Chemical Evaluation and Biological Activity of Olive Leaves Extract

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ABSTRACT:

Total phenolic content (TPC), total flavonoids content (TFC), phenolic compounds and antioxidant properties of extracts olive leaves were determined. Total phenolic compound for the extracted olive leaves were evaluated using the Folin-Ciocalteu method, while the total flavonoid contents were determined with an aluminum chloride colorimetric assay. The 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging effect of the herbs was measured by spectrophotometry. The antimicrobial activity and hemolytic activity of extracts were performed. The components of the extracted olive leaves were determined by EDX analysis and HPLC. The main components determined in the extracted olive leaves were Gallic acid, Vanillin, Rutin, Kaempferol, Naringenin, Ferulic acid, Coumaric acid, Catechin, Cinnamic acid, Syringic acid, Coffeic acid and Methyl gallate in El-Farafra leaves extract, and Gallic acid, Kaempferol, Ellagic acid, Querectin, Rutin, Catechin, Syringic acid, Ferulic and Naringenin in Borg El-Arab leaves extract. Results showed that the values of total phenols are higher than that within the total flavonoids. From the biological studies it was observed that Borg El-Arab extract has antimicrobial effect for gram-positive bacteria and Yeast higher that El-Frafra extract, while they have the same effect on the gram-negative bacteria.

Keywords: Olive leaves extract; biological activity; Total phenolic; Total flavonoids.

INTRODUCTION:

Since ancient times plants used to cure several kinds of human diseases. Different parts of plants such as leaves stem, bark, root, etc. Have been used for preventing; allaying symptoms or reverting anomalies back to usual [Sarkar *et a*l 2015]. Phytochemicals extracted from plants used as traditional medicines are considered as good alternatives for chemical drugs [Palombo 2011]. The olive tree is one of these plants has a great importance in ancient and Modern civilizations. It was used for human since prehistory for food, treatment, lighting...etc. scientific name of the olive tree =is *(Olea europaea* L*)* [Youssef *et al* 2010].

In Mediterranean region and Egypt, Olive tree has been cultivated for thousands of years. [Agamy *et al* (2017) and Ahmed (2019)]. Olive tree leaves represent about 10% of the total weight of olives yield [Ghanem *et al* 2019]. It is a potential inexpensive, renewable and abundant source of biophenols [Şahin and Bilgin 2018] and it is most of the contents of the leaves [Zorić *et al* 2016]. Phytochemical research has isolated flavonoids, sequoridiates, airidoides, pyofnols, triterpenes, benzoic acid derivatives, isocarman and other secondary categories of olive leaves [Hashmi *et al* 2015]. Therefore, olive leaves are used in both traditional medicine and the pharmaceutical industry and the phenolic compounds antioxidant compounds in olive leaves can have positive effects [Erbay and Icier 2010]. Olive leaves are a rich source of crude fiber and minerals especially calcium, followed by potassium, iron and contain all essential amino acids (except methionine) in favorable amounts [Ibrahim *et al* 2016].

Olive trees are the most cultivated plants in the world.They bear different environmental conditions because of its ability to be processed into oil and its importance for human health [Özer 2018]. Leaves are used in medicine to combat and treat diabetes, anticonvulsant, antioxidant, anti-inflammatory, immunomodulatory, analgesic, antimicrobial, antiviral, antihypertensive, anticancer, antihyperglycemic, antinociceptive, gastroprotective and wound healing activities. It is generally safe even at high doses [Acar-Tek and Ağagündüz (2020) and Mahmoud and Bayomey (2021)].

Scientists from divergent fields are investigating plants with an eye to their antimicrobial usefulness. Laboratories of the world have found literally thousands of

phytochemicals, which have inhibitory effects on all types of microorganisms in vitro [Cowan 1999]. Many researchers focused their studies on the extracts from many plants. It was used against bacteria for example *E. coli, Klebsiella pneumoniae, dermatophytes* and yeast, *S. enterica serovar Typhimurium, L. monocytogenes S. aureus, Salmonella anatum* and more Moreover. [Shan *et al* (2007) and Quinto (2019)] The use of antibiotics leads to multidrug resistant microorganisms reinforcing the focus of researchers on natural antimicrobials [Quinto 2019]. Olive leaves extract has been used as an antimicrobial [Liu *et al* 2017] and extract contain compounds that have significant antimicrobial properties [Hafez and Abdelrahman 2015].

