Zagazig J. Agric. Res., Vol. 48 No. (1) 2021 161-173

Food, Dairy and Home Economic Research

Available online at http://zjar.journals.ekb.eg

http:/www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master

# ANTIOXIDANT, ANTIMICROBIAL AND ANTI-INFLAMMATORY POTENTIAL OF OLIVE LEAVES

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**Received: 24/11/2020 ; Accepted: 06/12/2020**

**ABSTRACT:** Olive tree is one of the most important fruit trees because of its content of many potentially bioactive compounds and represent waste products from olive harvest and pruning of olive trees. However, the content of these compounds can differ between cultivars. The aim of the current study was to evaluate the antioxidant, antimicrobial and anti-inflammatory properties of three olive leaves cultivars (Picual, Shemlali and Tofahy) commonly cultivated in Egypt. Furthermore, determine their cytotoxicity for safety use in food applications. Three olive leaves cultivars were extracted with water and yield of extract was determined in each cultivar, the antioxidant activities, anti-inflammatory, cytotoxic and antimicrobial activity of 7 pathogenic bacteria, 1 yeast and 2 fungi species were determined. Of all the extracts tested, Picual had the highest content of each of phenolic (TPC) and flavonoid (114.79±4.19 µg GAE/mg,118.69±2.07 µg CE/g extract, respectively) and exhibited strong antioxidant potential in scavenging DPPH. Antibacterial potential method was against *Escherichia coli* ATCC25922, *Escherichia* coli BA12296, *Klebsiella pneumonia* ATCC12296, *Salmonella* spp., *Staphylococcus aureus* EMCC1351, *Streptococcus mutans* EMCC1815, *Bacillus cereus* EMCC1006, *Clostridium* perfringensEMCC1574. Antifungal activities of: *Aspergillus niger* EMCC72, *Aspergillus parasiticus* EMCC886T and *Candida albicans* EMCC15, were evaluated and the minimum inhibitory concentration (MIC) of all cultivars values for bacteria and yeast ranged from 12.5 to 100 mg/ml. The extracts from Picual, Tofahy and Shemlali of 5µg/ml had the highest anti-inflammatory effect. Cytotoxicity results indicated high safety use of the three olive leaves cultivars. The results confirmed convenient and safe use of extracts of three olive leaves cultivars as natural antimicrobial and antioxidant.

**Keywords:** Olives leave cultivar; Antioxidant; Antimicrobial; Anti-inflammatory; Toxicity potential.

## INTRODUCTION

Olive trees *(Olea europaea*) are widely distributed in Mediterranean basin states (**Benjeddou *et al*., 2019**). Olive oil industry and olive groves produce huge amounts of by-products and leaves constitute the most of these by-products. They contain simple phenols, secoiridoids and flavonoids, including them, oleuropein. Several previous studies showed interesting biological and pharmacological properties of oleuropein, in large part attributed to its putative antioxidant and anti-inflammatory activity (**Cavaca *et al*., 2020**). Production of value-added, innovative, and low-cost products based on the olive leaves by-products considered a good alternative way for developing a sustainable food production chain (**Tarchoune *et al*., 2019**). The Extracts of these

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by-products may contain different bioactive compounds with activity against bacterial or fungal pathogens (**Tiwari *et al*., 2011**). Several studies have revealed the strong antioxidant and antimicrobial activity of olive leaves extract due to its high content of different phenolic compounds (**Firat *et al*., 2010**) particularly Oleuropein which exhibited an antimicrobial activity against viruses, bacteria, yeasts, fungi, molds (**Markin *et al*., 2003**). Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as damaged cells or irritants or pathogens (**Ferrero-Miliani *et al*., 2007**). Inflammation has become the focus of global scientific research on account of its implication in virtually all human and animal diseases. The common drugs which might be used to relieve this phenomenon are either expensive or toxic and not generally available to the rural communities who constitute the major populace of the world (**Li *et al*., 2003**). The chemical composition of olive leaves differs according to several factors such as variety, climatic conditions, and tree age (**Niaounakis and Halvadakis, 2006**). The quantity and quality of bioactive compounds changes depending on the cultivar, maturation degree of the leaf, phenological stage, climate, and cultivation area (**Vita *et al*., 2018**). There are significant qualitative and quantitative changes in phenolic, flavonoids and antioxidant activity depending on the pretreatment of olive leaves before extraction (**Zeitoun *et al*., 2017**). So, the present study was focused on the determination of the phenolics and flavonoids present in olive leaves extracts of different cultivars to evaluate their antioxidant, antimicrobial and anti-inflammatory capacity as well as their cytotoxicity to determine the safety dose for food applications potential.

