leaves) against Salmonella and E. coli bacteria are (25, 22) respectively.

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Olive leaf extracts; antibiotics; Salmonella and E. coli bacteria.

1. Introduction

The olive tree (*Olea europaea, L.*), family Oleaceae, is one of the oldest trees cultivated by humans and one of the medicinal plants that has had a significant impact on pharmacology and medicine for many years, this tree is characterized by its elongated, evergreen leaves and is native to the Mediterranean basin (Cavaca et al., 2020; Al-Maliki et al., 2025). Olives and olive oil are an important part of the daily diet for many people around the world (Albini et al., 2023; Al-Musawi et al., 2025a). Olive leaves are one of the byproducts of olive cultivation, accumulating during tree pruning at a rate of 25 kg of byproducts (twigs and leaves) per tree annually. These can be found in large quantities in the olive oil industry after being separated from the fruit before processing, representing about 10% of the weight of the olives (Klikarová et al., 2019). Besides being an inexpensive raw material available year-round in many countries, olives have been used to treat various ailments in traditional medicine along European and Mediterranean coasts, interest in them has increased in recent years due to their high concentrations of phenolic compounds, the quantity and quality of which vary in olive fruits and leaves depending on the ripening and growth process, studies have indicated that olive leaf polyphenols are biologically active compounds. (Al-Musawi et al., 2025b). Recent studies have highlighted the importance of olive leaf extracts and their numerous medicinal properties in treating malaria, hypertension, high cholesterol, and diabetes. Furthermore, its compounds are used in preventative medicine as food additives with antioxidant, antibacterial, and anti-osteoporotic properties, and to treat colds and some bacterial and fungal infections, they can also be used in food preservation and packaging as antibacterial, antifungal, and antiviral agents, particularly against viruses that cause hepatitis, Olive leaves are rich in the polyphenols tyrosol and oleuropein, with concentrations ranging from 60 to 90 mg/g dry weight.

These compounds are responsible for the leaves' potent antimicrobial properties, inhibiting the growth of a wide range of pathogenic microorganisms, including *Staphylococcus aureus*, *Salmonella enteritis*, *Bacillus cereus*, *Escherichia coli*, *Bacillus cereus T* spores, fungi, viruses, and protozoan parasites (Rouibah et al., 2022). However, it has no effect against yeasts. In some countries, olive leaf extracts are marketed in capsule form as dietary supplements and syrups, due to the increasing resistance of some microorganisms to antibiotics used to inhibit bacterial growth, as well as to treat and prevent diseases, this has been linked to the development of resistance to these antibiotics in those bacteria (Ahmed et al., 2014; Mohammed et al., 2022). In addition to the side effects of using such antibiotics, it became necessary to search for safer alternatives to protect humans from many harmful and life-threatening diseases, among these alternatives are plant extracts and natural products used in many fungal and bacterial diseases, as well as other medical applications, studies indicate that the antimicrobial properties of olive leaf extract are well-known in traditional Mediterranean medicine, however, there are few studies on the antimicrobial properties of olives themselves, although some evidence suggests that olives may have an antimicrobial effect, the antimicrobial activity of olives may be related to the polyphenols they contain, such as oleuropein (Liu et al., 2017). Currently, microbial resistance to antibiotics is greater than ever before, Therefore, numerous recent studies have been published on alternative solutions to mitigate their impact on human health.

1.1. Study Aim

Our study focused on the synergistic effect of olive leaf extract, a plant containing important phenolic compounds widely used in our country, in conjunction with certain antibiotics against *Salmonella* bacteria. This bacterium is particularly dangerous because some strains of *Salmonella* are resistant to many antimicrobials, a fact that should be monitored by diagnosticians and microbiologists, as they are responsible for controlling both disease and food safety.

