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In Vitro Antioxidant, Antimicrobial and Anticancer Activities of Olive (*Oleaeuropaea* L.) Pomace

Wahdan, K. M. M. and T. F. Taha
Biochem. Dept., Fac. Agric., Zagazig Univ., Egypt

ABSTRACT

Egyptian olive pomace was collected from Presses Salehia Oils (Salhia, Sharkia, Egypt).The investigation was performed to study the composition (moisture, crude fat, crude protein, carbohydrate, fiber and total ash) of olive pomace. The qualitative phytochemical analysis stated the presence of flavonoid, tannins, and alkaloids, in acetone and methanolic70% extracts of olive pomace. Saponin and reducing sugar were found in methanol extract but not found in acetone extract. Starch, monosaccharide, reducing sugar and anthraquinone glycosides were not found in acetone and methanol extracts. Terpenoidswere found in acetone extract and not found in the methanol extract. Results stated that the phytochemicals recovery dependedupon the polarity of the used solvent. Total flavonoids and total polyphenols were determined. Glycones, aglycones and simple phenolic compounds in olive pomace acetone and methanol extracts were detected by HPLC. Antioxidant activity, antibacterial properties against gram-positive and gram-negative bacteria as well as fungi and anticancer activity of olive pomace extracts were studied. Olive pomace was found to possess a very potent inhibitory effect against hepatocellular carcinoma (HePG2)cell line, breast carcinoma (MCF7 and T47D)cells lines and colon carcinoma (HCT116) cell line.

Keywords: Phytochemicals, Agriculture by-products, Industrial wastes, Antimicrobial, Antifungal, Anticancer.

INTRODUCTION

Polyphenols (PPs) are constituents in thehuman diet wherein vegetables and fruits areconsumed. However, data on quantitative PPs intake is not sufficient, because of factors that modify PPs levels in foods and the wide range of PPs. PPs have high antioxidant potential*in vitro* being able to scavenge reactive chlorine, nitrogen, and oxygen species (peroxyl and hydroxyl radicals) as well as superoxide anion and hypochlorous acid (Petti and Scull 2009). In addition, PPs chelate metal ions to decrease their pro-oxidant properties. High PPs intake were associated with decreased risks of cardiovascular diseases, and cancers. Such biological traitswere *in vitro* evaluated on cultured cells, enzymes, or isolated tissues (Halliwell *et al.,* 2005). Olive pomace (OP) is an important industrial agricultural waste resulted from the olive processing. The pomace is often used for the extraction of OP oil with *n*-hexane, that has a considerable added-value (Saviozzi*et al.,* 1993). Some olive oil industries, owing to the environmental problems, are applying the biphasic systems of extraction (Civantos, 1999). Due to the retention of the moisture in the pomace, the obtained OP (*ca*. 80% w/w, with *ca*. 3% lipids) is usually wet (ca. 55-60% moisture). Because of the energy needed in drying steps, the olive pomace does not have a value (Civantos, 1999). Recovery of value-added products from this pomace is an important way of valorizing it. Olive contains high levels of phenolics, such as oleuropein, ligstroside, dimethyl-l oleuropein, and their hydrolytic derivatives, elenoic acid, oleuropeinaglycones, hydroxytyrosol, tyrosol, and oleoside-11-methyl ester (Mulinacci *et al.,* 2001). Olive also contain rutinglucosides, hydroxycinnamic acids derivatives, luteolin -7- glucoside, anthocyanins (i.e., cyanidin and delphinidin glycosides), and ahigh level of verbascoside (Mulinacci *et al.,* 2001). These phenolic compounds were detected in olive pomace, although the levels ofsecoiridoidscould be decreased by the malaxation during the production of olive oil (Romero *et al.,* 2002). Oleuropein and hydroxyl-tyrosol have important biological traits such as hypolipidemic, hypocholesterolemic ,antimicrobial, hypoglycaemic, and antiradical actions (Bisignano *et al.,* 1999).

The OP, possess high levels of organic compounds (14-15%) including volatile fatty acids, sugars, pectins, nitrogenous compounds, andalcohols (Lafka *et al.,* 2011) and high levels of phenolic compounds (Ranalli *et al.,* 2003). Therefore, disposal of these waste products has been an environmental issue in many olive-growing countries (Capasso *et al.,* 1992). Extraction of the phenolics from OP has the potential to limit the environmental problems and might provide a source of income for olive producers (Obied *et al.,* 2005).