# MATERIALS AND METHODS

## Materials

## Plant Material and Sample Preparation

Leaves of olive trees were collected on January 2019 from 3 cultivars (Picual, Shemlali and Tofahy) from the experimental Farm, City of Scientific Research and Technological Applications, New Burg El-Arab, Alexandria, Egypt. The leaves of different cultivars were washed and packed in polyethene bags and then stored at -80˚C until used to keep bioactive compounds

## Optimization Extraction of Phenolic Compounds from Olive Leaves

### Blanching and drying of olive leaves

To optimize extraction of total phenolic, olive leaves samples were also blanched for 2 min. at 90°C then cooled down by cold water at 15°C. The leaves were dried in an air oven for 3 days at 45°C (**Stamatopoulos *et al*., 2014**).

### Extraction and preparation from blanched and dried olive leaves

The samples were boiled with distilled water (1:10 *W/V*) at 100°C for 10 min., then centrifuged at 3000 X g for 10 min. at 20°C and filtered. The extract was lyophilized (Vacuum freeze dryer model: FDF 0350; Korea) (**Vongsak *et al*., 2013**).

### Chemicals and reagents

Solvents, chemicals, and reagents were obtained from El-Gomhouria Company, Alexandria, Egypt, and Sigma-Aldrich (Steinheim, Germany).

## Methods

## Total Phenolic Content (TPC)

Total phenolic content of olive leaves extract was determined according to the method of **Arabshahi-Delouee and Urooj (2007)**.

### Determination of total flavonoid content (TFC)

Total flavonoid content was measured according to **Dewanto *et al*. (2002)**.

## Antioxidant Activity Assays

### Scavenging activity on DPPH radicals

Free radical scavenging activity was determined by a DPPH radical assay, performed according to the method described by **Cheung *et al*. (2003)**.

### Reducing power

The spectrophotometric method of **Ferreira *et al*. (2007)** was used for the measurement of reducing power.

**ABTS radical scavenging assay**

ABTS radical scavenging assay was determined according to the method of **Re *et al.* (1999)**.

**Antimicrobial activity**

The antimicrobial activity was performed by agar well diffusion essay (**Kadaikunnan *et al*., 2015**), for all sample extracts. Seven pathogenic bacterial strains, one yeast strain, and two fungal spp. known to be pathogenic including *Escherichia coli* ATCC25922, *Escherichia coli* BA12296, *Klebsiella pneumonia* ATCC12296, *Salmonella* spp., *Staphylococcus aureus* EMCC1351, *Streptococcus mutans* EMCC1815, *Bacillus cereus* EMCC1006, *Clostridium perfringens* EMCC1574, and *Candida albicans* EMCC105 and Fungi strains were; *Aspergillus niger* EMCC72, and *Aspergillus parasiticus* EMCC886T. All strains were obtained from Microbiological Resources Center (MERCIN) in Egypt; the strains were maintained and stored at -80°C. The bacterial strains were grown in nutrient broth at 37°C, whereas the yeast strain was grown in Sabouraud dextrose broth at 28°C for 24 hr. A set of 5 concentrations of reconstituted plant water extracts (100, 75, 50, 25 and 12.5 mg/ml), were examined to determine the minimum inhibitory concentration (MIC) of each against a specific pathogenic strain. One hundred µl of the inoculums ($1\times10^8$ cfu/ml) were inculcated on agar media and poured into the Petri plate. A well was the tested compound was applied into the well. All the tested strains were incubated at 37°C for 24hr. the zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (mm), including the well diameter. The readings were taken in three different fixed directions in all triplicates and the average values were tabulated.

## *In vitro* Anti-Inflammatory Assay

*In vitro* anti-inflammatory activity of the extracts was assessed by the human red blood corpuscles (HRBCs) membrane stabilizing method of **Anandarajagopal *et al*. (2013)**.

## Cytotoxicity Assay

Neutral red cytotoxicity assay was used to determine the $LD_{50}$ on Peripheral blood mononuclear cells (PBMCs) (**Repetto *et al*., 2008**). Neutral red solution was freshly prepared from a 0.4% aqueous stock solution shielded from light. A 1:80 dilution of the stock solution was prepared in Roswell Park Memorial Institute Medium (RPMI), allowed to precipitate for 24 hr., at room temperature, and then centrifuged 10 min at 1500Xg, and then the clear red solution was used for the assay. The cells were washed with PBS (150 µl/well) and the plate were tapped then washing solution was aspirated, neutral red medium (100 µl) were then added to each well and the plate was incubated for 2 hr., at 37˚C. The neutral red medium was aspirated after 2hr. then cells were washed with 150 µl PBS. The plate was tapped gently then washing solution was aspirated. Neutral red destain solution (1% acetic acid, 49% de-ionized water and 50% ethanol) was added as 150 µl/well and the plates were shakes rapidly on a micro-titer plate shaker (Shaker PSU 2T plus, BOECO, Germany) for at least 10 minutes. The optical density (ȮḊ) of the neutral red extract was measured at 540 nm in a micro-titer plate reader spectrophotometer (Spectrostar$^{Nano}$, BMG Labtech), using blanks which contain no cells as a reference. Grubb's test for outliers, also called the ESD method (extreme student zed deviate), to determine whether one of the values in the list was a significant outlier from the rest (**Ryan and Deci, 2017)**. The percentage of cytotoxicity (inhibition) was calculated according to the following Equation:

$$\text{Inhibition (\%)} = 100\left(\frac{\text{O.D Control}-\text{O.D Treatment}}{\text{O.D Control}}\right)$$