The active substances in the extracts example phenolic compounds quantification were determined by HPLC, LC-mass and GC mass [J Rocha-Pimienta and R Ramírez 2020]. Therefore, the aim of this research is to decide the total content of phenols and flavonoids also to study the ability to inhibit free radicals in order to use these extracts natural antioxidants, antimicrobial and anti-hemolysis.

MATERIALS AND METHODS:

Plant Material:

Olive leaves were collected from an olive orchard located in El-Frafra and Borg El-Arab. Samples were taken from different parts of the plant and were mixed. Leaves were left to dry at room temperature for 1 week before use. The dried olive leaves were ground and 5 grams of each type of leaves were weighed and soaked in 100 ml 90% ethyl alcohol for 24 hours at room temperature followed by filtration using Whatman 47 mm x 9 0.45 µ filters. The filtrates were evaporated in a rotary evaporator. The extraction yield for El-Frafra and Borg El-Arab leaves were 7.69% and 10.42%, respectively.

EDX Analysis:

Patterns and percentage of the elements in olive leaves extracts were analysed by Electron dispersive X-ray was shown by Scanning Electron Microscope coupled with EDX-system (Jeol, JSM-IT 200, Japan).

HPLC analysis for the olive leaves extracts:

HPLC is used in literature for identification and detection of the polyphenol complex [Savournin 2001]. The HPLC system consists of low-pressure gradient preparative system (LC-20AP Prominence Liquid Chromatography), FCV-200 AL: a low-pressure gradient unit, a system controller (CB-20A). gradient mixer 14 mL for preparative applications, gradient mixer 4.5mL for analytical applications SPDM20A: Prominence photo diode array detector. (DGU-10B) Helium degassing unit, SLL-10AP autosampler (injection volume:1-5000µL), FRC-10A fraction collector and automatic rinsing pump, all from Shimadzu, (Japan). The analytical preparative HPLC was controlled remotely using LabSolution LC workstation Ver. 5.51 Malti LC-PDA (Japan). Minitab® 17.1.0 was used to establish the mathematical models for optimization. This instrument is available at "City of Scientific Research and Technology Application (SRTA-City)" in the Center of pharmaceutical & Fermentation Industries Development (New Borg El-Arab City Alexandria- Egypt).

About 1.5 g of the leaves from each cultivar were extracted with 20 ml ethanol/water 90% for 30 min. The solutions formed were filtered through GF/F filter paper. The extracts were further extracted with petroleum ether to remove chlorophyll, following filtration and centrifugation. 0.3 mL from the extracts were kept in vials prior to HPLC.

Determination of Total Phenol Contents (TPC):

The total phenolic content was determined using the Folin-Ciocalteu reagent [Singleton *et al* (1999) and Dewanto *et al* (2002)]. One mg extract was dissolved in 1ml deionized water and 200 µl of dissolved sample was taken and added to 600 µl of distilled water and 100 µl of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 minutes before addition of 2 ml of 2% Na_2CO_3. The solution was adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark for 30 min, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of Gallic acid (standard, from 0-100 µg/ml). Total phenol contents (TPC) were expressed as Gallic acid equivalent (GAE)/mg of dry weight and calculated using the following liner equation based on the calibration curve:

$y = 0.0095x - 0.0409$
$R^2 = 0.9953$
Where:
(y) is absorbance
(x) is the concentration (GAE) mg / g extract)
(R^2) is correlation coefficient.

All determinations were performed in triplicates.

Determination of Total Flavonoid Contents (TFC):

Total flavonoid content of the plant extract was determined by a modified colorimetric method [Sakanaka *et al* 2005], using catechol as a standard at concentrations of (20 – 200 µg/ ml). Extract or standard solutions (250 µl) were mixed with distilled water (1.25 ml) and 75 µl of 5% sodium nitrite ($NaNO_2$) solution followed by the addition of 150 µl of 10% aluminum chloride ($AlCl_3$) solution after 5 min later. After 6 min, 0.5 ml of 1 M sodium hydroxide (NaOH) and 0.6 ml distilled water were added. The mixture was then mixed, and absorbance was measured at 510 nm. Total flavonoids content was expressed as catechol equivalent (CE) and calculated using the following liner equation based on the calibration curve:

Y=0.0079 x - 0.109
R^2 = 0.986
Where:
(y) Is absorbance
(X) Is the concentration ((CE) mg /g extract).
(R^2) = correlation coefficient.

All determinations were performed in triplicate.