The goal of this work was to investigate the phytochemicals profile as well as the antioxidative, antimicrobial and anticancer properties of OP acetone and methanolic70% extracts.

MATERIALS AND METHODS

Pomace and chemicals

Olive pomace came from Presses Salehia Oils, Salehia, Sharkia, Egypt. Methanol and acetone were from E. Merck (Darmstadt, Germany). Standards of naringenin, quercetin, luteolin, hesperidin, apigenin and kaempferol were from Fluka (Germany). Standards were prepared (5 mg/50 mL) as stock solutions in methanol, except for apigeninand luteolin (5 mg/50 mL DMF: MeOH). Standards were prepared by diluting stock solutions in methanol to yield 2-4 µg/mL. Standards stock or working solutions were stored at -18°C in darkness. Other reagents were from Merck (Darmstadt, Germany)

Preparation of olive pomace extracts

Olive pomace dried in an oven at 50°C then powdered using a grinder and stored in air-tight jars at 4°C. Dried materials (250 g) were defatted using n-hexane then extracted successively with the following solvents: acetone then methanol (70%) with 2500 mL (1: 10 v/v) by soaking for 48 h. The extract was centrifuged at 2000 rpm 15 min (Jouan, MR 1822, France). The extraction and filtration were repeated till residue was colorless. The residue was re-extracted with methanol followingthe same steps. The solvent was evaporated under vacuum at 40ºCusing a Laborota 4000-efficient (Heildolph، Germany) rotary evaporator. The extracts were freeze-dried using lyophilizer. The obtained powdered extracts were kept at -18ºC in light-protected containers.

Chemical composition and phytochemical screening of olive pomace extracts

Moisture, crude fiber, ash, crude protein and crude lipid were determined in the extracts (AOAC, 2005). Total carbohydrate was determined according to Dubois *et al.,* (1960).The following constituents were determined in extracts; tannins (Trease *et al.,* 1978), flavonoids (Tadros, 1979), alkaloids and/or nitrogenous bases (Balbaa *et al.,* 1967), sterols and/or triterpenes and saponins(Tadros, 1979) unsaturated sterols and/or triterpenes(Amer *et al.,* 1994),and anthracenoside(Jagessar and Cox 2010).

Determination of total flavonoid content (TFC)

TFC was determined according to Ordon *et al.* (2006) with modification. A 1.5 mL of 20 g L^{-1} $AlCl_3$ ethanolic solution were added to 0.5 mL of extract solution (10 mg/10 mL solvent). After 60 min, the absorbance was measured at 420 nm. The extracts were assessed at a concentration of 1 mg/mL. TFC expressed as quercetin (QE), was computed according to the following equation:

$$y = 0.02248x$$
$$R^2 = 0.992$$

Where x is absorbance, y is concentration (µg QE), and R^2 is the correlation Coefficient.

Determination of total phenolics content (TPC) in extracts

The concentration of TPC in pomace extracts were determined by Jenway-UV–VIS Spectrophotometer, as described by Skerget*et al*. (2005).The used reagent was Folin-Ciocalteu (AOAS, 1990). Briefly, 200 µL of pomace acetone and methanol 70% extracts of, solution containing known concentrationweretaken separately in a test tube, and then 1 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water), after reacting for 8 min, 800 µL of 7.5% sodium carbonate were added, the mixture was allowed to stand for further 30 min. The phenols were measured at 765 nm.TPC expressed as gallic acid equivalent (GAE) was calculated using the following equation:

$$y = 0.0127x + 0.0505$$
$$R^2 = 0.9963$$

Where x is absorbance, y is the concentration (µg GAE), and R^2 is correlation coefficient.

