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Biological **activities and related phenolic compounds content of olive and plum stones ethanolic extract**

Heba E. Younis[a*], Walaa A. Elshalakany[a], Shimaa A. Amin[b], Mohammed A. T. Abdel-Reheem[a], Hemmat A. Ibrahim[a]

[a] *Agricultural Biochemistry Department, Faculty of Agriculture, Ain Shams University, P.O. Box 68, Hadayek Shubra, 11241 Cairo, Egypt*

[b] *Agricultural microbiology Department, Faculty of Agriculture, Ain Shams University, P.O. Box 68, Hadayek Shubra, 11241 Cairo, Egypt*

Abstract

The current study was conducted to assess the antioxidant, antibacterial, and anticancer activities of ethanolic extracts of defatted olive and plum stones (seeds and woody shells) and determined total phenolic compounds (TPCs), total flavonoids compounds content (TFCs) of extracts colorimetrically and identified by High-Performance Liquid Chromatography. Olive seed extract gave the highest content of TPCs (0.276g/100g.DW), while the highest content of TFCs was in plum seed extract (0.191g/100g.DW). There was a direct relationship between the antioxidant activity (reducing power, and scavenging activity of DPPH radical) and the extracts content of TPCs. Plum seed ethanol extract (EE) was the most effective extract against *Pseudomonas aeruginosa,* and *Escherichia coli*, while olive seed extract gave the most antimicrobial activity against *staphylococcus aureus* bacteria. On the other hand, olive and plum stone (seed, and woody shell) extracts have anticancer effect towards the two cell lines (HT-29, and MCF-7).

Keywords: Olive and plum plant, seeds and woody shells, EE, antioxidant activity, anticancer activity, antibacterial activity.

1. Introduction

Agro-based industries produce a huge amount of waste annually, the accumulation of these wastes in the environment without use may lead to pollution and harmful effects on human and animal health **[1].** Therefore, recent studies tended to take advantage of these wastes in many purposes to reduce environmental pollution in an environmentally safe and economically efficient manner **[2]**. There are many important uses for these wastes, including: fertilizer, animal feed and biofuel as well as heavy metal elimination of these wastes **[3].** Also, several effective compounds extracted from fruits waste can be used in cosmetic and pharmaceutical products **[4]** and also have therapeutic properties **[5].** The common compounds extracted from fruit by products are polyphenols which found in skins, pulp and seeds **[6].** So, the olive and plum stones (seeds and shelly) considered as fruit wastes which can be used in many purposes.

The olive tree (*Olea europeaea, Oleaceae*) produces 98 % of the world's olive oil, and its fruit is a significant crop in the Mediterranean region. Olive fruit consists of mesocarp (70% to 90%), endocarp (9% to 27%), and seed (2% to 3%). Furthermore, fruits collected during the normal harvest season have a mesocarp composition of 30% oil, 60% water, 4% carbohydrates, and 3% proteins, with the other ingredients primarily comprising fiber and ash. On the other hand, the endocarp is composed of 40%

*Corresponding author e-mail: hebaeid@agr.asu.edu.eg. (Heba E. Younis)
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other carbohydrates, 10% water, 30% cellulose, and 1% oil **[7,8].**

The whole olive stone is a rich source of useful components. Multiple previous studies have been written about olive oil importance, uses, and the chemical make-up of olives. However, just a few research have focused on the components and applications of the olive stone **[9].**

The Rosaceae family includes the plum (*Prunus Salicina Lindl*) which is a good source of anthocyanins and polyphenolic chemicals. Plums are employed in pharmaceutical, cosmetic preparations and other industries. The benefits of eating plums for health include anti-diabetic effect, cardioprotection **[10],** regulation of adipogenesis and inflammation **[11,12]** , and prevention of LDL oxidation **[13,14].** Plum's biological activities have been mainly attributed to its high content of phenolic compounds **[15].** Moreover, include significant levels of phenolic antioxidants, and include higher levels of the nitrogenous compound amygdalin, which use as a tumor chemotherapeutic agent **[16]**.

Plum stones contain a seed which constitutes about 5% of the total weight of the fruit. Along with making pellets, it can also be used to make pharmaceutical and cosmetic products **[17].** Determined the amygdalin content (3.791 g kg-1), which is effective in cancer patients' alternative therapies. Additionally, the plum seed contains phenolic compounds **[18,19]** ,proteins **[18,20]** and oil about 40% **[21,22].**

There was little work on olive and plum stones (seed and woody shell) biological activity, therefore this study was carried out to highlight the EEs of plum and olive stone as a source of important compounds that have many biological activities such as antioxidants, anticancer, and antibacterial.

2. Materials and methods

2.1. Chemicals

The used chemicals in the present work are ethanol (96%), dimethyl sulfoxide (DMSO), sodium carbonate, Folin-Ciocalteu reagent, gallic acid, sodium hydroxide, sodium nitrite, aluminum chloride, quercetin, 2,2-diphenyl-1picrylhydrazyl (DPPH), ferric chloride, monosodium dihydrogen phosphate, disodium hydrogen phosphate, potassium ferricyanide, and trichloroacetic acid, ascorbic acid and butylated hydroxy anisole (BHA). All chemicals used in this study were obtained from Piochem and Loba Chemie for Laboratory Chemicals and were purchased from the importing company Afak for chemicals and medical requisites (Cairo, Egypt).

2.2. Tested microorganisms

Tested microorganisms were obtained and identified from Agriculture Microbiology Department, Faculty of Agriculture, Ain Shams University which include the following standard strains: *Staphylococcus aureus* ATCC 29737, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

2.3. Cancer cell lines and cell culture

The cytotoxicity test was performed on two human cancer cell lines, HT-29 (Colorectal Cancer) and MCF-7 (Breast Adenocarcinoma). Nawah Scientific Inc. (Mokatam, Cairo, Egypt) supplied these cell lines. Cells were grown in Dulbecco's Modified Eagle medium (DMEM) supplemented with 100 mg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO_2 atmosphere at 37 °C **[23,24].**

2.4. Preparation of ethanol extracts of plum and olive stone

Plum (*Prunus salicina L.*) and olive (*Olea europaea L.*) stones as by-product were collected from local markets at Cairo, Egypt, and have been recognized by the Horticultural Research Center of the National Agricultural Research Center at Giza, Egypt.

The ethanol extracts (EEs) were prepared according to the method of **Abubakar and Haque [25]** as follows: Plum and olive stones were divided to seed and woody shell of each stone, then each part were dried by freeze drying then grounded into a fine powder. The powder samples were defatted by soaking it in hexane (1:4 w/v) for 24 h then filtered and the remained sample re-extracted with hexane about three times. The supernatants were discarded, while the residues were dried at 50ºC in oven for 12 h. The defatted samples were homogenized with ethanol 96% (1:4w/v) and then macerated for 72 h. The EEs were filtered and evaporated to dryness by rotary evaporator under vacuum at 50ºC. The total extracts were stored in deep freezer for further use.

2.5. Total phenol content of extracts

Total phenolic content was determined in all extracts by the colorimetric **Naczk and Shahid** method **[26].** The Folin-Ciocalteus reagent was used at 725nm. Adding 0.5 ml of each extract (0.1g /10ml

solvent), 0.5 ml Folin reagent, and 8 ml distilled water then shaking for 2 minutes then 1 ml saturated anhydrous Na_2CO_3 (33g) were dissolved in 100 ml distilled water at 70-80°C then cooled and kept overnight at room temperature, then filtered) was added, the developed blue color was determined using spectrophotometer (UV- visible spectrophotometer UV 9100 B, Lab Tech) after 1h at 725 nm against blank which prepared using by following the same technique but substituting an equal volume of solvent for the plant extract. The results were calculated as g gallic acid (GA/100 g of dry extract.