DPPH Radical Scavenging Activity:

The free radical scavenging activity of plant extracts was measured by the DPPH method as proposed according to the approval procedure [Brand-Williams *et al* 1995] with some modifications. A solution of 0.2 Mm DPPH in methanol (0.0078 g/100 ml) was prepared and 1 ml of this radical solution was added to 1 ml of sample or standard solution at different concentrations (1:1 V/V). The mixture was incubated for 30 min in the dark at room temperature and then the absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid solutions as standards in the concentration range of (5-200 µg/ml) were used to establish a standard curve. DPPH radical scavenging activity was expressed as mg ascorbic acid equivalent (AAE)/g dried sample. The percentage DPPH radical-scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (% inhibition) = ($Abs_{control}$-Abs_{sample})/ $Abs_{control}$ *100

For control, all reagents were added except plant extract and all determinations were performed in triplicate and the average values was tabulated.

Reducing Power:

A spectrophotometric method [Ferreira *et al* 2007] was used for the measurement of the reducing power of plant extracts in corresponding solvents were mixed with phosphate buffer 2.5 ml (0.2M, PH 6.6) and 2.5 ml of (1%) potassium ferricyanide. This mixture was incubated at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml freshly prepared ferric chloride solution (0.1%) and in similar manner excluding samples allowed to stand for 10 min. The absorbance was measured at 700 nm. Control was prepared. Ascorbic acid at various concentrations was used as standard for construction of the calibration curve and the reducing power was reported as EC50 (mg/ml): effective concentration at which the absorbance is 0.5.

Antimicrobial Activity:

The antimicrobial activity was performed by agar well diffusion assay [Kadaikunnan *et al* 2015], for all samples. Five microbial species known to be pathogenic including Gram negative Bacteria (*klebsila pneumonia ATCC700603, E.coli ATCC25922*), Gram positive Bacteria (*Staphylococcus aureus* ATCC25923 and *Bacillus cereus ATCC10876*) and yeast strain (*Candida albicans* EMCC105).The bacteria and yeast strains were grown in nutrient broth at 37°C for 24h. A set of 3 concentration of reconstituted tested material, were examined to determine the minimum inhibitory concentration (MIC) of each against a specific pathogenic strain. Hundred µl of the inoculums (1×10^8 cfu/ml) were inculcated on agar media and poured into the Petri plate and prepared in the plates with the help of a cork-borer (0.5 cm) and100 µl of the tested compound were applied into the well. All the tested bacteria were incubated at 37°C for 24 hr. 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.

Hemolytic Activity Assay:

The test was performed in 2mL microtubes following the method described according to the approval procedure [Farias *et al* 2013]. with some modifications. Firstly, a twofold serial

dilution of each extract was prepared with 0.9% NaCl ranging from1,000 to 1.9 $\mu g \cdot ml^{-1}$ and reserved. Then, 100 μL of a 1% red blood cells (A, B, and O human blood types or rabbit blood) suspension was added to a new microtube containing 900 μL of each extract dilution, which was then taken to be incubated at 37∘C for 1h. After that, the tubes were centrifuged at 3,000 ×g for 5min. The supernatant (200μL) was placed in a 96-well plate and led to a microplate reader to measure the absorbance at 540 nm. The cell suspensions of each human blood type or those of rabbits (100 μL) were mixed with distilled water or 0.9% NaCl (900 μL) to obtain the absorbance for 100 and 0% of cell lysis, respectively. The percentage of hemolysis was calculated as follows: % hemolysis = Abs test/Abs pc × 100, where Abs test = Abs540 of the 1% cell suspension treated with sample test and Abs pc = Abs540 of the 1% cell suspension treated with distilled water.

RESULTS AND DISCUSSION:

EDX Analysis:

The analysis of crushed olive leaves was done by energy dispersive X-ray (EDX). Figure (1) shows the typical EDX pattern for crushed olive leaves, a clear signal of the presence of C and O was observed. Furthermore, the presence small peaks represented Se were observed in both El Farafra and Borg El-Arab leaves with concentrations (0.37 ± 0.27 and 0.45 ± 0.19) respectively. Ca and Cs were detected only for the El-Farafra leaves, Table (1).