2. Materials and Methods

2.1. Plant parts tested in the study

The green leaves of the olive tree (*Olea europaea, L.*) were collected from home gardens throughout Baghdad. The leaves were then divided into two parts after being washed several times with distilled water to remove any dirt or dust, first part was used to prepare the aqueous extract, following the method described by (Zari & Al-Attar ,2011; Selim et al., 2022). Ten grams of washed green olive leaves were placed between two layers of filter paper to absorb the washing water, they were placed in an electric blender with the addition of one liter of distilled water. They were blended for 15 minutes, and the extract was filtered through several layers of medical gauze to ensure the removal of the uncrushed parts, it was also filtered through filter paper to remove the plant fibers, ensuring a crude extract. The filtrate was placed in an opaque bottle until use, the second part was left, after washing, to dry in the shade, away from moisture, in a well-ventilated place, not exposed to sunlight, and without applying any heat treatment to reduce the loss of active ingredients in the green parts of the plant, the dried leaves were then crushed using an electric grinder to obtain powders. The powder was placed in clean, airtight containers, away from light and heat, and placed in the refrigerator at a temperature of 4°C until use in preparing the alcoholic extract. This was done by weighing 10 grams of ground dried leaves with 100 ml of water, ethanol, and petroleum ether through soaking, extract was filtered through a Buchner funnel using Whatman Grade 1 filter paper under vacuum, yielding the crude alcoholic extract. The resulting extract was cryo-dried under vacuum using a lyophilizer supplied by Edward-high vacuum (UK) at 0-50°C. The crude extract was stored at 4°C until use.

2.2. Bacterial isolates

The *Salmonella* spp. And *E.coli* bacteria were obtained from some ready-to-eat meals that cause food poisoning, their affiliation was confirmed through confirmatory tests and they were used as a microscopic test organism to detect the inhibitory activity of plant extracts of olive leaves (Mohammed et al., 2026).

2.3. Inhibitory effect of olive leaf extracts in vitro

The well diffusion method was used to study the effect of olive leaf extracts on *Salmonella* spp. bacteria. The surface of Muller-Hinton agar medium was inoculated with experimental bacterial colonies using a sterile swab of bacterial suspension containing 1.5×10^8 cells/ml at an optical density of 0.98. Wells with a diameter of 4 mm were made in the surface of the culture medium using a cork borer. The prepared concentrations of cold aqueous, hot, and alcoholic extracts of olive leaves (25% and 35%, respectively) were placed in 0.1 ml of each extract into each well, ensuring that no spillage occurred on the surface of the medium (around the wells). A control well containing sterile distilled water for the aqueous extract and another containing sterile 25% ethyl alcohol for the alcoholic extract were kept as a control. The plates were left at room temperature for half an hour 10°C, they were then incubated at 37°C for 24 ± 2 hours, and the

diameter of the inhibition zone around each well was measured. Two replicates were performed for each treatment, and the experiment was repeated three times. Positive control plates containing only the bacterial culture were prepared on the nutrient medium. (Mohammed et al., 2022; Fijan et al.,2024).

2.4. Inhibitory effect of antibiotics

The sensitivity of *Salmonella* spp. bacteria was studied according to the Kirby, Bauer method mentioned in (Vandepitte et al., 1991; Chow et al., 2021) towards a group of commonly used antibiotics prepared by Bio-analyses, numbering seven, and whose details are listed in (**Table 1**). Note that the diameter of the inhibition zones was classified according to the clinical laboratory standards institute (CLSI) (CLSI, 2009). The surface of Muller-Hinton agar medium was inoculated with test bacterial colonies using a sterile swab of bacterial suspension containing 1.5×10^{8} cells/ml. The antibiotic discs under study were fixed in the middle of the medium, and each disc was individually placed with three replicates. The plates were then left at room temperature for 30 minutes, and then the plates were incubated at a temperature of 37°C for 24+2 hours. The diameter of the inhibition zone around each antibiotic disc was estimated and compared with the calibration plate of the bacterial isolate.

Table (1): Antibiotic concentration and inhibition zone diameter (Mm) of bacteria according to CLSI

Antibiotics	(Concentration µg)	Resistant	Intermediate	Susceptible
Amoxicillin (AX)	**25**	≤13	14–17	≥18
Gentamicin (CN)	**10**	≤12	13–14	≥15
Neomycin (NE)	**30**	≤12	13–16	≥17
Chloramphenicol (CH)	**30**	≤12	13–17	≥18
Doxycycline (DO)	**30**	≤10	11–13	≥14
Ampicillin (AM)	**10**	≤13	14–16	≥17
Ciprofloxacin (CIP)	**5**	≤15	16–20	≥21

2.5. The synergistic effect between olive leaf extracts and antibiotics

The extracts were reconstituted in 1% dimethyl sulfoxide (DMSO), except for the aqueous extracts, which were reconstituted in distilled water to 200 mg/mL. The reconstituted extract solution was sterilized by filtration through a 0.45 µm membrane filter before use in the bioassay (**25, 50, 75, 100** µg/mL).