2.6. Total flavonoid content of extracts

Flavonoids content were measured by the aluminum chloride colorimetric method according to **Marinova et al. [27]**. An aliquot (1 ml) of each extract or standard solution of Quercetin (with concentrations ranging from 10 to 100 µg/ml) was placed in a 10 ml volumetric flask containing 4 ml distilled water. 0.3 mL of $NaNO_2$ (5%) was added to the flask. After 5 minutes, 0.3 ml of 10% $AICl_3$ was added, followed by 2 ml 1M NaOH after 6 minutes, and the total volume was made up to 10 ml using distilled H_2O. After the mixture had been well mixed, the absorbance at 510 nm was measured in comparison to a blank using spectrophotometer (UV-visible spectrophotometer UV 9100 B, Lab Tech). Total flavonoids were measured in g of Quercetin (QUE) equivalent per 100 g of dry extract.

2.7. Antioxidant activity of extracts

2.7.1. Reducing power assay

Reducing power was determined using **Oyaizu** method **[28]**. Two and a half milliliters of each extract (from 0.5 to 15 mg/ml in ethanol) was combined with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide then the mixture was incubated at 50°C for 20 min. After adding 2.5 ml of 10% trichloroacetic acid (w/v), the mixture was centrifuged at 200 g for 10 minutes. From the upper layer, 1 ml was taken into a test tube and mixed with 1 ml of 1% ferric chloride then the total volume was made up to 10 ml with distilled H_2O. At 700 nm, absorbance was measured using a spectrophotometer (UV- visible spectrophotometer UV 9100 B, Lab Tech) against a blank. A higher absorbance indicates a greater reducing power. Ascorbic acid was used as a control.

2.7.2. DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the corresponding extract was determined from the bleaching of a purple colored ethanolic solution of 2,2'-biphenyl picryl hydrazyl (DPPH) according to **Güllüce et al. [29].** As a reagent, the stable radical DPPH is used in this spectrophotometric experiment. 2.5 ml from 0.5 to 15 mg/ml of each extract in ethanol was added to 2 ml of 0.004% (2mM) ethanolic solution of DPPH. The absorbance was measured at 517 nm using a spectrophotometer (UV- visible spectrophotometer UV 9100 B, Lab Tech) against a blank after 30 minutes of the incubation at room temperature. The following formula was used to calculate the percentage inhibition of the free radical DPPH (I%): %Antioxidant activity = $[(Ac - As)/Ac] \times 100$ where, Ac indicates the absorbance of the control and As denotes the absorbance of the sample, respectively. The plant sample was replaced with ethanol in the control.

2.8. Antimicrobial activity of extracts

The antibacterial activity of the plant extracts was tested by agar well diffusion assay **[30]** against pathogenic strains (*E. coli, P. aeruginosa, and S. aureus*). Nutrient Agar (NA) medium was poured into Petri dishes. Suspensions (100µl) of the target strain (previously incubated for 24 h) were spread on the plates, and wells of 10 mm in diameter were made with a sterile cork borer **[31,32].** The test samples (at various concentrations 0.5, 1.0, 2.5 and 5.0 mg/ml) were prepared in 5% Dimethyl sulfoxide (DMSO) and the negative control was 100µl of 5% DMSO for EEs, then were transferred into the wells in the agar plates previously inoculated with the target strain. The plates were let to stand until the plum and olive stone extracts were entirely absorbed before being incubated at 37°C overnight **[33]**. After 24 hours of incubation, the growth inhibition was evaluated with the naked eye, and the inhibition zone diameter (IZD) was measured using a ruler. The growth of the inhibition zone around the wells was used to determine antimicrobial activity.

2.9. Anticancer activity (cytotoxicity assay) of extracts

Cytotoxicity was determined in Nawah Scientific Inc., (Mokatam, Cairo, Egypt) according to the method described by **[23,24]**. The sulforhodamine B (SRB) assay was used to determine cell viability. In 96-well plates, aliquots of 100 µl cell suspension ($5x10^3$ cells) were inoculated and incubated in full medium for 24 hours. Cells were then treated with

another aliquot of 100 µl medium containing medicines at various concentrations (100, 200, 400, 800, and 1200 µg/ml) of plum and olive seed extracts (DMSO was used to dissolve the sample). Cells were fixed 72 hours after drug exposure by replacing medium with 150 µl of 10% trichloroacetic acid (TCA) and incubated at 4 °C for 1 hour. After removing the TCA solution, the cells were washed five times with distilled water. Aliquots of 70 µl SRB solution (0.4% w/v) were added and incubated for 10 minutes in a dark area at room temperature. Plates were cleaned three times with 1% acetic acid and left to air dry overnight. The protein-bound SRB stain was then dissolved in 150 µl of Tris buffer (10 mM), and the absorbance was measured at 540 nm with a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany).

2.10. Statistical analysis

All determinations were carried out at least three times (n = 3), and the statistical mean with ± SD was determined using the software IBM SPSS Statistics. A two-way ANOVA test was performed on the replicates, and mean values were compared using the Duncan test at the 5% level to compare between mean values **[34,35].**

2.11. HPLC Analysis of phenolic compounds in ethanolic extracts

To separate the polyphenolic components from extracts that had a higher level of activity, HPLC was used in National Research Center, Giza (An Agilent 1260 series). HPLC analysis was conducted according to **[36,37]**. A column called Eclipse C18 (4.6 mm x 250 mm i.d 5 µm) was used to carried out the separation. Water (A) and 0.05 % trifluoroacetic acid in acetonitrile (B) flowed through the mobile phase at a rate of 0.9 ml/min. The mobile phase was programmed in the following order: 0 min (82% A); 0-5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A); and 16-20 min (82% A). The multi-wavelength detector was employed at a wavelength of 280 nm. A volume of 5µl was injected into each of the sample solutions. The temperature in the column was kept constant at 40°C. Peaks were detected by comparing retention times and UV spectra to those of the standards.

3. Results and discussion

3.1. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Data in **Table (1)** showed (TPC) and (TFC) of olive and plum stones (seed and woody shell) EE. The highest TPC was found in the EE of olive seeds (OS) 0.276% followed by plum seeds (PS) 0.259% and olive woody shell (OWS) 0.112%, while the lowest TPC was found in plum woody shell (PWS) 0.089%. On the other hand, PS had the highest TFC (0.191%) followed by the extract of (OWS) 0.135% and (PWS) 0.110%, while (OS) showed the lowest TFC 0.093%.

Table 1: Total phenol and flavonoid contents in olive and plum stones (seeds and woody shells) EE as GAE and QE, respectively.

Plant ethanol extracts	TPC g GAE/100g DE	TFC g QE/100g DE
OS	**0.276±0.002^{a}**	**0.093±0.007^{d}**
OWS	**0.112±0.002^{c}**	**0.135±0.02^{b}**
PS	**0.259±0.007^{b}**	**0.191±0.04^{a}**
PWS	**0.089±0.008^{d}**	**0.110 ±0.03^{c}**

Where: OS is olive seed, OWS is olive woody shell, PS is plum seed, PWS is plum woody shell, and DE is dry extract.
All values present means of three replicates ± SD.
Different letters in the same column refer to significant difference ($p< 0.05$).

The Folic-Ciocalteu reagent is reduced when a phenol loses an H^{+} ion to generate a phenolate ion under the basic conditions of the reaction **[38,39]** .While the aluminum ion (Al^{+3}) forms complexes with C-4 keto and either C-3 or C-5 hydroxyl, or with ortho hydroxyl groups in the A or B ring in the total flavonoid assay **[40].** The change is measured using spectrophotometry.