HPLC Analysis of Phenolic Compounds:

The chromatographic profile of olive leaves extract was specified by HPLC. and Table (2) and Figure (2) show the phenolic compounds isolated from the olive cultivars El-Farafra and Borg El-Arab. Gallic acid (3924.71 µg/g in El-Farafra) and Vanillin (1388.54 µg/g in Borg El-Arab) were the main compounds followed by Vanillin, Rutin, Kaempferol, Naringenin, Ferulic acid, Coumaric acid, Catechin, Cinnamic acid, Syringic acid, Coffeic acid and Methyl gallate in El-Farafra leaves extract and Gallic acid, Kaempferol, Ellagic acid, Quercetin, Rutin, Catechin, Syringic acid, Ferulic and Naringenin in Borg El-Arab leaves extract. Levels of phenolic compounds layers found in olives and left in agreement with those in literature [Taamalli *et al* (2013), Tayoub *et al* (2012) and Abaza *et al* (2017)].

From these results we find that olive leaves collected from El- Farafra Oasis are richer in basic polyphenolic compounds than those collected from Borg El-Arab, with the exception of the following compound: Ellagic acid, Quercetin and Kaempferol.

Total phenolic and total flavonoids content of sample extracts:

Table (3) shows values of the total contents of phenolic and flavonoids for ethanolic olive leaf extracts. Results showed that the values of total phenols are higher than that within the total flavonoids, showing that these extracts oftotal phenolic recorded 115.42mg/g in Olive (Farafra), while they recorded 105.88mg/g in Olive (borg). Total flavonoids values were 77.07mg/g and 69.43mg/g in Olive (Farafra) and Olive (borg) respectively. These results are agreements with this in literature [AlShaal *et a*l 2019].

From Table (3), it was noticed that Total phenolic and total flavonoids content of El-Farafra Oasis are higher than that in Borg El-Arab.

Antioxidant Properties:

The extracted olive leaves gave good free-radical scavenging and ferric reducing antioxidant power [Meryem *et al* 2012]. The inhibition concentration values DPPH (IC_{50}) values of Borg El-Arab and El-Farafra extracts were (12.16±0.12) and (8.54±0.17), While the reducing activities were (73.53±0.17) and (67.57±0.13) respectively, Table (4,6), Figures (3,4). The percentage of inhibition of DPPH radical scavenging activity at different concentrations of the extracts was given in Table (5).

Antimicrobial Activity:

The effect of extracts with different concentrations on Five microbial species known to be pathogenic including Gram negative Bacteria (*klebsila pneumonia* ATCC700603, *E.coli* ATCC25922), Gram positive Bacteria (*Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC10876) and yeast strain (*Candida albicans* EMCC105) were studied, Table (7), Figure (4). The two extracts showed nearly the same inhibition zones for gram negative bacteria and yeast, while the olive leaves that extracted from Borg El-Arab showed inhibition zone higher than that extracted from El-Farafra for gram positive bacteria. The effect of plant extracts as antibacterial is due to phenolic compounds [Takó *et al* 2020] and it is the most important component of olive leaf extracts [Ghomari *et al* 2019] these extracts explain the research progress of antibiotics activity and these results obtained are in agreement with those in

literature [Ghomari *et al* (2019), J Rocha-Pimienta and R Ramírez (2020) and Borjan *et al* (2020)] mentioned that the olive leaf extracts have the ability to inhibit germs and bacteria.

Hemolytic activity assay:

The hemolytic activity from the Olive extracts whit different concentrations of RBCs membrane was showed in Table (8). The result showed the haemolytic activity of the crude olive extracts on rabbit blood. Result presented that the extracts acted as protectors against direct hemolysis when concentration increased. The result revealed that Olive (Farafra) was the highest haemolytic activity compared to Olive (borg). When IC_{50} (µg/ml) record 55.56±0.11 and 117.19±0.12 in Olive (Farafra) and Olive (borg) respectively.

CONCLUSION

The current study showed that:

The total phenolic compounds, antioxidant activity and the reducing power for the extract from Borg El-Arab was higher that from El-Farafra.

From the biological studies it was concluded that Borg El-Arab extract has antimicrobial effect for gram-positive bacteria and Yeast higher that El-Frafra extract, while they have the same effect on the gram-negative bacteria**.**

Result presented that the extracts acted as protectors against direct hemolysis when concentration increased.

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Table 1: EDX analysis of crushed olive leaves.

Element	% Mass El-Farafra Leaves	% Mass Borg El-Arab leaves
C	48.0 ± 0.,36	67.27 ± 0.30
O	48.43 ± 0.82	32.28 ± 0.58
Ca	2.39 ± 0.12	ND
Se	0.37 ± 0.27	0.45 ± 0.19
Cs	0.21 ± 0.08	ND
Total	100.00	100.00

Table.2: The concentration of the main components of the El-Farafra and Borg El-Arab olive leave extracts by HPLC chromatograms at 280 nm.