Radical scavenging activity (RSA) of methanol extract

RSA of the methanol 70% extract was tested with DPPH· radicals in methanol. A methanolic DPPH· was prepared at 10^{-4} M. 100 µL of methanol extract was mixed with 5 mL DPPH· methanol solution and the mixture was vortexed for 20 sec. against a blank of methanol , the absorption was measured at 517 nm after zero, 30, 60 and 120 min, using Jenway-UV–VIS Spectrophotometer. RSA was calculated from the differences in absorbance of methanolic DPPH· with or without sample. The% of inhibition percent was calculated according to Mensor *et al.* (2001).% Inhibition = [(absorbance of control-absorbance of test sample)/absorbance of control] × 100

High-performance liquid chromatography (HPLC) of phenolics

HPLC was used to separate and identify phenolics in the pomace extracts. The analysis of pomace extracts was carried out with Hewlett Packard Series 1050 (USA) HPLC, equipped with Hypersil BDS 5 um C18 column, and sampling injector using quaternary HP pump (series 1100). Iso-gradient separation at 35°C was performed with methanol and acetonitrile at a flow rate of 1 mL/min. The UV detector was set at 280 and 330 nm for phenolics and flavonoids, respectively. Retention times and peak areas were applied to calculate phenolics and flavonoidslevels by the data analysis of HEWLETTpackard software (Goupy *et al.,* 1999).

***In vitro* antimicrobial activities of extracts**

Antimicrobial traits of the tested OP extracts were tested using a modified Kirby-Bauer (Bauer *et al.,* 1996) disk diffusion method. Agar-based techniques like E test and disc diffusion are faster and simpler than broth-based technique (Liebowitz *et al.,* 2001; Matar *et al.,* 2003).

Hundred µL of the tested bacteria or fungi were grown in 10 mL of fresh media till they reached about 105 cells/mL for fungi or 108 cells/mL for bacteria (Pfaller *et al.,* 1988). Hundred µL of the microbial suspension spread onto the agar plates corresponding to the broth that was maintained. Isolated colonies that might play a pathogenic role must be tested for susceptibility by disc diffusion method (NCCL, 1993; 1997). Mueller-Hinton agar was recommended due to its good batch-to-batch reproducibility. Disk diffusion methods for filamentous fungi tested using standard method (M38-A) approved by the NCCLS (2002) to evaluate the susceptibility of fungi to antifungal factors. Disc diffusion methods for yeasts were applied using standard method (M44-P) approved by NCCLS (2003). Plates were inoculated with fungi (i.e. *Aspergillus flavus*) for 48 h at 25°C; gram positive bacteria (i.e. *Bacillus subtilis*, and *Staphylococcus aureus*); gram negative bacteria such as *Pseudomonas aeuroginosa* and *Escherichia coli*at 35-37°C for 24-48 h, and yeast (i.e. *Candida albicans*) were incubated for 24-48 h at 30°C.

The diameters (mm) of the inhibition zone were recorded (Bauer *et al.,* 1996). Standard discs of amphotericin B (an antifungal agent) and tetracycline (antibacterial agent) used as antimicrobial controls. Filter discs impregnated with 10 µL solvent (chloroform, distilled water, and DMSO) served as a negative control. Agar utilized is Mueller-Hinton agar that was rigorously examined for pH and composition. The technique was well documented and standard inhibition zones were determined for resistant and susceptible values. For the disc diffusion, the zone diameters were recorded with slipping calipers (National Committee for Clinical Laboratory Standards NCCLS , 1993).

Measurement of cytotoxicity

MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) *assay*

Cell viability was evaluated by the mitochondrial-dependent stenography (Mosmann, 1983) of yellow MTT to purple formazan. The procedure was achieved in a sterile area using a laminar stream cabinet (biosafety class II level, Baker, SG403INT, ME, USA). Cells were suspended in RPMI 1640 medium for HePG2, HCT116 and MCF7, 1% antibiotic-antimycotic mixture (10,000 µg/mL streptomycin sulfate, 10,000 U/mL potassium penicillin, and 25 µg/mL amphotericin B) and L-glutamine (1%) at 37 ºC under 5% CO_2. Cells are cultured for 10 days, then seeded at $10x10^3$ (cells/well) in a growth medium in 96-well microtiter plastic plates at 37ºC for 24 h