## Statistical Analyses

The data was statistically analyzed using SPPs version 16, USA. One-way analysis of variance with $p<0.05$ was performed to identify significant differences among all studies parameters by Duncan's test. All experiments were carried out in triplicate.

# RESULTS AND DISCUSSION

## Extraction Yield

The extraction yield is a measure of the ability of the solvent to extract specific compounds from the original material. The yield

of extraction of different cultivars was shown in Table 1. The yield of Picual was (17.00±2.19%), the yield of Tofahy was (16.50±1.88%) and Shemlali was (17.00±2.36%). No significant differences were noted among the three cultivars in the extraction yield ($p>0.05$). These results were in line with the results obtained by **Al-Attar and Shawush (2014)** where the extract yields mean of water extract of olive leaves was (18.70%). For confirming the suitability of the used method for obtaining an active compound without any adverse on their chemical structure or altering in their nature, the antioxidant and antimicrobial activities has been determined.

## Total Phenolic Content (TPC) and Flavonoids

Total phenolic content in the leaves extracts of the three cultivars was expressed as micrograms of gallic acid equivalents per g of extract (µg GAE/g extract). Table 1 show that, the highest phenolic content was obtained in Picual cultivar extract (114.79 ± 4.19 µg GAE/g) followed by Tofahy (63.88 ± 0.888 GAE/g and Shemlali 60.62±4.36 µg GAE/g extract ($p<0.05$). Total phenolic content of the three cultivars of olive leaves differ depending on the origin and variety of the plant material. These results agree with **Boudhrioua *et al.* (2009)** who reported that the amount of the total phenolics content was varied according to the olive variety. These values are in line with those reported by **Molina Alcaide and Nefzaoui, (1996)** for Picual espagnol olive leaves variety. Olive cultivar or genotype may influence the quantity of the extracted phenolic compounds of olive leaves extract **Rigacci and Stefani (2016)**. The extracts in the present study were found to have various levels of flavonoids. The content of flavonoids in different cultivars of the leaves extracts is significantly different (Table 1). Therefore, these levels vary considerably, from 31.28±1.78 to 118.69±2.07 µg CE/g in the extracts obtained from the olive leaves. Picual had the highest flavonoids content (118.69±2.07 µg CE/g extract) while Shemlali showed the lowest content (31.28±1.78 µg CE/g). Tofahy exhibited total flavonoids of (89.65±0.93 µg CE/g extract. These results agree with **Vagiri *et al.* (2013)** who reported that the quality and composition of different bioactive compounds may be influenced by many factors, such as climate, genotype, ripening and storage conditions, of which the effect of genotype was larger than that of location for the content of most of the bioactive compounds studied. Flavonoids content of olive leaves was ranged from 56.57±6.00 to 125.64±3.36 µg CE/g and there is a significant difference was found in the content of total flavonoids of leaves among the cultivars (**Salah *et al.*, 2012**). The obtained results in the current study emphasized that the olive leaves extract is a good source of phenolic compounds and flavonoids which may differs according to the cultivar, ripening stage, and cultivation conditions.

## Antioxidant Activity

### DPPH radical scavenging activity

DPPH radical scavenging assay is a widely used parameter for evaluating the antioxidant potential of natural compounds in a relatively short period (**Adegborioye *et al.*, 2018**). Results of DPPH radical scavenging activity presented in Table 2 as $IC_{50.}$ The obtained results showed that, ascorbic acid as standard presented the highest scavenging ability with the lowest $IC_{50}$ value (11.16±0.43 µg/ml), followed by Picual ($IC_{50}$=45.14±2.34 µg/ml), Tofahy ($IC_{50}$= 55.45 ± 1.99 µg/ml), and Shemlali ($IC_{50}$= 55.82 ± 2.89 µg/ml), respectively. There were significant differences in an antioxidant power among the three varieties of olive leaves extracts ($p<0.05$) (Table 2), at the same time all tested varieties showed a considerable antioxidant capacity. These findings are consistent with those reported by **Saija *et al.* (1998)** who emphasized that olive leaves extract may possess a strong DPPH radical-scavenging ability at relatively low concentrations. **Khlif *et al.* (2015)** revealed that the olive leaves extract showed a significant antioxidant capacity with $IC_{50}$ of 45.00 µg/ml which is remarkably close to the results in current study. Phenolic antioxidant activity depends not only on the concentration level of polyphenols but also on their kind (**Cyboran *et al.*, 2014**). The strong antioxidant activity of olive leaves could be attributed to their high total polyphenolic and flavonoids contents (**Bouaziz *et al.*, 2008; Salah *et al.*, 2012**). The antioxidant capacity of the olive leaves extract may encourage its utilization in formulating health-promoting foods and/or supplement other food products to improve their functionality.