Statistical Analysis

The ANOVA method was used in the statistical analysis of the current study at probability levels of **(0.001, 0.01, and 0.05**) to evaluate the results of this study as well as to compare the results of the synergistic effect.

3. Results

3.1. The inhibitory effect of olive leaf extracts in vitro

Table (1) shows the results of the inhibitory activity of olive leaf extracts on *Salmonella* spp. Bacteria, it is noted that there is a variation in the inhibitory activity of these extracts on the test bacteria with increasing concentration of the extract, as the highest inhibition reached 6.9 mm at a concentration of 35% for the hot aqueous extract, while at the same concentration it reached an inhibitory power of 5.0 mm for the cold aqueous extract. The areas of inhibition of bacteria increased to (11.5 and 16.8) mm respectively for the alcoholic extract at concentrations of (25 and 35%) respectively.

Table (1): Results of the inhibitory activity (Mm) of olive tree leaf extracts on *Salmonella* spp. bacteria.

Treatment and concentration	Diameter rates of inhibition zones (mm)
Control	**Zero**
Cold aqueous extract 25%	**3.5**
Hot aqueous extract 25%	**4.0**
Cold aqueous extract 35%	**5.0**

Hot aqueous extract 35%	6.9
Alcoholic extract 25%	11.5
Alcoholic extract 35%	16.8

3.2. The inhibitory effect of antibiotics

Most isolates were sensitive to Ampicillin (75%), Amoxicillin (75%), and Gentamicin (75%). All isolates were susceptible to ciprofloxacin (100%), chloramphenicol (100%), and doxycycline (100%). Most isolates showed moderate sensitivity to neomycin (50%).

Table (3): Susceptibility of bacteria against some antibiotics

Antibiotics	*Salmonella*		*E. coli*	
	S%	R %	S%	R%
Amoxicillin (AX)	75	25	75	25
Gentamicin (CN)	75	25	75	25
Neomycin (NE)	50	50	50	50
Chloramphenicol (CH)	100	0	100	0
Doxycycline (DO)	100	0	100	0
Ampicillin (AM)	75	25	75	25
Ciprofloxacin (CIP)	100	0	100	0

(S): sensitive (R): Resistant

3.3. The synergistic effect between olive leaf extracts and antibiotics

The synergistic between olive leaf extracts and antibiotics against *salmonella* and *E.coli* , found highest inhibition zone was found in Gentamicin with Alcoholic extract 35% (25,22) ,while the lowest inhibition zone was found in Ampicillin with Alcoholic extract 35% (13,14) showed in (Table 4) .

Table (4): synergistic effect against bacteria on this study

Antibiotics	Olive leaf extracts	Inhibition zone (mm)	
		Salmonella	*E. coli*
Amoxicillin	Hot aqueous extract 35%	19	17
	Alcoholic extract 35%	20	21
Gentamicin	Hot aqueous extract 35%	16	18
	Alcoholic extract 35%	25	22
Neomycin	Hot aqueous extract 35%	19	17
	Alcoholic extract 35%	20	21
Ampicillin	Hot aqueous extract 35%	11	10
	Alcoholic extract 35%	13	14

4. Discussion

4.1. The inhibitory effect of olive leaf extracts in vitro

The superiority of hot water extraction over cold water extraction may be attributed to the release or extraction of the majority of the active compounds present in the plant leaves. Additionally, hot water inhibits enzymes present in plant extracts (Harborne, 1984). Olive

leaves are a rich natural source of bioactive compounds such as oleuropein, verbascoside, rutin, apigenin, kaempferol, luteolin, caffeic acid, tyrosol, and hydroxytyrosol, all of which exhibit antioxidant and antimicrobial properties. The antibacterial activity of olive leaf extract is attributed to its high phenolic content, this study was agreement with (Cavaca et al., 2020), found activity is likely due to the combined effects of polyphenol absorption on bacterial membranes, leading to membrane rupture and subsequent leakage of cellular contents, as well as the generation of hydroperoxides from polyphenols, the inhibitory effect of phenolic compounds can also be explained by their interaction with enzymes and substrates, and by the deprivation of metal ions.