Components	Conc.(µg/g) El-Farafra	Conc.(µg/g) Borg El-Arab
Gallic acid	3924.71	1023.85
Catechin	306.61	135.54
Methyl gallate	71.04	ND
Caffeic acid	117.66	ND
Syringic acid	143.81	121.83
Rutin	965.53	467.63
Ellagic acid	ND	788.29
Coumaric acid	398.15	ND
Vanillin	2064.73	1388.54
Ferulic acid	413.25	89.93
Naringenin	613.89	72.35
Quercetin	ND	767.01
Cinnamic acid	249.56	ND
Kaempferol	723.09	980.20

Table 3: Total phenolic and total flavonoids content concentrations in mg/g of extracted samples

Extracts	Total phenolic (mg/g)	Total flavonoids (mg/g)
Olive (borg)	105.88±0.99	69.43±0.53
Olive (Farafra)	115.42±0.47	77.07±0.14

- Reported values are the mean ± SD of three replicates. Total phenolic was expressed as Gallic acid equivalents (GAE) mg/g sample. Total flavonoids were expressed as quercetin mg /g sample.

Table 4: The inhibition concentration values DPPH (IC_{50}) value of sample extracts

Extracts	DPPH (IC50) µg/ml
Ascorbic	3.64±0.01
Olive (borg)	12.16±0.12
Olive (Farafra)	8.54±0.17

- IC_{50} (µg/ml): inhibitory concentrations at which 50%of DPPH radicals are scavenged.

Table 5: % of inhibition of DPPH radical scavenging activity.

Concentration (µg/ml)	Ascorbic	Concentration (µg/ml)	Olive (borg)	Olive (Farafra)
5	68.64	10	41.11	58.54
10	72.13	20	67.25	72.47
15	75.61	40	72.13	74.22
		80	77.00	75.26
		100	77.35	76.66

Table 6: The reducing power (EC_{50}) value of sample extracts.

Extracts	Reducing power EC_{50} (mg/ml)
Ascorbic	16.82±0.01
Olive (Borg)	73.53±0.17^{b}
Olive (Farafra)	67.57±0.13^{d}

- EC_{50} (mg/ml): effective concentration at which the absorbance is 0.5.

Table (7): Diameter of inhibition zone against *klebsila pneumonia, E. coli, Staphylococcus aureus, Bacillus cereus* and *Candida albicans* (mm) with 0.025, 0.050 and 0.075 mg/ml of different concentrations of extracted olive leaves.

Pathogenic strain	Tested material	Inhibition zone diameter (mm)**/	Sample concentration (mg/ml)	
	0.075	0.050	0.025	MIC
Gram negative Bacteria				
klebsila pneumonia ATCC700603				
Olive (borg)	17	15	13	0.025
Olive (Farafra)	17	15	11	0.025
E.coli ATCC25922				
Olive (borg)	17	11	ND	0.050
Olive (Farafra)	15	11	13	0.025
Gram positive Bacteria				
Staphylococcus aureus ATCC25923				
Olive (borg)	19	15	13	0.025
Olive (Farafra)	17	10	ND	0.050
Bacillus cereus ATCC10876				
Olive (borg)	20	18	15	0.025
Olive (Farafra)	15	12	10	0.025
Yeast				
Candida albicans EMCC105				
Olive (borg)	20	15	11	0.025
Olive (Farafra)	19	15	13	0.025

ND: Not Detected.

MIC: Minimum inhibitory concentration inhibition zone including 5 mm well diameter.

Tab. 8: In vitro hemolytic activity and (IC50) value of sample extracts:

Concentration	% Of Hemolysis	
(µg/ml)	Olive extract (borg)	Olive extract (Farafra)
10	32.67	40.67
20	33.33	43.33
40	34.67	45.33
60	40.67	54.00
80	41.33	65.33
100	42.67	86.00
IC_{50} (µg/ml)	117.19±0.12	55.56±0.11

- Reported values are the mean ± SD of three replicates.
- IC_{50} (µg/ml): Extracts concentration that causes 50% hemolysis of RBCs