**Table 1. Total phenolic content (TPC) and flavonoids (µg GAE/g extract) of olive leaves extract**

| Olive cultivar | Yield (%) | Total phenolic content (µg GAE/g extract) | Total flavonoids (µg CE/g extract) |
|---|---|---|---|
| **Picual** | 17.00 ± 2.19a | 114.79±4.19a | 118.69 ±2.07a |
| **Tofahy** | 16.50 ± 1.88a | 63.88±0.88b | 89.65 ±0.93b |
| **Shemlali** | 17.00± 2.36a | 60.62±4.36b | 31.28 ±1.78c |

Each reported value is the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$).

**Table 2. Antioxidant activity (DPPH, reducing power and ABTS radical scavenging assay) of three cultivars olive leaves extract.**

| Olive cultivar | (DPPH) $IC_{50}$ (µg/ml) | (Reducing power) $EC_{50}$ (µg/ml) | (ABTS) $IC_{50}$ (µg/ml) |
|---|---|---|---|
| **Ascorbic acid** | $11.16\pm0.43^{c}$ | $15.57\pm1.22^{c}$ | $11.60\pm1.49^{d}$ |
| **Picual** | $45.14\pm2.34^{b}$ | $53.98\pm1.84^{a}$ | $50.98\pm2.66^{b}$ |
| **Tofahy** | $55.45\pm1.99^{a}$ | $47.39\pm1.37^{b}$ | $41.05\pm2.16^{c}$ |
| **Shemlali** | $55.82\pm2.89^{a}$ | $55.17\pm1.65^{a}$ | $70.29\pm1.96^{a}$ |

$IC_{50}$: Effective concentration which achieve 50% DPPH radical scavenging activity.$EC_{50}$: Effective concentration at which the absorbance is 0.5. ABTS•+ radical assay expressed as percentage activity as a function of concentration of extracts. Each reported value is the mean ± SD of three replicates. Means in the same column followed by different letters are significantly different ($p<0.05$).

**Reducing power**

In Table 2, the reducing capacities of three cultivars of olive leaves extract were measured against ascorbic acid as standard. Tofahy variety showed a significant reducing power with $EC_{50}$= 47.39±1.37 µg/ml following that of ascorbic acid ($EC_{50}$=15.57±1.22µg/ml) ($p<0.05$), while there was insignificant difference between Picual (53.98 ± 1.84 µg/ml) and Shemlali (53.98 ±1.84 µg/ml) ($p>0.05$). In general, the results obtained by reducing power confirmed that obtained by DPPH, as all three cultivars showed significant reducing power but at concentrations higher than ascorbic acid. The order of reducing power was as follows: ascorbic acid > Tofahy > Picual > Shemlali, respectively. These results were in line with the findings of **Kubola and Siriamornpun (2008)**. The greater abundance of total phenols in olive leaves extract indicates that it can donate electrons to the reactive free radicals more effectively, converting them into stable components and terminating the free radical chain reaction. These findings encourage utilization of olive leaves extract as a good source of antioxidant in food applications such as functional foods and food preservatives.

**ABTS radical scavenging assay**

The quantitative determination of the radical scavenging activity of the ABTS free radical involved measuring the disappearance of the colored free radical and the blue colored (**Adegborioye *et al.*, 2018**). ABTS radical converts to its colorless form when it reacts with antioxidants. Scavenging of the ABTS derived nitrogen-centered radical cation (ABTS•+) was applied to match the total antioxidant activities of the three cultivars of olive leaves compared to ascorbic acid in Table 2. Tofahy extract showed the highest antioxidant activity ($IC_{50}$ 41.05±2.16 µg/ml) followed by Picual ($IC_{50}$ 50.98±2.66 µg/ml) while Shemlali had the least scavenging capacity ($IC_{50}$ 70.29 ± 1.96 µg/ml) ($p>0.05$). Once again, the ABTS radical scavenging assay confirms the previous results obtained by DPPH and reducing power. The obtained results agreed

with **Abdel-Razek *et al*. (2017)** who reported the value of $IC_{50}$ of olive leaves extract which valued 57.52 µg/ml while $IC_{50}$ for ascorbic acid was recorded as 14.00 µg/ml and **Adegborioye *et al*. (2018)** who reported that the percentage inhibition values were noted to increase as the concentration of the extracts increased in the assay.

