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Research Article
Inhibitory Effect of Olive Leaf and Palm Pit Extracts Against Bacterial Species Related to Food Poisoning

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Abstract

Background and Objective: Food poisoning is a common cause of illness and death in developing countries. This study evaluates inhibitory, synergistic and cytotoxic effects and antioxidant properties of olive leaves and palm pit extracts. **Materials and Methods:** Olive leaf and palm pit extracts were tested for antibacterial activity against selected bacterial species (*Salmonella pullorum*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). The well-diffusion method was adopted to study the bacterial inhibition against different concentrations of both plant extracts (25, 50, 75 and 100 mg mL^{-1}). Antioxidant activity of plant extracts was evaluated using (DPPH) assay whereas (MTT) assay was conducted to assess their cytotoxicity in human lymphocytes. **Results:** The OLE ethanolic extracts exhibited the highest antibacterial activity at a concentration of 100 mg mL^{-1} against *E. coli*, whereas, the lowest activity was noted against *S. pullorum*. Contrarily, a moderate antibacterial activity of palm pit extract was noted against all tested bacteria and *S. pullorum* species exhibited resistance to the extracts. The OLE and palm pit extracts exhibited significant antioxidant activity by inhibiting 81.3 and 78.5% free radicals, respectively. **Conclusion:** The results demonstrated that OLE and palm pit extracts can serve as a good source of natural antioxidants. The cytotoxic effects of both extracts were evaluated using human lymphocyte cells. The results depicted significant cell growth inhibition at various concentrations (50, 100, 200 and 250 mg mL^{-1}) of the ethanolic extracts. The cell growth inhibition percentages against different concentrations of OLE remained as 14.1, 30.1, 56.2 and 63.2%, respectively whereas, cell growth inhibitions of 11.5, 20.2, 39.9 and 43.2% were noted against different concentrations of palm pit extract.

Key words: Antibacterial activity, plant extract food poisoning, olive leaf, palm pit, antioxidant, cytotoxicity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Foodborne illnesses area broad spectrum of diseases that are responsible for major global morbidity and mortality. During 2005, 2 million deaths were linked to gastrointestinal illness worldwide. Different pathogens such as bacteria, fungi and viruses and their toxins are associated with more than 250 types of foodborne illnesses[1]. Food poisoning-related diseases and deaths are particularly common in developing countries[2]. Chemical preservatives such as sulfur dioxide and nitrites are known to effectively prevent and control the outbreak of diseases associated with food poisoning. However, continuous applications of these chemicals over the years have led to the rise of certain problems such as the accumulation of residues in foods and food chains, microbial resistance and unpleasant side effects on human health[3]. Microbial resistance to existing antibiotics urges to search for effective and nontoxic antimicrobial drugs from natural materials[4]. The appearance of irresponsive bacterial strains to antibiotics has worsened the situation and is raising serious health concerns due to untreatable bacterial infections. Therefore, new natural antimicrobial agents are urgently required[5].

Antioxidants play a key role in maintaining good health. The antioxidant activity of plant materials is being increasingly investigated due to their higher potency and lower toxicity as compared to synthetic materials[6]. Many plants traditionally used in herbal medicines are potentially mutagenic, toxic and carcinogenic[7]. Isolation and characterization of plant bioactive compounds can facilitate the synthesis of more potent drugs with reduced toxicity. Olive leaves are a promising source of bioactive phytochemicals, which are obtained as biomass after the burning of olive trees[8]. Date palm plants are rich in minerals and bioactive compounds and possess beneficial antibacterial and antioxidant properties for human health. The presence of higher amounts of phenolic compounds in palm pits might help to prevent human diseases such as diabetes and cancer[9].

This study was aimed to estimate antimicrobial, antioxidant and cytotoxic activities of olive leaf and palm pit extracts against selected bacterial isolates associated with food poisoning.