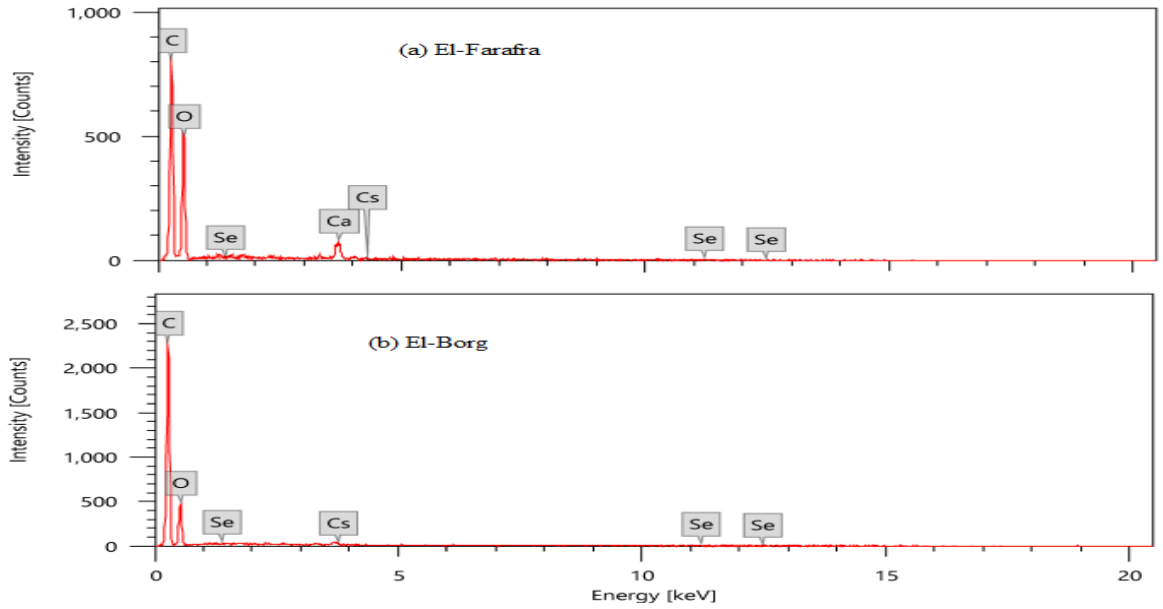

Figure 1: Energy dispersive X-ray (EDX) analysis of in crushed olive leaves from (a) El-Farafra and (b) Borg El-Arab.