From all the above results in **Table (1)** we can concluded that EE of (OS) was a better extract for phenolics and (PS) extract was a better extract for flavonoids.

Phenolic and flavonoid compounds consider biologically active compounds which showed anti-carcinogenic, antibacterial, anti-inflammatory, and antioxidant properties, as well as a reduction in cardiovascular disease **[41].**

3.2. HPLC Analysis

Nineteen phenolic compounds had been identified by HPLC in ethanolic extracts of which a compound

belonging to simple phenols (pyrocatechol), 5 compounds are considered benzoic acid derivatives (gallic, methyl gallate , syringic acid, ellagic and vanillin), as well as 5 cinnamic acid derivatives (chlorogenic, caffeic, coumaric, ferulic and cinnamic acid) and also 8 flavonoid compounds (catechin, rutin, naringenin, diadzein, quercetin, apigenin, kaempferol and hesperetin) were represented in (Table 2) and (Figs 1-5).

Table 2: Identified polyphenolic compounds in EE of olive and plum stones (seed and woody shell) by HPLC analysis.

peak	compounds	R.T[min]	Conc. (µg/g DW)			
			OS	OWS	PS	PWS
1	Gallic acid (GA)	3.390	283.35	120.31	253.79	81.36
2	Chlorogenic acid (CHA)	4.264	1024.78	381.44	797.48	219.92
3	Catechin (CAT)	4.645	0.00	10.90	106.87	107.39
4	Methyl gallate (MG)	5.620	225.49	47.09	195.92	12.26
5	Caffeic acid (CAA)	6.072	833.34	73.42	125.04	206.25
6	Syringic acid (SYA)	6.592	1480.22	14.38	106.16	9.52
7	Pyro catechol (PCAT)	6.796	506.43	8.06	78.87	9.39
8	Rutin (RU)	8.002	15.20	26.06	111.67	36.92
9	Ellagic acid (EA)	8.845	4075.71	811.24	403.97	90.68
10	Coumaric acid (COA)	9.163	75.89	18.82	30.42	10.40
11	Vanillin (VA)	9.761	2037.49	18.52	173.85	9.20
12	Ferulic acid (FA)	10.250	837.58	1910.37	82.34	10.73
13	Naringenin (NAR)	10.495	102.10	40.68	65.09	7.71
14	Daidzein (DAI)	12.265	432.38	0.00	24.07	26.59
15	Quercetin (QUE)	12.753	935.56	21.27	102.11	0.00
16	Cinnamic acid (CA)	14.053	45.53	10.57	12.38	7.05
17	Apigenin (API)	14.515	61.39	0.00	0.00	0.00
18	Kaempferol (KAE)	15.026	171.11	0.00	0.00	43.29
19	Hesperetin (HES)	15.608	0.00	6.46	0.00	0.00

Where: OS is olive seed, OWS is olive woody shell, and PS is plum seed, PWS is plum woody shell.

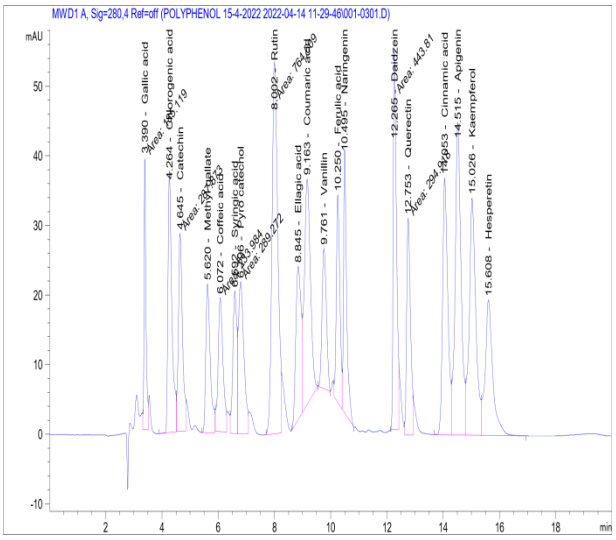

Fig. 1: High performance liquid chromatography chromatogram of 19 typical phenolic compounds; gallic acid GA(1), chlorogenic acid CHA(2), catechin CAT (3), methyl gallate MG(4), caffeic acid CAA (5), syringic acid SYA (6), pyrocatechol PCAT(7), rutin RU(8), and ellagic acid EA(9), coumaric acid COA (10), vanillin VA(11), ferulic FA acid (12), naringenin NAR(13), daidzein DAI(14), quercetin QUE (15), cinnamic acid CA(16), apigenin API (17), kaempferol KAE(18), hesperetin HES (19).

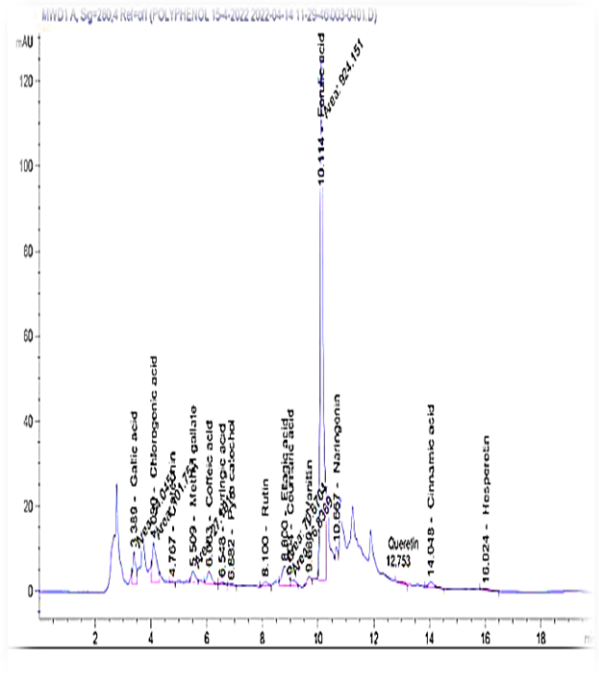

Fig. 2: High- performance liquid chromatography of EE of olive seed (OS).

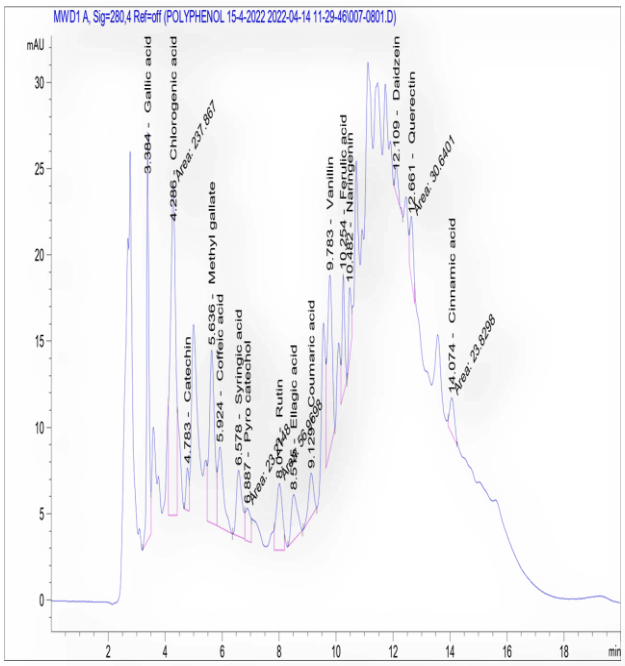

Fig. 3: High- performance liquid chromatography of EE of olive woody shell (OWS).