MATERIALS AND METHODS

Time and location: Olive (*Olea europaea*) leaves and palm (*Phoenix dactylifera*) pits used in this study were carried out at Tikrit University in, College of Agriculture in August, 2017.

Preparation of plant extracts: Plant parts were washed with distilled water, oven-dried at 50°C and ground in a blender to achieve a fine powder. About 100% absolute methanol, ethanol and acetone were used as organic solvents for the extraction. Soxhlet extraction was carried out by using 25 g powder of olive leaves or date palm pits. The samples were placed in a thimble-holder containing a filter paper inside the main chamber. About 200 mL of fresh condensed extraction solvent was gradually added from a distillation flask. As the solvent reached the overflow level, a siphon aspirated the solutes from the thimble-holder and unloaded it back to the distillation flask. The Soxhlet extraction was carried out at 40-80°C and took about 10-12 hrs for each sample. A rotary evaporator was used to remove the solvents and yield the extracted compounds. The remaining non-soluble portions of the plant samples in thimble were discarded[10].

Antibacterial activity assay: Four concentrations (25, 50, 75 and 100 mg mL^{-1}) were prepared from the crude extract of each extraction solvents (methanol, ethanol and acetone). The bacterial isolates included Gram-negative bacteria (*Salmonella pullorum*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram-positive bacterium (*Staphylococcus aureus*). Most of these bacteria have been implicated in food poisoning. Bacterial isolates were characterized and identified by the central health lab in Baghdad, Iraq. Nutrient cultures were prepared and bacterial inoculum was incubated at 37°C for 24 hrs[11]. Agar well-diffusion assay was performed to evaluate the efficiency of olive leaves and date palm pit extracts against the bacterial isolates. Agar well-diffusion medium was prepared by pouring Muller-Hinton agar on the Petri dishes and solidified. Bacterial inoculums were seeded into Muller-Hinton agar and poured on the surface of the solidified agar. The 100 µL of each plant extract concentration (25, 50, 75 and 100 mg mL^{-1}) was pipetted onto the holes. The combined efficacy of OLE and palm pit extracts was assessed at different ratios (1:1, 2:1 and 1:2). The presence of the inhibition zone was measured and recorded as antibacterial activity. All tests were carried out in triplicate.

Antioxidant activity assay: 1-1-Diphenyl-2-picrylhydrazyl (DPPH) assay was carried out to determine the antioxidant activity of olive leaf and date palm pit extracts. The DPPH (0.1 mM) solution was prepared in methanol. The 0.2 mL of plant extract was mixed in 2.8 mL of DPPH in the test tube and placed in the dark for 30 min at room temperature. The DPPH absorbance in methanol was measured at 517 nm using a

spectrophotometer (SP 3000 Plus Optima)[12]. The following equation was used to calculate the antioxidant activity:

$$\text{Antioxidant activity (\%)} = \left(1 - \frac{\text{As}}{\text{Ac}}\right) \times 100$$

Where:
As = Absorbance of the sample with DPPH
Ac = Absorbance of DPPH

Ascorbic acid was used as a positive control to compare with the antioxidant activities of olive leaf and palm pit extracts. The experiments were conducted in triplicate.

Cytotoxicity assay: Human cells were cultured in Roswell Park Memorial Institute Medium (RPMI-1640) for the cytotoxicity assay. Complete Culture Medium (CCM) consisted of RPMI-1640 medium supplemented with 10%. A solution of 10% fetal calf serum containing streptomycin about 100 µg mL^{-1} and penicillin about 100 unit mL^{-1}, thin sterilized the medium using millipore filters (0.22 µm)[13]. Phosphate buffered saline (PBS) was prepared according to the manufacturer's instructions (Sigma), autoclaved and stored at 4°C.

MTT dye: Two mg of the 3-[4,5-dimethylthiazol]-2,5-diphenyltetrazolium bromide was dissolved in 1 mL of PBS (10 mM, pH = 7.2). The solution was sterilized using a millipore filter (0.22 µm) and stored at 4°C until used.