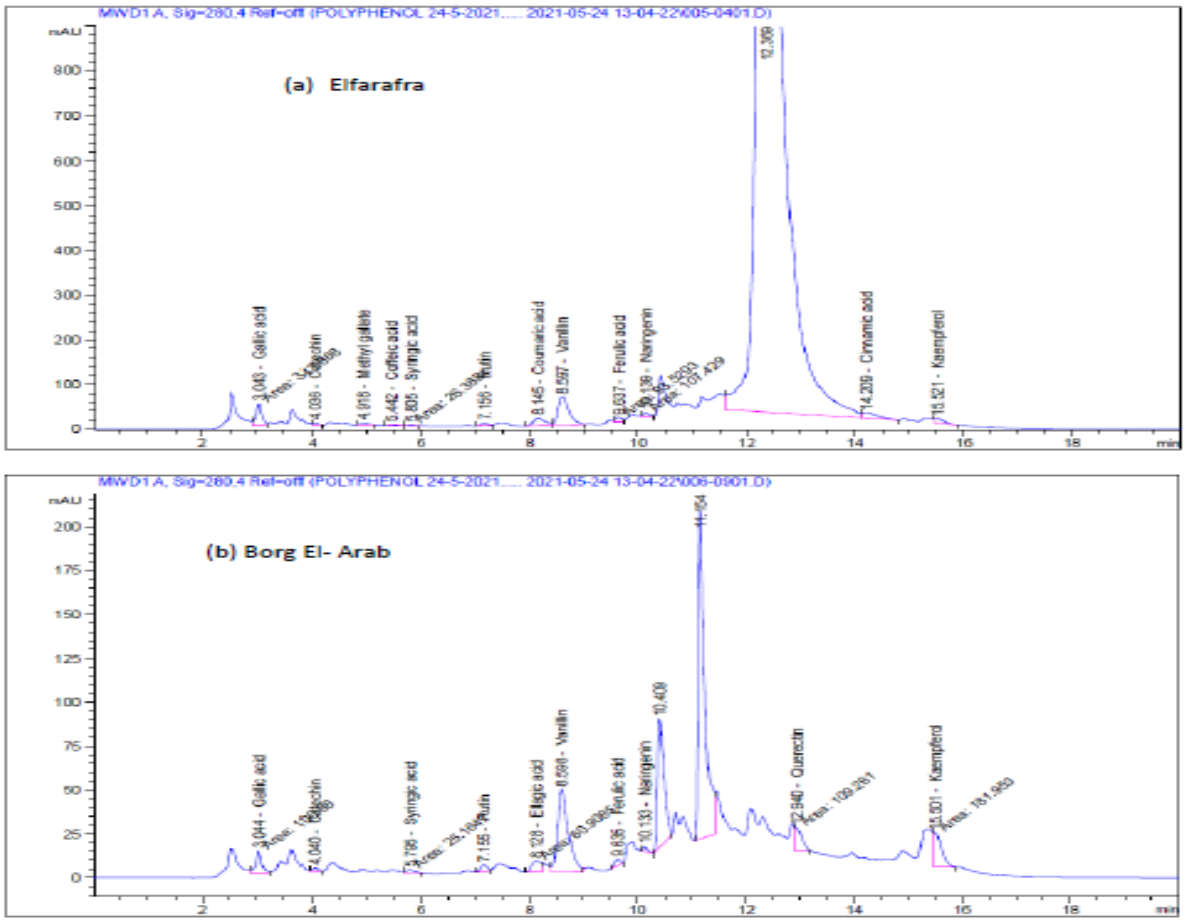

Figure2: HPLC chromatograms of the El-Farafra and Borg El-Arab olive leave extracts at 280 nm.

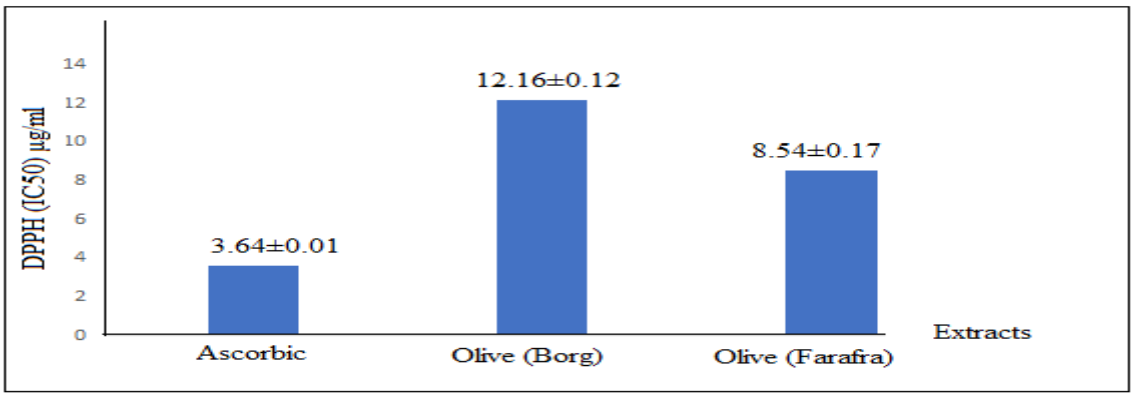

Figure 3: The inhibition concentration values DPPH (IC_{50}) value of sample extracts.