The study was agreement with Oulahal & Degraeve ,(2022) ,evaluated the antimicrobial activities of some phenolic compounds in olive leaves against microorganisms and reported that the most effective compound was oleuropein. Their results showed a high oleuropein content (6.05 mg/kg) in the acetone extract.

The results indicate that the type of olive leaf cultivar had a significant effect on inhibiting *S. typhimurium* similar to that found by (Borges et al. 2020 ; Prevete et al. 2024).

This study was agreement with (El and Karakaya 2009; Papoti and Tsimidou 2009; Bilgin and Sahin 2013). They reported that the aqueous extract of olive leaves had no antibacterial effect against the tested microorganisms, while the acetone extract showed a significant inhibitory effect. They also noted that the type of solvent affected the phenolic distribution and concentration in the extracts, as well as their antimicrobial activity. It is known that the phenolic profile of olive leaves is influenced by several agricultural and technological factors, such as climate, farming practices, leaf age and degree of maturity, geographical origin, variety, excess yield on the tree, moisture content, degree of soil contamination, and extraction procedures .

4.2. The inhibitory effect of antibiotics

This study was agreement with (Ahmed et al., 2014) found olive leaf extract is known for its antiviral effects, primarily as a preventative and supportive agent. It may help boost the immune system, making the body more resistant to viral invasions. Some studies suggest that olive leaf extract may reduce viral load and symptoms of conditions like the common cold and flu. The extract's ability to interfere with viral replication may slow the progression of viral infections, giving the immune system more time to mount an effective response. While olive leaf extract is effective against both bacterial and viral infections, it should not be considered a substitute for prescribed medications. A good antibiotic is one that has high selectivity so that it affects the targeted pathogen without affecting the host.

Study was agreement with (Mohammed et al., 2023;Fijan et al., 2024) found many cases, selective toxicity is relative and not absolute, most important factors affecting this are concentration, selective toxicity depends on the presence of receptors or groups that interact with the antibiotic on the surfaces of pathogenic cells, next in importance is the ability of the antibiotic to prevent the occurrence of reactions or biochemical pathways in pathogens and not the host, most important outlines of the effect of antibiotics fall into one of the following areas: inhibiting the synthesis of cell walls of pathogens, interfering with the functions of cell membranes, inhibiting protein synthesis, and inhibiting the synthesis of nucleic acids,the leaves contain oleuropein, a compound that breaks down in the body to produce calcium oleate, which prevents the oxidation of bad cholesterol, thus preventing the accumulation of blood platelets inside the arteries and protecting against cardiovascular diseases.

4.3. The synergistic effect between olive leaf extracts and antibiotics

Study was agreement with (Salman et al., 2008; Muslims & Al-Zein, 2023) found many studies have confirmed that plant compounds have a synergistic effect with antibiotics, even against some antibiotic-resistant species, this activity is sometimes described as a modulatory or resistance-modifying effect, plant compounds change the rate of antibiotic efflux into the cell, as bacterial resistance to most antibiotics comes through the process of antibiotic efflux, a process that is genetically controlled, these efflux systems are proteins that are not specific to a particular type of drug and can clearly distinguish a wider spectrum of unwanted chemicals without changing or breaking down the compound. Meanwhile, recent studies have found that some plants produce substances that inhibit the action of pumps, in addition to the active compound, which facilitates the entry of the active substance into gram-positive and gram-negative bacteria, even with the presence of those defense systems, there is evidence of the possibility of using plant extracts to increase the sensitivity of bacteria to antibiotics, especially those that are resistant to them (Al-Musawi ,2026**)** found the antibiotic gentamicin is widely used and affects Gram-negative anaerobic bacilli and some Gram-positive bacteria. It belongs to the aminoglycoside group, and its effectiveness is attributed to the inhibition of protein synthesis in bacteria. However, it also has side effects with frequent use.

Declarations

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Authors' Contribution

SJM contributed to writing the research paper. ATM contributed to data analysis, ASA contributed to writing and revising the draft sources. All authors thoroughly discussed and approved the manuscript.

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Conflict of interest

There is no conflict of interest

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