Cytotoxic effects of the extracts on human lymphocytes: Cytotoxic effects of the extracts (olive leaves and palm pits) on human lymphocytes were estimated at Al-Nahrain University Lab Research Center. Peripheral venous blood was used in all experiments. The blood was donated by a healthy 26 years old male donor. About 10 mL of blood was added to a 50 mL sterile test tube containing 500 µL of heparin. The blood was diluted by adding 10 mL of PBS solution and 20 mL of the diluted blood was carefully added into a test tube containing 20 mL of ficoll separation fluid. The test tubes were centrifuged at 400 rpm and 4°C for 30 min. The upper plasma layer was discarded. The lymphocytes layer was carefully removed using a 3 mL sterile Pasteur pipette and transferred to a sterile test tube.

These cells were washed by adding 2 mL RBCs lysis buffer and centrifuged at 400 rpm and 4°C for 10 min to remove the RBCs and other debris. Then, the lymphocyte pellets were washed by adding 1 mL RPMI-1640 and centrifuged at 400 rpm and 4°C for 10 min. The washing step was repeated three times and the supernatant was discarded. Finally, the isolated lymphocyte cells were again collected and suspended in the CCM medium. The suspended cells were transferred into the microtiter plate and incubated for 24 hrs at 37°C in a CO_2 (5%) incubator. The viability and the number of lymphocytes were determined using the trypan blue hemocytometer method[14].

Assessment of cell count and viability: The cell count and viability were determined according to Freshney[15]. About 10 µL of both Trypan blue stains and lymphocyte cell suspension were mixed for 30 sec and then 10 µL of the mixture was gently applied into the edge of the grooves on two sides of the hemocytometer chamber. Cells were counted on the top and left sides touching the middle line of the perimeter of each square. Cell concentration (cell mL^{-1}), total cell count and viable cell count (%) was calculated as follows:

$$\text{Cell viability (\%)} = \frac{\text{Total viable cells (unstained)}}{\text{Total cells (stained+unstained)}} \times 100$$

Cell/mL = Average count per square × dilution factor × 10^4
Total cells = Cells per mL × Original volume of the fluid from where the cell sample was removed

Cytotoxicity assessment on lymphocytes: The suspension of cultured human lymphocytes was adjusted to a cell count of 1×10^4 cells mL^{-1}. About 100 µL of the cell suspension was dispensed into each well of 96 well-microtiter plates to achieve a final cell count of 1000 cells/well. Then, the plates were incubated at 37°C for 24 hrs in an incubator supplemented with CO_2 (5%). After incubation, 100 µL of each concentration (50, 100, 200 and 250 mg mL^{-1}) of both extracts were transferred to separate wells of the microtiter plate. The lymphocytes were exposed to the extracts for 24 hrs and lymphocytes without any treatment served as the negative control. Three replicates of each treatment were carried out. About 50 µL of MTT dye (2 mg mL^{-1}) was added to each well and incubated for a further 4 hrs. The MTT-formazan crystals, formed only by live cells, were dissolved by adding 100 µL DMSO to all the wells. The optical density of each well was measured using an ELISA reader at a transmitting wavelength of 620 nm[15,16]. The rate of cell growth inhibition was measured according to Wang *et al.*[17] as follows:

$$\text{Cell growth inhibition (\%)} = \frac{\text{OD of concentration-OD of sample}}{\text{OD of control}} \times 100$$

Statistical analysis: Three replicates were carried out for each experiment and the results were expressed as average±SD (standard deviation). Statistical Package for Social Sciences (SPSS) software version 18.0 was used for the data analysis. One-way ANOVA with Tukey *post hoc* and paired t-test were used to compare the means of tested groups at $p \leq 0.05$.