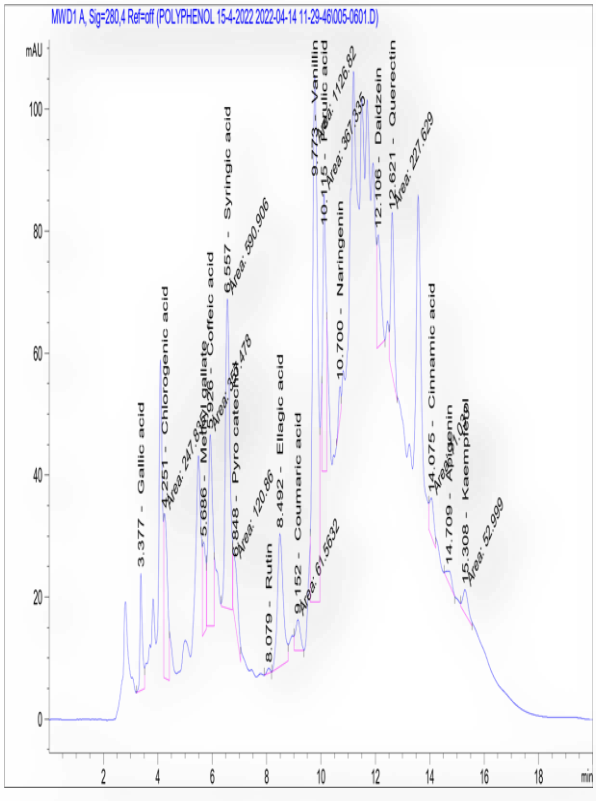

Fig. 4: High- performance liquid chromatography of EE of plum seed (PS).

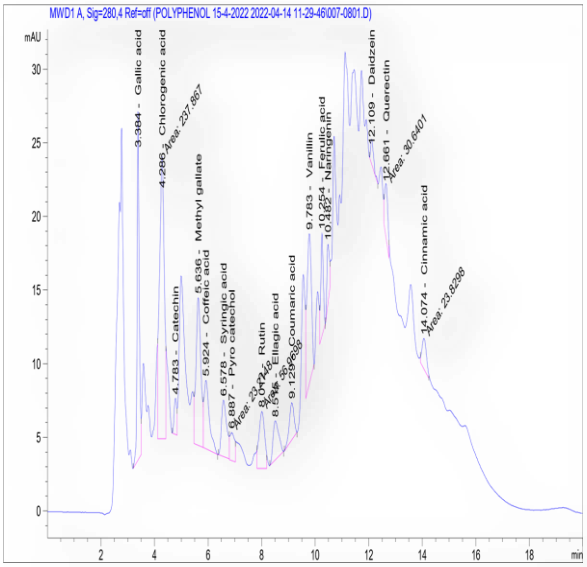

Fig. 5: High- performance liquid chromatography of EE of plum woody shell (PWS).

OS extract had the highest concentrations of most compounds which identified by HPLC as follows: ellagic acids was the highest compounds (4075.71 µg/g DW) followed by VA (2037.49 µg/g DW), SYA (1480.22 µg/g DW), CHA (1024.78 µg/g DW), QUE (935.56 µg/g DW), FA (837.58 µg/g DW), CAA (833.34 µg/g DW, PCAT (506.43 µg/g DW), DAI (432.38 µg/g DW), GA (283.35 µg/g DW), MG (225.49 µg/g DW), KAE (171.11 µg/g DW) and NAR (102.10 µg/g DW),. Whereas CAT and HES were disappeared, on the other hand, RU, COA, CA and API were presented in low concentrations.

While, OWS extract contained high concentrations of ferulic acid (1910.37 µg/g DW), EA (811.24 µg/g DW), CHA (381.44 µg/g DW) and GA (120.31 µg/g DW), while DAI, API and KAE were absent and the other compounds were found in low concentrations.

Also, PS was the second extract after OWS extract in high concentration of phenolic constituents which identified by HPLC, where PS contained high levels of CHA (797.48 µg/g DW), followed by EA403.97 µg/g DW), GA (253.79 µg/g DW) MG (195.92 µg/g DW), VA (173.85 µg/g DW), CAA (125.04 µg/g DW), RU (111.67 µg/g DW), CAT (106. 87 µg/g DW), SYA (106.16 µg/g DW) and QUE (102.11 µg/g DW), while API, KAE and HEP were absent, but COA, FA, NAR, DAI and CA were found in low concentrations.

Finally, PWS extract was the lowest extract in its content of phenolic compounds which separated by HPLC, where PWS extract contained high levels of CHA (219.92 µg/g DW) followed by CAA (206.25 µg/g DW), CAT (107.39 µg/g DW), while QUE, API and HES were absent, but the other compounds were found in low concentrations. CAT absent in OS extract only, while QUE disappeared only from PWS extract whereas, DAI was not found in OWS extract only. On the other hand, API appeared in OWS only, also KAE was found in OS and PWS extracts, while HES was found in OWS extract only.

These results were matched with the investigation of **Boskou, Boskou et al. & Uylaşer and Yild [42-44],** they concluded that table olives contain six different phenolic compounds classes as a maior components as follows: phenolic alcohols (hydroxytyrosol and tyrosol), flavones (luteolin, luteolin-7-O-glucoside, API and apigenin-7-O-glucoside), flavonols (RU), anthocyanins (cyanidin-3-O-glucoside), phenolic acids (5-O-caffeoylquinic acid) and a hydroxycinnamic acid derivative (verbascoside). there were several biologically active compounds were identified in plum fruits such as phenolic acids (chlorogenic, neochlorogenic, protocatechuic, caffeic, trans-*p*-coumaric, and ferulic acids), flavonoids (anthocyanins, flavonols, and flavan-3-ols), and vitamins (ascorbic acid, tocopherols, and carotenoids) **[45,46].** Also, plums contained neochlorogenic acid (3-O-caffeoylquinic acid) as predominant polyphenols while, CHA (5-O-caffeoylquinic acid) and cryptochlorogenic acid (4-O-caffeoylquinic acid) were found at lower concentrations **[47,48].** Chlorogenic acid has antioxidative properties for human low-density lipoprotein, antimutagenic and anticarcinogenic effects**,** and glucose lo**we**ring properties **[49,50].**

3.3. Antioxidant activity

3.3.1. Reducing Power (or antioxidant capacity)

Reducing power of OS, OWS, PS and PWS extracts and ascorbic acid as control, were displayed in **Table (3)**. There were positive relation between extracts concentration and reducing power value, the extract with the highest reducing power had the lowest value of IC 0.5.

Table 3: Reducing power of ascorbic acid, olive and plum stones extracts

Ethanolic extracts	0.5 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml	15 mg/ml	IC 0.5 mg/ml
OS	0.57±0.005^{m}	0.72±0.001^{jk}	1.33±0.02^{e}	1.99±0.002^{c}	2.68±0.005^{a}	2.60±0.01^{a}
OWS	0.52±0.004^{p}	0.56±0.002^{n}	0.71±0.003^{k}	0.85±0.007^{h}	1.06±0.006^{g}	6.11±0.03^{c}
PS	0.66±0.006^{l}	0.73±0.02^{j}	1.23±0.005^{f}	1.77±0.005^{d}	2.26±0.01^{b}	2.98±0.01^{b}
PWS	0.52±0.002^{p}	0.55±0.005^{o}	0.66±0.001^{l}	0.78±0.01^{i}	0.86±0.01^{h}	7.06±0.05^{d}
Ascorbic acid (ST)	**0.2 mg/ml** 0.8	**0.4 mg/ml** 1.87	**0.6 mg/ml** 2.469	**0.8 mg/ml** 2.98	**1 mg/ml** 3.61	**IC 0.5 mg/ml** 0.137

Where: OS is olive seed, OWS is olive woody shell, and PS is plum seed, PWS is plum woody shell.
All values present means of three replicates ± SD.
Different letters in the same column refer to significant difference ($p< 0.05$).
IC 0.5 = the concentration which exhibited absorbance value 0.5.