## Antimicrobial Activity

Antimicrobial activity of each extract was studied individually against seven pathogenic bacteria strains, one yeast strain and two fungal species, the obtained results are present in Table 3. A significant bacterial inhibition was obtained by Picual extract against Gram- negative strains *Escherichia coli* BA12296, *Escherichia coli* ATCC25922 and *Salmonella* spp. with MIC of 12.5 mg/ml. On the other hand, moderate bacterial inhibition power with a higher concentration (MIC= 25 mg/ml and 100 mg/ml) was recorded against Gram- positive strains, *Clostridium perfringens* EMCC15 and *Staphylococcus aureus* EMCC1351 (Table 3). In the same time, antibacterial effect was obtained by Tofahy extract against Gram-negative *strains Escherichia coli* BA12296, ATCC25922 and *Salmonella* spp. with MIC of 12.5 mg/ml, while the bacterial inhibition ability against Gram-positive strains with MIC of 50 mg/ml was recorded against *Clostridium perfringens* EMCC15 (Table 3). Shemlali showed antibacterial activity against Gram-negative strains of *Klebsiella pneumonia* ATCC12296 and *Escherichia coli* BA12296 with MIC of 25 mg/ml, while the effective concentration against Gram-positive strains (*Clostridium perfringens* EMCC15 and *Staphylococcus aureus* EMCC1351) was higher with MIC of (50 mg/ml, and 75 mg/ml, respectively). Results showed a reasonable inhibition power against *Candida albicans* EMCC105 strain with MIC of 12.5 mg/ml for Shemlali extract, while the MIC for Picual, and Tofahy was tow times higher (MIC= 50 mg/ml). Results presented in Table 4 revealed antifungal activity against *Aspergillus niger* EMCC 72 with MIC of 200 mg/ml for both Picual and Tofahy extracts, while MIC value was 300 mg/ml for Shemlali extract. On the other hand, a significant inhibition ability against *Aspergillus parasiticus* EMCC 886 was noted for Picual and Tofahy extracts with MIC 100 mg/ml, while MIC was 200 mg/ml for Shemlali extract. The obtained results in the present investigation agreed with the findings of **Lee and Lee (2010)** who reported the combined of phenolic mixture prepared from the olive leaves extract exhibited inhibition effects against *B. cereus* and *S. enteritidis.* Olive leaves extract showed antimicrobial activity against *E. coli, S. aureus, B. cereus, and S. typhi* (**Owen *et al*., 2003**). The present findings are in line with the findings previously reported by **Pereira *et al*. (2007) and Hussain *et al*. (2015)** who revealed the pharmacological properties of olive leaves extract such as antibacterial, antifungal activity. In the current study, polyphenolic compounds presented in olive leaves extract showed a strong antimicrobial activity against a broad spectrum of microorganisms such as Gram-positive, Gram-negative, Yeast and fungi which emphasize their potential application in pharmaceutical and field functional foods.

## Cytotoxicity Assay

A cytotoxicity assay was measured as the capacity of the extracts to decrease cell viability. Peripheral blood mononuclear cells (PBMCs) were used as normal cells to determine the safe dose of three olives leaves cultivars. Cell lines were cultured in the presence of graded concentrations of olive leaves extracts for 24 hr., (Fig. 1). The ability of olive extracts to decrease (PBMCs) cell viability in a dose-dependent manner differs among all the tested cultivars. Thus, $LD_{50}$ values (the concentration of olive leaves extracts needed to decrease cell viability by 50% relative to untreated control cells). The cytotoxicity assay showed the $LD_{50}$ value of Picual, Tofahy and Shemlali (0.204, 0.390 and 0.940 mg/ml, respectively). The obtained results revealed insignificant toxicity of the olive leaves extract for the living cells which confirm the high safety of the extract when used to the food and pharmaceutical applications. **Guex *et al*. (2018)** reported that olive leaves are known to possess beneficial effects on metabolism when used as a therapeutic agent, treatment with olive leaves in a single dose of 2000 mg/kg caused no signs of toxicity and no mortality was recorded. The present findings was agreed with