RESULTS

Antibacterial activity of the extracts: The extraction of olive leaves and palm pits were carried out with three solvents including methanol, ethanol and acetone. Antibacterial activities of different concentrations (25, 50, 75 and 100 mg mL^{-1}) of the extracts were estimated against selected bacterial species by measuring the diameter (mm) of inhibition zones. Analysis of Variance (ANOVA) was carried out to analyze the data.

The results of methanolic and ethanolic olive leaf extracts against *E. coli* showed significant differences among the three concentrations of 25, 50 and 100 mg mL^{-1} but the results at the concentrations of 50 and 75 mg mL^{-1} and 75 and 100 mg mL^{-1} were not significantly different as shown in Table 1. The acetonic extract also exhibited significant differences among the three concentrations of 25, 50 and 75 mg mL^{-1} but no significant differences were observed between 75 and 100 mg mL^{-1}. The ethanolic extract showed the highest bacterial inhibition zones followed by methanol and acetone. The results of palm pit extract against *E. coli* showed significant differences among the concentrations of 25, 50 and 100 mg mL^{-1} of methanolic extract but there were no significant differences between the two concentrations of 50, 75 mg mL^{-1}. Ethanolic extracts depicted significant differences among all the concentrations (25, 50, 75 and 100 mg mL^{-1}). The acetonic extract showed significant differences among the concentrations of 25, 50 and 75 mg mL^{-1} but no significant differences were noted between 75 and 100 mg mL^{-1}. The ethanolic extract exhibited the highest bacterial inhibition zones against *E. coli* followed by methanol and acetone.

The results of olive leaf and palm pit extracts against *S. pollurom* revealed that there were no inhibition zones at the concentrations of 25, 50 and 75 mg mL^{-1} of all the solvents (methanol, ethanol and acetone) as shown in Table 2. Small inhibition zones were noted at 100 mg mL^{-1} that was significantly different from other concentrations (25, 50 and 75 mg mL^{-1}).

The results of olive leaf extract activity against *P. aeruginosa* revealed the absence of inhibition zones at 25, 50 and 75 mg mL^{-1} concentrations of methanolic and acetonic extracts (Table 3). An inhibition zone was only observed at the concentration of 100 mg mL^{-1}. The ethanolic extract did not show significant differences among the concentrations of 75 and 100 mg mL^{-1}. The results of palm pit extract activity against *P. aeruginosa* presented significant differences among the concentrations of 25 and 50 mg mL^{-1} and 75 and 100 mg mL^{-1}, respectively. The difference was noted to be significantly higher in the ethanolic extract concentrations of 75 and 100 mg mL^{-1} as compared to other extracts.

The results of olive leaf extract activity against *K. pneumoniae* in Table 4 depicted the absence of inhibition zones at the concentration of 25 mg mL^{-1} whereas, the results at 50 and 75 mg mL^{-1} were not significantly different. The diameter of the inhibition zone at 100 mg mL^{-1} was significantly different from other concentrations except 75 and 100 mg mL^{-1} of methanolic extract. Inhibition zones were not observed on *K. pneumoniae* against three concentrations (25, 50 and 75 mg mL^{-1}) of palm pit extract. A smaller inhibition zone was observed at 100 mg mL^{-1} that was significantly different from other concentrations.

The results of olive leave extract activity against *S. aureus* showed significant differences among concentrations in all solvents used in extraction. Palm pits extract showed presented significant differences among concentrations except for concentrations (50 and 75) there was no significant difference between them (Table 5).

Table 6 presents significant differences among the combined ratios of olive leaves and palm pit extracts against different bacteria. *Escherichia coli* was found to be the most susceptible against combined treatments followed by *S. aureus*, *P. aeruginosa* and *K. pneumoniae* whereas, *S. pullorum* appeared as the least susceptible bacteria to the combined treatments. The ratio of 1:1 exhibited better antibacterial activity followed by the ratios of 2:1 and 1:2. There is no previous literature about the combined effects of olive leaf and palm pit extracts on microbial species.