The highest reducing power was shown in the (OS) EE (IC 0.5: 2.60) followed by the EE of (PS) (IC 0.5: 2.98) and OWS (IC 0.5: 6.11), while the EE of (PWS) showing the lowest reducing power (IC 0.5: 7.06).

In this assay, the Fe^{+3}/ferricyanide complex is reduced to the ferrous form due to the presence of reducers (i.e., antioxidants).

As a result, the Fe^{+2} content can be monitored by monitoring the production of Perl's Prussian blue at 700 nm **[51]**.

There was direct relation between reducing power value and TPC, where the extract that containing the highest TPC had the highest reducing power value. So, the order of the extracts is descending from the highest TPC to the lowest TPC, which is the same order from the highest reducing power to the lowest as followes: (OS) > (PS) >OWS > PWS **Table (3)**. The correspondence made mention of the high antioxidant potential of phenolics and flavonoids.

When the extracts were compared to ascorbic acid (**Table 3**), results illustrated that 15 mg/ml of OS (2.68) gave higher reducing power than 0.6 mg/ml of ascorbic acid (2.469), also 15mg/ml of PS (2.26) gave higher reducing power than 0.4 mg/ml of ascorbic acid (1.87). 15 mg/ml of OWS (1.06) and PWS (0.86) had higher reducing power than 0.2 mg/ml ascorbic acid (0.8).

3.3.2. DPPH Radical Scavenging

The DPPH radical scavenging assay was used to determine the antioxidant of the EEs of olive and plum stones. The results are shown in **Table (4)**.

Table 4: scavenging activity of ascorbic acid, olive and plum stones EEs on DPPH radical.

Ethanolic extracts	0.5 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml	15 mg/ml	IC 50 mg/ml
OS	19.57±1.31^{gh}	32.82±1.41^{e}	46.17±2.12^{d}	58.04±1.77^{b}	63.63±0.88^{a}	8.47±0.41^{a}
OWS	14.85±0.19^{i}	21.52±2.05^{g}	33.24±4.48^{e}	45.67±0.48^{d}	58.97±0.79^{b}	11.57±0.44^{c}
PS	18.42±0.81^{h}	29.96±0.21^{f}	45.41±1.56^{d}	52.96±0.42^{c}	63.20±0.75^{a}	9.21±0.02^{b}
PWS	13.71±1.33^{i}	21.78±0.31^{g}	34.57±1.22^{e}	45.35±0.93^{d}	58.48±1.76^{b}	11.65±0.48^{c}
Ascorbic acid (ST)	0.002 mg/ml 10.65	0.004 mg/ml 18.42	0.006 mg/ml 28.28	0.008 mg/ml 36.55	0.01 mg/ml 48.2	IC 0.5 mg/ml 0.011

Where: OS is olive seed, OWS is olive woody shell, and PS is plum seed, PWS is plum woody shell.
All values present means of three replicates ± SD.
Different letters in the same column refer to significant difference ($p< 0.05$).

The EE of (OS) and (PS) showed almost equal potency in the assay, while the EE of (OWS) and (PWS) also showed almost equal potency. The antioxidant activity was also measured as a function of concentration and was found to steadily rise with concentration. In comparing between EEs and ascorbic acid activities, the results showed that 15 mg/ml of all extracts had higher scavenging activity than ascorbic acid at all concentration (**Table 4**).

These results were in agreement with the investigation of **Ibrahim and Hikal [52]** who found that *Syzygium cumini* fruit EE had the highest reducing power and scavenging activity against DPPH radical, than *Eriobotrya japonica*, *Ziziphus spina-christi* and *physalis peruviana* L. fruits EEs because of *Syzygium cumini* (Sc) extract had a higher concentration of total phenolic , flavonoids, tannins and flavonols than other extracts. Antioxidant substances interact with free radical by giving an electron or a hydrogen atom to the free radical; therefore, it inhibits the effect of this free radical **[53].** Several fruits and vegetables components such as flavonoids, polyphenols compounds, terpenoids (β-carotene) and vitamins like vitamin C and tocopherol had high antioxidant activity **[54,55]**.

Plum biological activity depend on its high content of phenolic compounds **[56].** Plum had antioxidant capacity and can protect cells from oxidative damage which happen by free radicals. This activity may due to high concentration of phytochemicals such flavonoids, phenols, anthocyanins, etc. **[57].** Plum extract gave the highest scavenging activity, against DPPH and had higher reducing power than peach, nectarine, pear, and other stone fruits extracts **[58-60]**, this is due to the presence of rutin, vanillic, syringic, and chlorogenic acid **[61].**

There was direct correlation between the high scavenging ability, reducing power and high concentrations of TPC and TFC **[52, 62-64]** due to phenolic compounds and flavonoids contain an aromatic hydroxyl group, which in turn gives the hydrogen atom to harmful free radical, which becomes in active, while the phenolic ring is transformed into more stable radical through delocalization around the π- electron system **[65,66].** On the other hand, triphenols are higher reducer than diphenols and momophenol because o-position forms an intramolecular H bond between the hydroxyl groups by stabilizing the phenoxy free radical and elevating the molecule reducing properties **[67].** Furthermore, greater delocalization of the unpaired electron caused by antioxidative activity improves the reducing abilities of phenol derivatives: for example, caffeic acid is a stronger reducer than protocatechuic acid, and quercetin is a better reducer than catechin **[68].**

3.4. Anticancer Activity

Cancer is a complex disease that develops over time and is affected by gene-environment interactions. These interactions can lead to abnormal changes in many systems, such as DNA repair, apoptotic, and immunological responses **[69].** The anticancer activity of EEs of olive and plum stones on HT-29 (Colorectal Cancer) and MCF-7 (Breast Adenocarcinoma) were shown in (**Table 5**) and (**Table 6**) **respectively.** Generally, all extracts had significant anticancer activity on two kinds of cell lines, where it was noted that the effect of all extracts on HT-29 cells was higher than the effect on MCF-7 cells.

The anticancer activity of the extracts increased with increasing concentrations. when comparing anti-cancer activity of extracts at the highest concentration (1200 µg/ml) on HT-29 cells, the results showed that OS had the highest anticancer activity (99.6%) followed by PS (98.28%), OWS (92.69%), whereas PWS extract gave the relatively lowest anticancer activity (56.24%) (**Table 5**). On the other hand, there was an inverse relationship between the high anticancer activity of the extracts and the concentration that inhibits 50% of cancer cells (IC 50 value).

Table 5: Anticancer activity of olive and plum stones EEs on HT-29 cell line.

Extracts	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	1200 µg/ml	IC 50 µg/ml
OS	**12.58±0.59^{n}**	**21.02±0.61^{k}**	**37.89±0.59^{h}**	**71.24±1.07^{d}**	**99.60±0.26^{a}**	**580.96±3.97^{a}**
OWS	**11.79±0.53^{n}**	**19.12±0.53^{l}**	**33.80±0.56^{i}**	**63.26±0.51^{f}**	**92.69±0.64^{c}**	**634.81±4.85^{c}**
PS	**14.87±0.57^{m}**	**22.48±1.40^{j}**	**37.59±0.56^{h}**	**68.23±0.62^{e}**	**98.28±1.07^{b}**	**591.49±2.66^{b}**
PWS	**7.91±0.58^{o}**	**12.39±0.63^{n}**	**21.19±0.63^{k}**	**38.78±0.47^{h}**	**56.24±0.57^{g}**	**1038.08±5.43^{d}**

Where: OS is olive seed, OWS is olive woody shell, and PS is plum seed, PWS is plum woody shell.
All values present means of three replicates ± SD.
Different letters in the same column refer to significant difference ($p< 0.05$).