**Table 3. Inhibition zone diameter of aqueous extracts against bacterial and yeast strains**

| Pathogenic strain | Herbal infusion | Inhibition zone diameter (mm)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | 100* | 75* | 50* | 25* | 12.50* | MIC |
| **Gram negative bacteria** | | | | | | | |
| ***Escherichia coli* ATCC25922** | **Tofahy** | 25 | 20 | 18 | 11 | ND | 25 |
| | **Shemlali** | 11 | ND | ND | ND | ND | 100 |
| | **Picual** | 27 | 25 | 20 | 19 | 12 | 12.50 |
| ***Escherichia coli* BA12296** | **Tofahy** | 24 | 22 | 20 | 18 | 15 | 12.50 |
| | **Shemlali** | 19 | 17 | 15 | 10 | ND | 25 |
| | **Picual** | 34 | 30 | 27 | 24 | 12 | 12.50 |
| ***Klebseilla pneumonia* ATCC12296** | **Tofahy** | 19 | 16 | ND | ND | ND | 75 |
| | **Shemlali** | 28 | 25 | 23 | 19 | ND | 25 |
| | **Picual** | ND | ND | ND | ND | ND | ND |
| ***Salmonella* spp.** | **Tofahy** | 24 | 21 | 19 | 15 | 11 | 12.50 |
| | **Shemlali** | 20 | 14 | 12 | 10 | 9 | 12.50 |
| | **Picual** | 30 | 28 | 25 | 20 | 19 | 12.50 |
| **Gram positive bacteria** | | | | | | | |
| ***Streptococcus mutans* EMCC1815** | **Tofahy** | 27 | 25 | 17 | 13 | ND | 25 |
| | **Shemlali** | 25 | 23 | 15 | 10 | ND | 25 |
| | **Picual** | ND | ND | ND | ND | ND | ND |
| ***Staphylococcus aureus* EMCC1351** | **Tofahy** | 10 | 9 | ND | ND | ND | 75 |
| | **Shemlali** | 19 | 10 | ND | ND | ND | 75 |
| | **Picual** | 13 | ND | ND | ND | ND | 100 |
| ***Clostridium perfringens* EMCC15** | **Tofahy** | 20 | 15 | 11 | ND | ND | 50 |
| | **Shemlali** | 22 | 20 | 16 | ND | ND | 50 |
| | **Picual** | 32 | 24 | 17 | 12 | 10 | 12.50 |
| **Yeast** | | | | | | | |
| ***Candida albicans* EMCC105** | **Tofahy** | 18 | 16 | 10 | ND | ND | 50 |
| | **Shemlali** | 27 | 23 | 20 | 14 | 12 | 12.50 |
| | **Picual** | 24 | 21 | 10 | ND | ND | 50 |

*Concentration of extracts and MIC are in mg/ml.
**Diameter include 5 mm well diameter.
ND; inhibition not detected.
MIC; Minimum inhibition concentration.

**Table 4: Inhibition zone diameter of aqueous extracts against fungi strains**

| Fungi | Cultivar | Inhibition zone diameter (mm)** | | | |
|---|---|---|---|---|---|
| | | 100* | 200* | 300* | MIC |
| ***Aspergillus niger* EMCC 72** | Tofahy | ND | 20 | 27 | 200 |
| | Shemlali | ND | ND | 15 | 300 |
| | Picual | ND | 13 | 20 | 200 |
| ***Aspergillus parasiticus* EMCC 886** | Tofahy | 10 | 19 | 30 | 100 |
| | Shemlali | ND | 10 | 15 | 200 |
| | Picual | 14 | 19 | 27 | 100 |

*Concentration of extracts and MIC are in mg/ml. **Diameter include 5 mm well diameter.
ND; inhibition not detected. MIC; Minimum inhibition concentration.