Antioxidant activity: The DPPH assay demonstrated marked antioxidant activity in the radical scavenging at a concentration of 2 mg mL^{-1} as illustrated in Table 7.

Cytotoxicity of plants extracts: The MTT assay was performed to assess the cytotoxicity of olive leaf and palm pit extracts against human lymphocyte cells. The effects of plant extracts on cell inhibition are illustrated in Table 8 and 9.

Table 1: Antibacterial activity of different concentrations of the extracts against *E. coli*

		Concentration (mg mL^{-1})			
Plant parts	Extraction solvent	25	50	75	100
Olive leaves	Methanol	9±2^{c}	12±1.73^{b}	13.5±0.76^{ab}	14±1.73^{a}
	Ethanol	19±3.46^{c}	26±2^{b}	27±1^{ab}	28±0.5^{a}
	Acetone	NA	10±2.64^{b}	12±1.5^{a}	13±1^{a}
Palm pits	Methanol	8±1.73^{c}	12±2^{b}	12±0.5^{b}	14±2^{a}
	Ethanol	8±1.5^{c}	9±1^{bc}	10± 1.73^{b}	15±1^{a}
	Acetone	NA	8±1.5^{b}	10±2^{a}	11±1.5^{a}

Means with similar letters in a row are not significantly different, values represent the diameter of inhibition zones (mm) and NA: No activity

Table 2: Antibacterial activity of different concentrations of the extracts against *S. pullorum*

		Concentration (mg mL^{-1})			
Plant parts	Extraction solvent	25	50	75	100
Olive leaves	Methanol	NA	NA	NA	7±1.5^{a}
	Ethanol	NA	NA	NA	8±2^{a}
	Acetone	NA	NA	NA	6±0^{a}
Palm seed	Methanol	NA	NA	NA	7±1^{a}
	Ethanol	NA	NA	NA	7±1^{a}
	Acetone	NA	NA	NA	6±0^{a}

Means with similar letters in a row are not significantly different, values represent the diameter of inhibition zones (mm) on *S. pullorum* and NA: No activity

Table 3: Antibacterial activity of different concentrations of the extracts against *P. aeruginosa*

		Concentration (mg mL^{-1})			
Plant parts	Extraction solvent	25	50	75	100
Olive leaves	Methanol	NA	NA	NA	11±2^{a}
	Ethanol	NA	NA	11±0.5^{a}	12±2.64^{a}
	Acetone	NA	NA	NA	11±1.73^{a}
Palm seed	Methanol	NA	NA	10±1.5^{a}	10±0.5^{a}
	Ethanol	NA	NA	10±2^{b}	14±1^{a}
	Acetone	NA	NA	8±1.5^{a}	9±2^{a}

Means with similar letters in a row are not significantly different, values represent the diameter of inhibition zones (mm) on *P. aeruginosa* and NA: No activity

Table 4: Antibacterial activity of different concentrations of the extracts against *K. pneumoniae*

		Concentration (mg mL^{-1})			
Plant parts	Extraction solvent	25	50	75	100
Olive leaves	Methanol	NA	8±2^{b}	10±2^{a}	11±2.64^{a}
	Ethanol	NA	9±2.5^{b}	10±0.5^{b}	13±2^{a}
	Acetone	NA	8±1.73^{b}	8±1^{b}	11±0.5^{a}
Palm seed	Methanol	NA	NA	NA	7±1^{a}
	Ethanol	NA	NA	NA	8±1.5^{a}
	Acetone	NA	NA	NA	6±0^{a}

Means with similar letters in a row are not significantly different, values represent the diameter of inhibition zones (mm) on *K. pneumoniae* and NA: No activity

Table 5: Antibacterial activity of different concentrations of the extracts against *S. aureus*