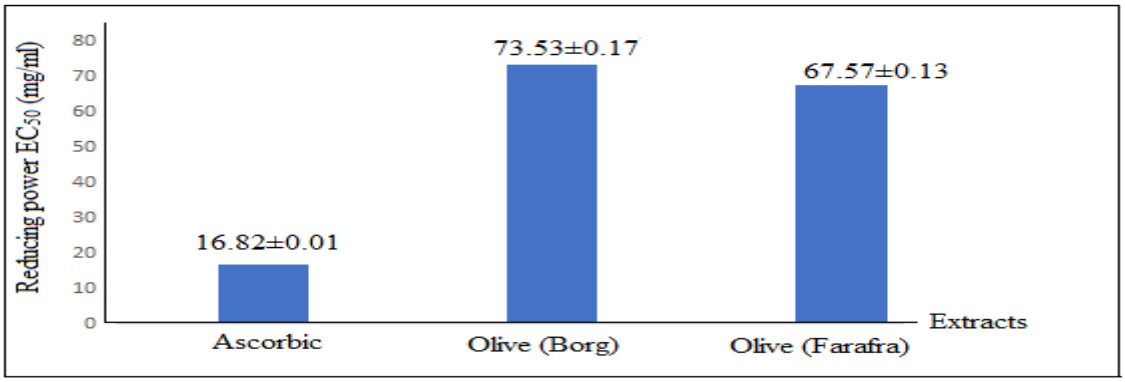

Figure 4: The reducing power (EC_{50}) value of sample extracts

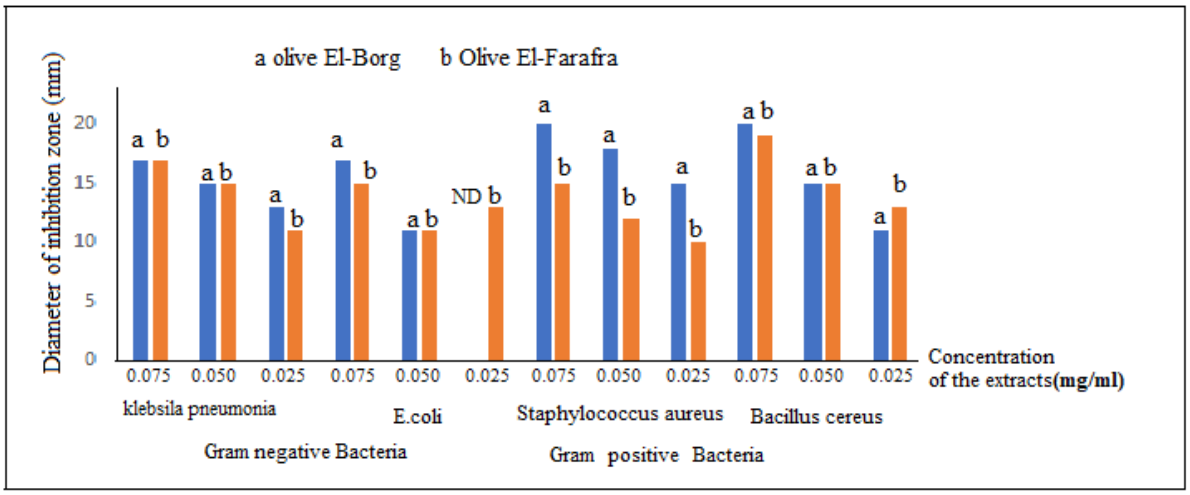

Figure 5: Diameter of inhibition zone against *klebsiella pneumonia, E. coli, Staphylococcus aureus* and *Bacillus cereus* (mm) with 0.025, 0.050 and 0.075 mg/ml of different concentrations of extracted olive leaves.

التقييم الكيميائي والنشاط البيولوجي لمستخلص أوراق الزيتون

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الملخص العربي:

تم تقديرالمحتوى الفينولي الكلي، والمحتوى الكلي من الفلافونويدات، والمركبات الفينولية، وخصائص مستخلصات أوراق الزيتون المضادة للأكسدة. تم تقييم إجمالي المركبات الفينولية لمستخلصات أوراق الزيتون باستخدام طريقة Folin-Ciocalteu، في حين تم تحديد إجمالي محتوى الفلافونويدات باستخدام aluminum chloride colorimetric assay. وقد تم قياس تأثير المستخلصات في الكنس الجذري لجزيئ 2,2-diphenyl-1-picryl hydrazyl (DPPH) بواسطة القياس الطيفي الضوئي (spectrophotometry). تم إجراء النشاط المضاد للميكروبات والنشاط الانحلالي للمستخلصات. تم تحديد مكونات أوراق الزيتون المستخرجة من خلال تحليل EDX و HPLC .وكانت المكونات الرئيسية الموجودة في أوراق الزيتون المستخلصة هي Gallic acid, Vanillin, Rutin, Kaempferol, Naringenin, Ferulic acid, Coumaric acid, Catechin, Cinnamic acid, Syringic acid, Coffeic acid and Methyl gallate في مستخلص أوراق (الفرافرة). و

Gallic acid, Kaempferol, Ellagic acid, Querectin, Rutin, Catechin, Syringic acid, Ferulic and Naringeninفي مستخلص أوراق (برج العرب). وأظهرت النتائج أن قيم الفينولات الكلية أعلى من قيم الفلافونويدات الكلية. وخلصت الدراسات البيولوجية إلى أن مستخلص أوراق زيتون (برج العرب) له تأثير مضاد للميكروبات على البكتيريا الموجبة للجرام وعلى الخميرة أعلى من مستخلص أوراق زيتون (الفرافرة)، في حين أن لهما نفس التأثير على البكتيريا السالبة للجرام.

الكلمات الاسترشادية: مستخلص أوراق الزيتون، النشاط البيولوجي, الفينولات الكلية, الفلافونيدات الكلية.