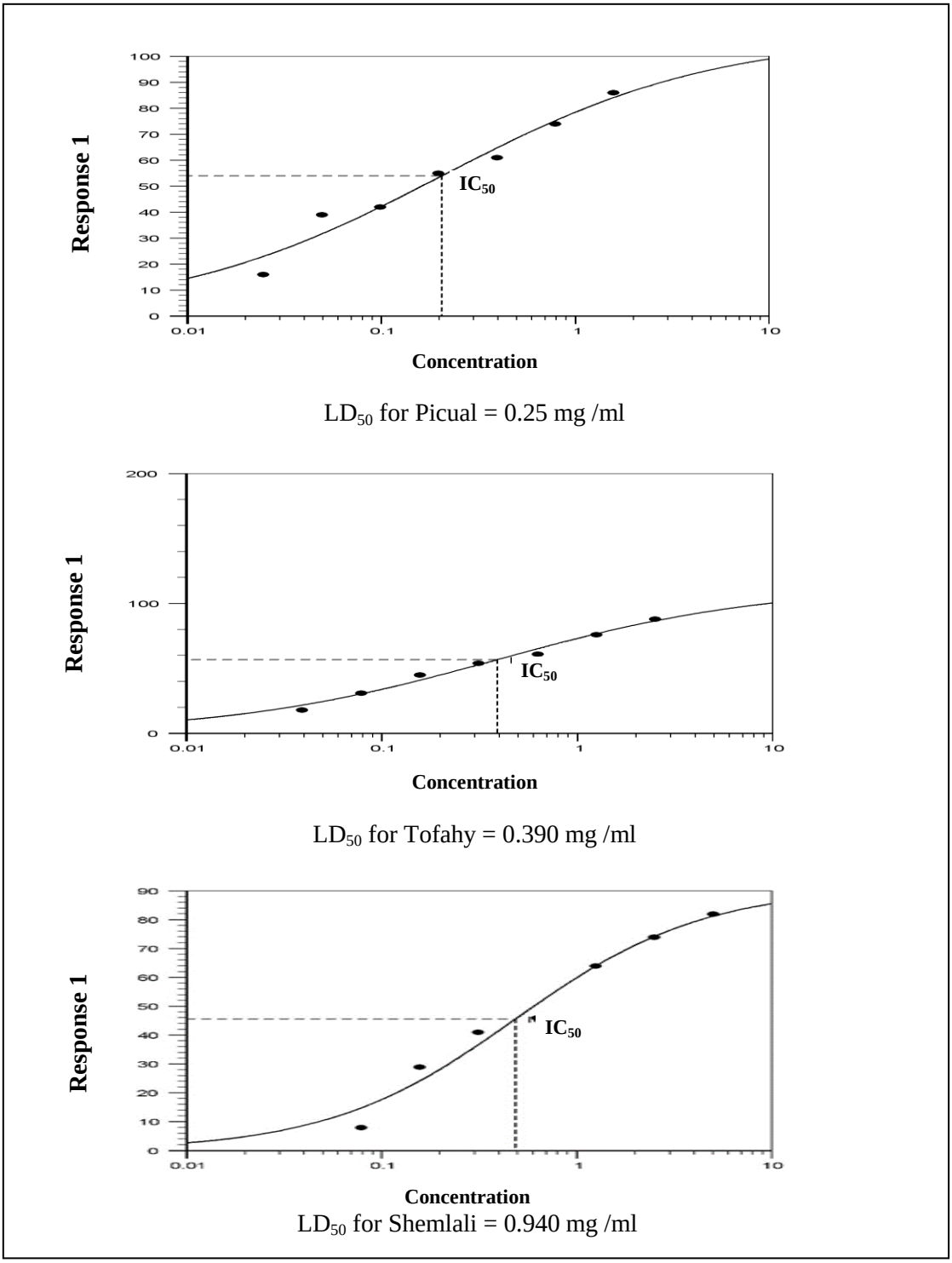


**Fig. 1. A Cytotoxicity assay of three cultivars of olive leaves extract**

the findings of **Rashidipour and Heydari (2014)**, the cytotoxicity activities of the synthesized Silver nanoparticles and olive leaves extract containing Silver nanoparticles against human breast ($IC_{50}$) was found to be 50 and 0.024 µg/ml at 24 hr., incubation, respectively. So, the cytotoxicity results emphasized the safety of olive leaves extracts even at high concentration which indicates the ability of utilizing them in food application without specific precautions or restrictions.

## *In vitro* Anti-Inflammatory Activity

The *in vitro* anti-inflammatory activity of the tested extracts was assessed by HRBC membrane stabilizing method (Fig. 2). The extracts from Picual, Tofahy and Shemlali of 5 µg/ml (the lowest concentration used in this study) showed the highest anti-inflammatory effect as the HRBC membrane stability. The key role of anti-inflammatory substances is to inhibit the cyclooxygenase enzyme which responsible for conversion the arachidonic acid to prostaglandin when it released extracellularly by pain receptors in lysosome. As the HRBC membrane is similar to the membrane of lysosome therefore the stabilizing ability of HRBC membrane by influence of the phenolic compounds existed in the extract will emphasize its ability to protect the lysosomal membrane and then inhibit the conversion arachidonic acid to prostaglandin in lysosomal membrane consequently inhibit the inflammation. The obtained results in the current study evidenced that the tested three olive leaves extracts exhibited significant stabilizing ability of HRBC. The presence tannins, flavonoids, and phenolic compounds are present in extracts, they were exhibited a significant anti-inflammatory activity (**Huang *et al.*, 2009**). The bitter compound oleuropein which the main predominant phenolic compound in olive leaves extracts might be a potent antioxidant endowed with anti-inflammatory properties (**Benavente-Garcıa *et al.*, 2000; Pereira *et al.*, 2007**).