		Concentration (mg mL^{-1})			
Plant parts	Extraction solvent	25	50	75	100
Olive leaves	Methanol	8±1^{c}	13± 1^{a}	11±1.73^{b}	12±1^{ab}
	Ethanol	9±2^{c}	10±2^{c}	12±2.17^{b}	13±1^{a}
	Acetone	NA	6±0^{c}	8±2^{b}	13±1.5^{a}
Palm seed	Methanol	7±1^{c}	10±2.5^{b}	11±2.64^{b}	13±1.73^{a}
	Ethanol	9±1.5^{c}	11±0.5^{b}	11±1^{b}	14±2^{a}
	Acetone	NA	6±0^{b}	7±0^{b}	11±2^{a}

Means with similar letters in a row are not significantly different, values represent the diameter of inhibition zones (mm) on *S. aureus* and NA: No activity

Table 6: Antibacterial activity at different combined ratios of plant extracts

	Extract Extraction ratio	Concentration (mg mL^{-1})			
Bacteria	Olive: Palm	25	50	75	100
E. coli	1:1	7±1.32^{ab}	11±2^{a}	15±1.73^{a}	15±0.25^{b}
	2:1	8±2^{a}	10±2.64^{ab}	14±2^{a}	17±1^{a}
	1:2	6±0^{b}	9±1^{b}	12±0.75^{b}	12±1.5^{c}
S. aureus	1:1	8±0.5^{a}	10±2.5^{a}	10±2.59^{b}	13±0.5^{a}
	2:1	7±1^{a}	8±1.5^{b}	12±1.52^{a}	12±1.73^{ab}
	1:2	NA	NA	9±2.5^{b}	11±2^{b}
S. pullorum	1:1	NA	NA	NA	12±2.59^{a}
	2:1	NA	NA	NA	11±1^{a}
	1:2	NA	NA	NA	11±0.75^{a}
P. aeruginosa	1:1	NA	NA	9±2.64^{a}	12±2.17^{a}
	2:1	NA	NA	7±1^{b}	11±0.5^{a}
	1:2	NA	NA	9±2^{a}	11±2^{a}
K. pneumoniae	1:1	NA	NA	8±0.28^{b}	10±1.73^{ab}
	2:1	NA	NA	11±1.73^{a}	11±0^{a}
	1:2	NA	NA	9±0.5^{b}	9±2^{b}

Means with similar letters in a column are not significantly different, values represent the diameter of inhibition zones (mm) and NA: No activity

Table 7: Scavenging of free radicals by olive leaf and palm pit extracts and ascorbic acid

Sample	DPPH radical scavenging (%)
Olive leaves	81.3^{a}
Palm pits	78.5^{b}
Ascorbic acid (positive control)	91.7

Values are the mean of three independent experiments, means with similar letters in a column are not significantly different

Table 8: Lymphocytes growth inhibition (%) after treatment with olive leaf extract

Concentration (mg mL^{-1})	Cells growth inhibitions (%)
50	14.01
100	30.11
200	56.09
250	63.22

Values represent the cell growth inhibition (%) after treatment with different concentrations of olive leaf extract

Table 9: Lymphocytes growth inhibition (%) after treatment with palm pit extract

Concentration (mg mL^{-1})	Cells growth inhibitions (%)
50	11.54
100	20.21
200	39.90
250	43.15

*Values represent the cell growth inhibition (%) after treatment with different concentrations of palm pits extract

DISCUSSION

Significant differences were observed among the activities at all the concentrations of olive leaf extracts in different solvents (methanol, ethanol and acetone) against *S. aureus*. Palm pit extracts also exhibited significantly different activities among various concentrations except 50 and 75 mg mL^{-1}. Indu *et al.*[18] studied the microbial inhibition efficiency according to the inhibition zones. Inhibition diameter of less than 12 mm represents slower antibacterial activity and the diameters between 12-16 mm exhibit moderate activities whereas, compounds with the inhibition zone diameter of 16 mm are considered highly active. According to these parameters, the olive leaf extracts showed higher inhibition efficiency against *E. coli*, moderate inhibition activity against *S. aureus*, *S. aeruginosa* and *K. pneumonia* whereas, lower inhibition activity was noted against *S. pullorum*. Palm pit extracts showed moderate inhibition efficiency against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae* and low inhibition activity against *S. pullorum*.