Table 6: Anticancer activity of olive and plum stones EE on MCF-7 cell line.

Extracts	100 µg/ml	200 µg/ml	400 µg/ml	800 µg/ml	1200 µg/ml	IC 50 µg/ml
OS	**9.82±0.61^{m}**	**14.88±0.64^{k}**	**25.34±0.62^{g}**	**45.77±0.66^{c}**	**65.97±0.60^{a}**	**881.86±6.22^{a}**
OWS	**3.76±0.63^{p}**	**6.46±0.72^{no}**	**12.14±0.58^{l}**	**23.36±0.61^{h}**	**34.39±0.58^{e}**	**1720.22±9.06^{c}**
PS	**5.71±0.61^{o}**	**10.14±0.58^{m}**	**18.91±0.59^{j}**	**36.26±1.09^{d}**	**54.24±0.62^{b}**	**1098.11±5.03^{b}**
PWS	**4.35±0.59^{p}**	**6.96±0.67^{n}**	**11.84±0.61^{l}**	**21.45±0.60^{i}**	**31.36±0.53^{f}**	**1868±4.03^{d}**

Where: OS is olive seed, OWS is olive woody shell, and PS is plum seed, PWS is plum woody shell.
All values present means of three replicates ± SD.
Different letters in the same column refer to significant difference ($p< 0.05$

When studying the effect of extracts on MCF-7 cells (**Table 6**), it was found that the extracts take the same behavior that they had on HT-29 cells in the same order, but less effective, where OS had the highest anticancer activity (65.97%), followed by PS (54.24%) and OWS (34.39%), while PWS had a comparatively low anticancer activity (31.36%) at the highest concentration 1200 µg/ml.

There were direct relation between antioxidant activity and anticancer activity, where the extract that gave highest antioxidant activity also gave the highest anticancer activity. Also, the anticancer activity was associated with the high content of phenolic compounds. These results were consistent with **Rotelli et al. [70]** who investigated that some biological components of olive tissue and olive oil by-products showed excellent efficacy against CRC. Where the phytochemicals presented in olive tissue and olive oil by-products may be contribute in prevention of colorectal cancer. Phenolic compounds such as flavonoids, tannins, saponins, as well as of some terpenoids had cytotoxic activity against human colorectal carcinoma **[71].** In contract, **Gigante et al. [72]** mentioned that although grape skin extracts from 'Egnatia N.' had lower TPC and total antioxidant capacity than Autumn Royal N, it has greater potency to influence on cell proliferation and apoptosis, as well as to exert a growth arrest in the S phase of the cell cycle, particularly in the Caco2 human colon cancer cell line. Flavan-3-ols which a precurcers of proanthocyanidins had the highest positive correlation with the anticancer activity of extracts. Plum extracts phenolic compounds which were purified could slow the growth of colon cancer cells and stimulate its differentiation *in vitro* **[69]**, and *in vivo* **[73]**, the presence of anthocyanin cause decrease risks of cancer, diabetes, arthritis, and cardiovascular diseases, that's was due to antioxidant and anti-inflammatory activities of anthocyanins**[74]. Mendonca et al. & Russo et al. [75,76]** mentioned that Plums' anti-cancer activities are correlated with its antioxidant effects.

Stone fruits' anti-inflammatory and antioxidant activities can both be correlated with their anticancer activity, these may be due to antioxidants can prevent oxidative damage to important cells macromolecules (proteins, DNA, and lipids), and therefore reducing cell death and neoplasia, which are the origin of many human diseases **[77].** Stone fruits can prevent the inflammatory processes in chronic diseases such as cancer, Alzheimer's disease, and diabetes**[78-81].** Plum extract had more pronounced antiproliferative effects on colon cancer cells (HT29) than estrogen-dependent breast cancer cells **[82].** Also, when plum extract cause increase in BAX protein expression and a decrease in (B-cell lymphoma 2) BCL-2 in cell lines which consequently led to an increase of apoptosis **[83].**

The anticancer activity of phenolics is primarily due to their antioxidant activity; they are potent free radical scavengers, metal chelators, modulators of self- defense mechanisms as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPx) **[84,85].** Furthermore, phenolics compounds anticancer effects are related to its ability to inhibit cell proliferation (extracellular signal-regulated kinase (Erk)1/2, D-type cyclins, and cyclin-dependent kinases (CDKs), and protein kinase B (Akt)), which leads to apoptosis, and preventing cellular migration and metastasis **[84,86]. Also,** the therapeutic activities of phenolic acids are augmented by their roles as epigenetic regulators and promoters of adverse events or resistance associated with conventional anticancer therapy **[87].** The anticancer activity of phenolic compounds depends on the presence of the following: the aromatic ring, double bonds in side chains and the number and position of free hydroxyl groups **[88].**

It's clear from the results that the extracts are rich in phenolic compounds that have high biological activity. As the antioxidant and anticancer activities of the extracts were associated with the concentration of phenolic compounds, also its may be due to the presence of, the following compounds: CHA, GA, MG, CAA, SYA, COA, VA, NAR, QUE and CA, so there were a positive relation between the concentration of the pervious extracts and their activities as antioxidant and anticancer.

FA appears antioxidant at low concentrations only, while at high concentration it play as prooxidant **[89].** FA exhibits cytotoxic properties against colorectal cancer Caco-2 cell line through elongating S/G2 phase and reducing G1 phase. Also, FA also seems antioxidant activity, where it turns off production of superoxide anion formation and prevents cancer migration via decreasing cell adhesion in adenocarcinoma lung A549 and colon HT29-D4 **[90].**

CAA, as well as its ester (CHA) have anti-mutagenic, anti-inflammatory, and anti-carcinogenic properties, which related to its antioxidant activity **[91].** It can decrease viability of HT-29 cell lines, by promotion of specific cell cycle alterations and induced apoptosis in a time and dose-dependent manner **[92].**

Interestingly, the antioxidant activity of CA can prevent formation of free hydroxyl radicals by its chelating iron properties and thus inhibiting the Fenton reaction that occurs as result of oxidative damage **[91].** Also, CA can prevent ROS -SOD-production and stop cancer progression and migration by reducing cell adhesion by decrease connection to extracellular matrix (ECM) in human lung A549 and colon adenocarcinoma HT29-D4 cells **[90].**

SYA displayed scavenging activity ,which may be attributed to the presence of two methoxy groups at positions 3 and 5 of phenyl ring **[85].** SyA's antioxidants, anti-cell proliferation, and apoptosis-inducing activities were mediated via the down-regulation of TBARS, LOOH, enzymatic (SOD, CAT and Gpx) and non-enzymatic antioxidants (vitamin E and GSH) and a decrease in PCNA, Cyclin D1, and mutant p53 expression **[93].**

It also shows antiapoptotic and chemosensitizing activity in human colorectal cancer SW1116 and SW837 cell lines through time-dependent activation of cell-cycle arrest at S/G2-M, G1/G2-M, and S/G2-M phases, as well as apoptosis, suppression of cell migration and NF- _B, and DNA binding **[94].**

GA has potent anti-inflammatory, and anticancer activities. It fights cancer by limiting cellular growth, promoting and producing reactive oxygen species (ROS), and stopping the cell cycle in the G2/M phase **[95].**

GA led to apoptosis via induction of ROS and GSH accompanied by the loss of mitochondrial membrane potential **[96].**

The ROS-dependent pro-apoptotic effects of GA have also been shown in declined viability of HCT-15 colon cancer **[95].** The capacity of GA to cause G1 phase arrest and death via the p38 mitogen-activated protein kinase/p21/p27 axis in MDA-MB-231 cells demonstrated that GA has therapeutic effects in triple-negative breast cancer (TNBC) **[97].** Cinnamic acid had many biological activities such as: anticancer, antifungal, antimicrobial, and antioxidant **[84].**

Also, p-COA has been demonstrated to inhibit HT 29 and HCT-15 colon cancer cell lines by causing mitochondrial mediated apoptosis via increased ROS levels and inhibition of the cell cycle at the sub G1 phase [88]. Furthermore, p-CA treatment inhibited glucose related protein 78, which is frequently unregulated in colon cancer, and activated apoptosis mediated by unfolded protein in colon cancer in vitro and in vivo models [98].