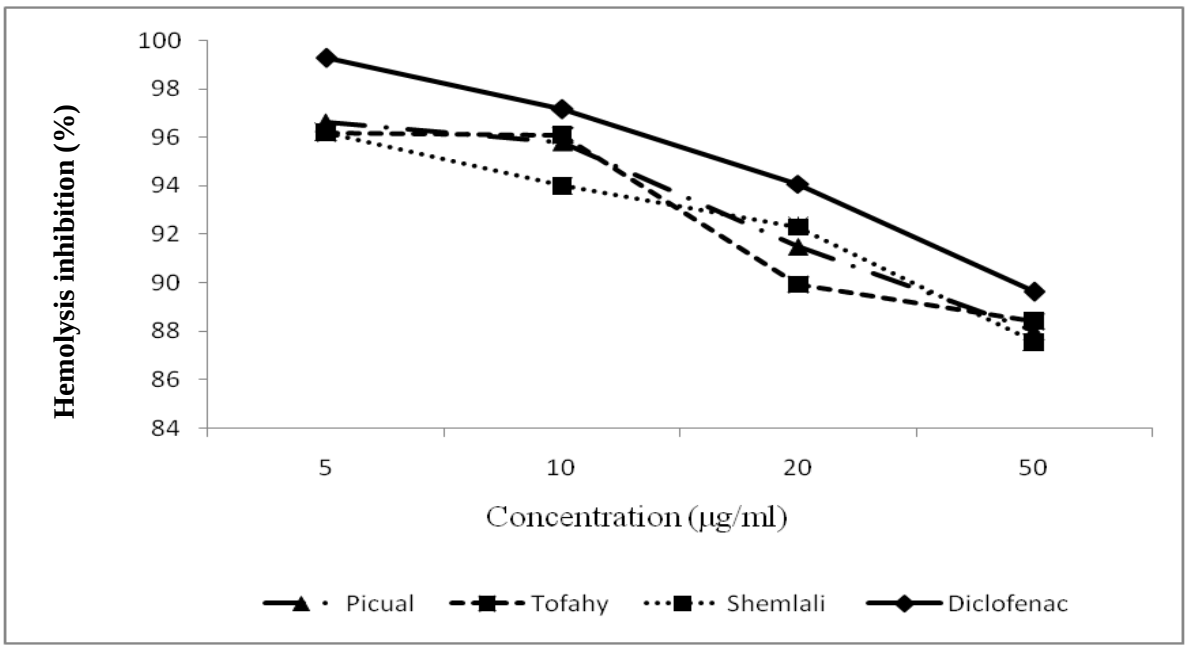


**Fig. 2. *In vitro* anti-inflammatory activity of three cultivars olive leaves extract.**

## Conclusions

Olive leaves contain interesting bioactive compounds that may exhibit an antioxidant and antimicrobial power. The current investigation focused on the measuring of the antioxidant, antimicrobial and anti-inflammatory activity of three cultivars grew under Egyptian climate conditions (Tofahy, Shemlali and Picual). Extracts of three olive leaves cultivars are good sources of phenolic compounds and flavonoids which may differs according to the cultivar, ripening stage, and cultivation conditions. The olive leaves extracts exhibited a significant antioxidant capacity which confirmed by three different common widely used methods (DPPH, Reducing power and ABTS radical scavenging assay). At the same time, the three different extracts showed a significant antimicrobial activity against both Gram-positive and Gram-negative bacteria and yeasts and fungi as well. The obtained emphasized that the tested three olive leaves extracts exhibited significant stabilizing ability of HRBC and their effectiveness as anti-inflammatory agents. The conclusion of the findings of the current investigation showed the high potential of olive leaves extracts in food and pharmaceutical application which is adding value for these by-products to be a low-cost source of some beneficial bioactive compounds.

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# مضادات الأكسدة ومضادات الميكروبات والالتهابات المحتملة لأوراق الزيتون

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شجرة الزيتون من أهم الأشجار المثمرة لاحتوائها على العديد من المركبات النشطة بيولوجيًا و تعتبر اوراق الزيتون مخلفات ثانوية ناتجة عن عملية تقليم الأشجار، يختلف محتوى هذه المركبات بين الأصناف، كان الهدف من الدراسة الحالية هو تقييم الخصائص المضادة للأكسدة والميكروبات والمضادة للالتهابات لأصناف أوراق الزيتون الثلاثة (بيكوال، شملالي، تفاحي)، وأيضًا تحديد سميتها الخلوية للاستخدام الآمن في التطبيقات الغذائية، تم استخلاص الأصناف الثلاثة بالماء وتم تحديد كمية المستخلص في كل صنف، تحديد النشاط المضاد للأكسدة، المضاد للالتهابات، المضاد للميكروبات لسبع أنواع من البكتيريا الممرضة، نوع واحد من الخميرة ونوعان من الفطريات، من بين جميع المستخلصات التي تم اختبارها، كان صنف بيكوال الأعلى محتوى من الفينول والفلافونويد وأظهر نشاطًا قويًا كمضاد للأكسدة، وكان الحد الأدنى للتركيز المثبط للبكتيريا والخميرة من 12.5 إلى 100 مجم/مل للأصناف الثلاثة، أعطى تركيز 5 ميكروجرام/مل لمستخلصات البيكوال والتفاحي والشملالي أعلى تأثير مضاد للالتهابات، أشارت نتائج السمية الخلوية امكانية الاستخدام الآمن لأصناف أوراق الزيتون الثلاثة كمضادات طبيعية للميكروبات ومضادات للأكسدة والالتهابات.

**الكلمات الاسترشادية:** أصناف الزيتون، مضادات الأكسدة، مضادات الميكروبات، مضاد التهاب، احتمالية السمية