Gokmen *et al.*[19] adopted the disk diffusion method to reveal higher antibacterial properties of olive leaf extract against *S. aureus*, *E. coli* and *P. aeruginosa*. Liu *et al.*[20] demonstrated that olive leaf extract almost completely inhibited the growth of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella enteritidis* pathogens at a concentration of 62.5 mg mL^{-1}. Pereira *et al.*[21] explained the antimicrobial mechanism of olive leaf extract as the denaturation of the protein that further affects the cell membrane permeability. Some researchers have reported various pharmacological properties of oleuropein including antioxidant, antimicrobial, anti-inflammatory, antiatherogenic, anticarcinogenic and antiviral activities[22-25].

Phytochemical screening of palm seeds crude extract has revealed the presence of alkaloids and carbohydrates in palm pit extract. Secondary metabolites such as steroids, flavonoids, tannins and saponins have also been reported in palm pit extract. The tannins are particularly known for their astringent and antimicrobial property[26]. The phenolic profile of palm pits has revealed the presence of cinnamic acid, flavonoids, glycosides, flavonols, four free phenolic acids and nine bound phenolic acids[9]. Polyphenols exert antibacterial properties by

participating in the protein precipitation and enzyme inhibition of the microorganisms. Therefore, the antibacterial properties of palm pits extract might be based on the activity of phenolic compounds[27].

Saddiq and Bawazir[28] reported the antimicrobial activity of aqueous extract of date palm pits against Gram-negative bacteria (*K. pneumonia* and *E. coli*). Farah[29] studied palm pit extract against some bacterial species and demonstrated high antibacterial activity against *S. aureus*. Al-daihan[30] also revealed the antibacterial activity of palm pit extract against *S. aureus* with an inhibition zone diameter of 11.6 mm. Contrarily, Yassein[31] did not observe inhibition zones against *K. pneumoniae* at 100 and 200 mg mL^{-1} concentrations of palm pit extract whereas, Maged and Abbas[32] found moderate inhibition zones against *Salmonella* sp., *P. aeruginosa*, *E. coli*, *S. aureus* and *K. pneumoniae*.

Phytochemicals may inhibit microbial growth through different mechanisms such as interference with microbial metabolic processes, cellular membrane perturbations, gene expression pathways, or modulation of signal transduction[33]. The antimicrobial properties of phenolic compounds might depend on their ability to change microbial cell permeability that leads to the loss of macromolecules including Na glutamate and ribose. They could also interfere with nutrient uptake, protein structure, enzyme activity, nucleic acid synthesis and electron uptake of the membrane[34].

The results demonstrated significant free radical scavenging efficiency of OLE (81.3%), palm pits (78.5%) and ascorbic acid (91.7%) against DPPH at a concentration of 2 mg mL^{-1}. The OLE presented significantly higher antioxidant activity than palm pit extract. These results are in line with the findings of most of the previous studies where the antioxidant activity of plant extracts against DPPH free radicals has been reported. The DPPH radical scavenging assay was carried out to evaluate the antioxidant activity of OLE according to Enujiugha *et al.*[35]. The data showed that the radical scavenging activity of OLE increased in a concentration-dependent manner and the extract concentration of 0.6 mg mL^{-1} caused 50% inhibition of the free radicals (IC_{50}). Hayes *et al.*[36] reported that 0.035 mg mL^{-1} concentration of OLE inhibited 50% DPPH radicals. Al-Farsi and Lee[37]. demonstrated that the antioxidant activity of palm pits is due to the presence of various phenolic compounds such as procyanidins p-coumaric, flavonoids and sinapic and ferulic acids. Benavente-Garcia *et al.*[38] proposed that the polyphenol synergism might provide better activity against radicals as compared to individual phenolic compounds. Adeosun *et al.*[39] studied palm pit extract to assess the antioxidant activity and noted the IC_{50} value as 10.21 mg mL^{-1}.