Furthermore, p-CA demonstrated a powerful antioxidant response and detoxifying mechanism, shielding the colon from genotoxic assault, with 100mg/kg body weight being the best dose.

3.5. Antibacterial Activity

Staphylococcus aureus, which are the mean reason for food-borne diseases, leading to a worldwide public health issue **[100].** *P. aeruginosa* can be found on the skin and in the physiological microflora of human intestines. In addition to it able to produce several harmful proteins which damage the tissue and cause an immunological insufficiency **[101].**

Many human diseases were caused by *E. coli* pathogenic strains **[102],** 90% of urinary tract infections that leads to inflammation of kidney are caused by *E. coli*. Also , it can lead to hemolytic uremic syndrome and colitis in humans **[103].**

The EEs of olive and plum stones were tested as an antimicrobial against genus *Staphylococcus aureus* ATCC 29737 (as Gr^{+ve}), and *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 (as Gr^{-ve}).

The results in **Table (7)** demonstrated that inhibition zone was increased by increasing extract concentration from 0.5 to 5mg/ml.

Table 7: Antibacterial activity of EEs of olive and plum stones (seeds and woody shells) and ampicillin against P. aeruginosa, E. coli and S. aureus bacteria

Treatment	Pathogenic bacteria/ Concentration(mg/ml)											
	P. aeruginosa				*E. coli*				*S. aureus*			
	0.5	**1**	**2.5**	**5**	**0.5**	**1**	**2.5**	**5**	**0.5**	**1**	**2.5**	**5**
OS	5.67± 0.28^{pq}	9.67± 0.28^{no}	11.67 ±0.28^{m}	19.67 ±0.57^{g}	NA	NA	NA	NA	6.33± 0.28^{p}	9.5± 0.5^{o}	10.33 ±0.2^{n}	11.83 ±0.28^{m}
OWS	3.17± 0.28^{s}	6.17± 0.28^{pq}	15.67 ±0.28^{i}	19.83 ±0.28^{fg}	NA	NA	11.33 ±0.57^{m}	14.33 ±0.57^{k}	1.83± 0.28^{t}	3.83± 0.28^{r}	5.5± 0.5^{q}	9.67± 0.28^{no}
PS	20.5± 0.5^{f}	23.33 ±0.28^{d}	25.67 ±0.28^{b}	26.83 ±0.28^{a}	14.67 ±0.7^{jk}	19.67 ±0.57^{g}	21.67 ±0.57^{e}	23.33 ±1.44^{d}	NA	NA	NA	NA
PWS	15.17 ±0.28^{ij}	20.33 ±0.28^{fg}	24.17 ±0.28^{c}	26.33 ±0.28^{a}	10±1no	12±0^{m}	12.67 ±0.28^{l}	18.17 ±0.28^{h}	NA	NA	NA	4±0^{r}
Ampicillin (10 µg)		8.0±0.25				9.0±0.54					12.0± 0.4	

Where: OS is olive seed, OWS is olive woody shell, and PS is plum seed, PWS is plum woody shell. Where: NA= No Activity.All values present means of three replicates ± SD. Different letters in the same column refer to significant difference ($p< 0.05$).

OS, OWS, PS, PWS extracts inhibited *P. aeruginosa* at all concentrations, the highest zone of inhibition was found in the EE of PS followed by PWS and OWS, while the lowest zone of inhibition was found in the EE of OS, since the inhibition zones were 26.83, 26.33, 19.83, and 19.67mm, respectively at concentration 5mg/ml. Also, the *E. coli* bacteria scored the highest inhibition by PS extract followed by PWS extract at all concentrations with inhibition zones of 23.33 and 18.17mm at concentration 5mg/ml, while OWS extract caused inhibition zone 11.33 and 14.33mm only at 2.5 and 5mg/ml, respectively. But the EE of OS did not possess any antibacterial activity when tested against *E. coli* bacteria.

On the other hand, the highest inhibition zone of *staphylococcus aureus* was caused by OS extract followed by OWS extract at all concentrations with inhibition zone of 11.83 and 9.67 mm at concentration 5mg/ml, while the extract of PWS had little inhibition zone 4.00mm only at 5mg/ml. In contrast, the extract of PS did not show any activity against *staphylococcus aureus* bacteria.

An important conclusion that an extract of PS may be able to treat infections caused by *P. aeruginosa* and *E. coli* and this agreement with data obtained **Lee et al. [104]**, while an extract of OS may have the potential to treat infections caused by *s. aureus*, according to further controlled experiments **[105,106].** This is caused by the presence of multiple different types of compounds (flavonoids, polyphenolic acids, and their esters), which most likely exert synergistic effects **[107]**.When comparing between antimicrobial activity of EEs and ampicillin , the results showed that the 2.5 and 5 mg/ml of EEs which gave higher inhibition zone against *P. aeruginosa* and *E.coli* than 10 µg of ampicillin.

The results were in agreement with the finding of **Markín et al. [108]** who mentioned that olive leaf water extract had bactericidal effects against *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pnebdmonia* and *B. subtilis*. On the other hand **Knobloch et al. [109]** reported that olive EE exhibited antimicrobial activity against *E. coli*. These antimicrobial activity due to phenolic compounds which present in extract such as oleuropein, RU, hydroxytyrosol, and CAA **[110-113].** Also, *S. aureus* growth was inhibited by CA, oleuropein, RU, and verbascoside **[114,115,112,116],** where phenolic compounds had effect on the secretion of exoprotein by this organism **[117]**.

Plum seed EE inhibited Gram (-) bacteria (*P. aeruginosa* and *E. coli*) growth by 96% **[118]**. These effect may be due to presence of flavonoids, polyphenolic acids, and their esters, which have the synergistic effects**(Savic et al. & Aziz et al. [103,119]** concluded that VA, SYA, and QUE consider a good antimicrobial compounds. Also, GA has high influence on several pathogenic microbes **[120].** Also, **Debnath et al. [121]** said that EE of

plum seeds had inhibitory effects against (*Pseudomonas aeruginosa* NCIM5029 and *Escherichia coli* NCIM2118). Gr^{-ve} bacteria is highly resistance bacteria that may be due to their outer cell membrane which composed of lipopolysaccharides (hydrophilic) so limits the diffusion of hydrophobic molecules **[122,123]**. While Gr^{+ve} bacteria had lipophilic ends of the lipoteichoic acids of their outer cell membrane it allow penetration of hydrophobic compounds **[124]. Rhayour et al. [125]** mentioned that aromatic compounds group had an antibacterial activity that may be due to its ability to inhibit production of amylase and protease by *bacteria* , deteriorate cell wall, and cause cell lysis **[126]**.