Kiritsakis *et al.*[40] investigated the antioxidant activity of Olive Greek cultivars *Koroneiki*, *megaritiki* and *Kalamon*. The results of the cultivars were significantly different from each other and all showed a positive correlation between antioxidant activity of extracts and total phenol content. Abd El-Rahman and Al-Mulhem[41] reported the antioxidant activity of palm fruit, pits and shell against DPPH radicals, which exhibited 91.87, 81.85 and 63.77% inhibition, respectively.

The results revealed that the cytotoxicity of olive leaf and palm pit extracts were directly related to their concentrations. OLE treatment at different concentrations 50, 100, 200 and 250 mg mL^{-1} inhibited the growth of human lymphocytes by 14.01, 30.11, 56.09 and 63.22%, respectively whereas, the palm pit extract inhibited the cell growth by 11.54, 20.21, 39.90 and 43.15% at above-mentioned concentrations. The toxicity of extracts was found to be higher at higher concentrations. The production of toxic material by medicinal plants for their defence against insects, infections and herbivores has been reported[42]. Several studies have elaborated that herbal or traditional medicines can be potentially toxic, carcinogenic and mutagenic[7]. Such toxicity could lead to the alteration in cell membrane permeability and apoptosis. The loss of cell membrane integrity is a typical phenotypic characteristic of cytotoxicity[43]. A study has shown that OLE inhibited the cell proliferation of human breast adenocarcinoma (MCF-7), Bovine Brain Capillary Endothelial (BBCE) and human urinary bladder carcinoma (T24)[44]. Han *et al.*[45] reported based on the MTT assay that 200 μg mL^{-1} of Oleuropein or 50 μg mL^{-1} of hydroxytyrosol reduced the cell viability of MCF-7 cells. Oleuropein or hydroxyl tyrosol decreased the number of MCF-7 cells by inhibiting cell proliferation and inducing cell apoptosis. The effectiveness of date pit (100 μL mL^{-1}) against human colon cancer cells (53.65% viability) *in vitro* has been reported. However, its Anti-carcinogenic effect against human hepatocellular carcinoma was comparatively lower (79.95% viability)[46]. Samet *et al.*[47] demonstrated the anti-leukaemia effects of OLE on the human chronic myeloid leukaemia cells for the first time. The OLE has also been reported to inhibit the proliferation of K562 cells by inducing cell cycle arrest. OLE significantly inhibited the growth of human lymphocytes.

CONCLUSION

The OLE presented higher antibacterial activity against *E. coli* and moderate activity against *K. pneumoniae*, *S. aureus* and *P. aeruginosa* whereas, lower activity was observed against *S. pullorum*. Palm pit extract exhibited moderate antibacterial activity against *E. coli*, *S. aureus*, *K. pneumoniae*

and *P. aeruginosa* and lower activity against *S. pullorum*. The combined treatments of both extracts at different ratios were found to be less effective against bacteria as compared to individual treatments. The DPPH assay revealed that the antioxidant activity of olive leaf extract was higher than palm pits. The OLE and palm pit extracts also significantly inhibited the growth of human lymphocytes but the cell growth inhibition activity of olive leaf extract was comparatively higher than palm pit extract.

SIGNIFICANCE STATEMENT

This study discovers the effect of olive leaves and palm pit extracts against some bacterial species and the effect on human cells. In our society with less knowledge and awareness of the side effect of accumulation and overused plant extracts the study shall help consumers to aware of the benefits and harmful sides. The study will help the researcher to uncover the critical area that many researchers were not able to explore especially the toxic side of extracts. Thus, a new theory on the chemical constituents and their benefits and harm sides arrived at.

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