The results illustrated that the antimicrobial activity against gram negative bacteria (*P. aeruginosa* and *E. coli*) may be related to the presence of rutin and catechin where the concentration of these compounds in the extracts gradient with the same gradient of antimicrobial activity. So, PS had the highest activity against *P. aeruginosa*, followed by PWS, OWS and OS which gave the lowest activity. The same effect of extracts was observed against *E. coli*, except that OWS extract gave growth inhibition only at high concentration (2.5 and 5 mg/ml), while OS extract didn't give any activity against *E. coli*, these results may be due to the lower catechin content of OWS and its disappearance in OS extract. Rutin had antimicrobial activity against Gr- bacteria (*P. aeruginosa* and *E.coli) these effect due to* anti-biofilm potential against drug-resistant biofilm-producing bacteria **[127,128].** Catechin may be inhibit the function of the cytoplasmic membrane and that licochalcones A and C prevent metabolism of energy **[128].** CAT and GA (belong to pseudo tannins) may be inhibit or reduce catalase activity in microorganism, where this enzyme was necessary to reduce H_2O_2 level which was harmful for many pathogens because it can convert to reactive oxygen species (ROS). ROS induce oxidative or nonoxidative damage; these cause bactericidal effect **[129]. Aksun et al. [130]** reported that gallic acid had highly antimicrobial activity against Gram-negative pathogens. Also, GA, QUE, CAA, COA and catechol were effective in inhibiting the growth of *E. coli* and *P. aeruginosa*

On the other hand, the antimicrobial activity of OS, OWS, and the highest concentration of PWS on gram positive bacteria (*S. aureus*) where OS extract gave the highest activity these may be due to the presence of caffeic acid, ferulic acid, daidzein, apigenin, kaempferol (total content of this compounds 2.335 mg/g DW), while OWS activity was may be due to the presence of high concentration of FA which compensated for CAA decrease, as well as the presence of HES. The others compounds in each extracts other than the ones mentioned participate in antimicrobial activity also through synergistic effect as follows: **Bhawna et al., Kosina et al., Mandalari et al., & Aksun et al. [131-133,130]** showed that CAA, NAR, p-COA and SYA have antimicrobial activity towards Gram-positive like *Bacillus spp*., and Gram-negative bacteria including *E. coli*. CAA and its ester (CHA) exhibits antibacterial properties. **[118]. Cushnie and Lamb [134]** investigated that QUE had antibacterial activity where they inhibit production of bacteria lipase **[135]** and inhibit activity of d-alanine ligase which occurs in production of peptidoglycans **[136].**

The phenolic compound daidzein has no activity against *E. coli, P. aeruginosa* and *S. aureus* **[137].** While, the HES compound showed high scavenging activity against DPPH radical, high anti-inflammatory effect in RAW264.7 cells and had higher antibacterial activity against Gr^{+ve} bacteria than on Gr^{-ve} (125 µg/mL against *S. aureus*, 250 µg/mL *E. coli* and 500 µg/mL against and *P. aeruginosa*) this may be due to HES is an aglycone form with high Log P value (poorly water soluble) showed high antibacterial activity against the Gram-positive and Gram-negative bacteria **[138].**

Flavonoids interact with lipid bilayers through two ways **[139].** The first is related with the more hydrophobic part of the membrane which is more non polar chemicals, while the second one the more hydrophobic flavonoids can form hydrogen bonds with polar head groups of lipids at the membrane. Also, flavonoids can interact nonspecifically with phospholipids and change structure features of the membrane (e.g., thickness and fluctuations) and indirectly altering membrane proteins functions **[140].** The flavonoids and lipid bilayers interaction influenced by -OH groups number and distribution, the polymerization degree, as well $–OCH_3$ groups as the presence in the C ring, can influence the type of interactions that occur between lipid bilayers and

flavonoids **[141].** Moreover, lacking –OH groups on their B Rings increase the activity against microbial membranes than those with the –OH groups **[142].** This is due to opposite correlation between the hydrophobicity of flavonoids and the -OH groups present. There are another mechanism by which flavonoids inhibit bacterial growth, where it cause aggregation of bacterial cells, this results in the formation of biofilm and bacteria become more resistant by 10 to 1000 times than single **[143].** Also, flavonols cause bacterial cell aggregation **[144].** Flavonoids cause bacterial aggregation by their partial lysis, which leads to membrane fusion, and consequently decrease the active nutrient uptake via a smaller membrane area, thus it cannot be stated that flavonoids support biofilm formation **[144].** In the contrary, some flavonoids inhibit biofilms formation **[145].** Such as QUE inhibit enteroaggregative *E. coli* EAEC 042 biofilm **[146].**

CAT had antimicrobial effects, where CATs bind with the lipid bilayer of bacterial membrane and by inactivating or inhibiting intracellular and extracellular enzymes synthesis **[147].** On the other hand, CAT can generate ROS that that cause alteration in the membrane permeability and membrane damage **[148].** CATs have less effect on Gr $^{-ve}$ bacteria due to negatively charged Liposome of the outer bacterial membrane **[149].** So, catechins have lower antibacterial activities against Gr $^{-ve}$ bacteria than Gr $^{+ve}$ bacteria **[150].** Moreover, the membrane disruption by catechins causes potassium leakage in methicillin-resistant *S. aureus* strain (MRSA). CAT cause pseudomulticellular aggregates of *S. aureus* cells **[151].** Among antimicrobial mechanism of flavonoids is inhibition of peptidoglycan synthesis, where it is essential component of bacterial cell wall **[152].** Catechins can interfere with bacterial cell wall biosynthesis by binding with the peptidoglycan layer. **[153]**

Naringenin (flavanone) had antibacterial activity against MRSA may be due to it can reduce the fluidity in hydrophilic and hydrophobic regions of the both inner and outer cellular membrane **[154].** Flavonols quercetin and rutin decreased the bilayer thickness, furthermore rutin disrupted the lipid monolayer structure **[155]**

QUE (flavonol) can decrease of proton-motive force in *S. aureus* , that cause increase membrane permeability **[156].** Moreover, **Ollila et al. [157]** showed that apigenin caused membrane structure destabilization via disordering and disorientation of the membrane lipids and induced leakage from the vesicle. QUE and API inhibit D-alanine-D-alanine ligase, which necessary for the production of dipeptide of peptidoglycan precursor, where two flavonoids bind to the active center of D-alanine-D-alanine ligase. However, QUE had poorer activity compared to API, which is attributed to its additional -OH groups at C ring that enforce its affinity to the enzyme. **[158].** Furthermore, the hydrophilic nature of QUE limits its penetration into the bacterial cell. In addition to that QUE and API inhibit DNA gyrase from *E. coli* which is an essential enzyme for the DNA replication **[159].** Where, QUE associates to the B subunit of gyrase and forms hydrogen bonds via 5, 7 and 3' –OH groups to the amino acid residues of DNA gyrase thus preventing the association of ATP with the enzyme **[160].** KAE greatly inhibited DNA gyrase from *E. coli* **[161].** OUE inhibited alginate production; this results in reduced biofilm formation. Also, QUE inhibited N-acyl homoserine lactones (AHL)-mediated QS. Most notably, QUE regulates several iron siderophore proteins expression limiting the amount of Fe^{+3} which required for the biofilm formation in *P. aeruginosa* **[162].**

Conclusion

This study concluded that, the OS extract contained the greatest concentration of antioxidant compounds, and it had the highest antioxidant activity. The PS extract had high content of TFC and high antioxidant activity, but it ranked second when compared with OS extract. While, the extracts of OWS and PWS had the lowest TPC and antioxidant activities. The pathogen *P. aeruginosa and E. Coli* scored the highest inhibition zone by PS and PWS extracts, while *S. aureus* was inhibited by OS and OWS only. On the other hand, the OS extract has high effect toward HT-29 cells more than MCF-7cells. “This work will provide a foundation for further phytochemical and pharmacological research.

Conflicts of interest

There are no conflicts to declar

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