under CO_2 using a water-jacketed CO_2-incubator (Sheldon, OR, USA). Media was aspirated, fresh medium without serum was added then cells were incubated either alone (negative control) or with different samples concentrations to give 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL concentrations. The medium was aspirated after 48 h, 40 uL MTT salt (2.5 ìg/mL) were added to each well and incubated under CO_2 for 4 h at 37ºC. 200 ìL of 10% SDS in deionized water added to h well and incubated overnight at 37ºC to stop the reaction and dissolving the formed crystals. A positive control (100 µg/mL) was utilized (Thabrew *et al.,* 1997; Menshawi *et al.,* 2010). The absorbance was recorded at 595 nm (reference wavelength of 620 nm) using a microplate multi-well reader (model 3350, Bio-Rad Laboratories, CA, USA). A statistical significance between samples and negative control was analysed using independent t-test (PSS 11 program). DMSO used to dissolve the OP extracts (concentration on the cells less than 0.2%). The percentage of the adjustment in viability was determined[(Reading of extract / reading of negative control) -1] x 100

Srb Assay

Potential cytotoxicity of the olive pomace extract was tested for breast cancer carcinoma cell line T47D and normal cell line (human dermal fibroblast cell line (HFB4) according to Skehan *et al.* (1990). Cells T47D and HFB4 were plated in 96-multiwell plate (104 cells/well) for 24 h before the treatment with the extract to allow the attachment of cells to the wall of the plate. Several concentrations of the tested extract (12.50, 25.00, 50.00 and 100.00 ìl/mL) added to the cells monolayer, 6 replicates wells were performed for dose. Monolayer cells were incubated with OP extract for 48 h at 37ºC and in 5% CO_2. Cells were constant after 48 h and washed then stained with sulfo-Rhodamine-B stain. Color intensity was estimated with ELISA reader. The relation between drug concentration and surviving portion was plotted to get the survival bend of tumor cell-line after the predetermined compound. IC_{50} of the extract against both HFB4 and T47D cell lines was figured utilizing the survival curves.

Statistical analysis

A probit analysis was performed for IC_{90} and IC_{50} determinations using SPSS 11 program.

RESULTS AND DISCUSSION

Composition and phytochemical profile of olive pomace

The moisture content of olive pomace was 5.49%. Protein content was in the range of 5.17%, total lipids were 18%. The ash content was 8.44%. The fiber content of both studied plant was 57.5 %. Finally, the carbohydrate contentswere11.5%. Table 1 presents the qualitative results for the presence of some bioactive chemicals in olive pomace. Preliminary or qualitative phytochemical analysis showed the presence of flavonoid, tannins, and alkaloids, in acetone and methanol extracts. Saponin and reducing sugar were found in methanol extract but not found in acetone extract. Starch, monosaccharide, reducing sugar and anthraquinone glycosides were not found in acetone and methanol extracts. Terpenoidswere found in acetone extract and not found in the methanolic extract. Results stated that the phytochemicals recovery depended on the polarity of the used solvent.

Table 1. Phytochemical profile of *Oleaeuropaea*L pomace

Compound	Acetone extract	Methanol extract
Flavonoids	++++	+
Tannins	+green	light green
Alkaloids	+	+
Saponine	-	+
Reducing sugar	-	+
Starch	-	-
Barfoids	-	-
Anthraquinone glycosides	-	-
Terpenoids	+	-

Total flavonoid contents were 15.19 g/100g dried acetone extract and were 20.052 g/100g dried methanol extract. Total polyphenol content was 22.746 g/ 100gdried acetone extract and was 34.12 g/100g dried methanolic extract. Methanolic extract was characterized by a higher content of total flavonoids and total polyphenol.

Phenolics profile of olivepomace acetone and methanol extracts

Data illustrated in Tables 2 and 3 cleared that thirty-six compounds were tentatively identified of glycones, aglycones and natural organic compounds in olive pomace. Rutin was absent in olive pomace acetone extract.Syringic acid was the major natural organic compounds being 0.63698 and 4.08239 g/100 g dried acetone or methanol extracts, respectively.

Radical scavenging activity (RSA) of olive pomace extracts againstDPPH· free radicals

RSA is utilized to test the antioxidant traits in a short time (Schwarz *et al.,* 2000). The results of RSA for methanolic extract after 15 min, 30 min and 60 min with DPPH· and control are shown in Table 4. Methanolic extract had high values of RSA, it was 17%, 79%,80.7 % and 83 % for methanolic extract of pomace, respectively.

The results of radical scavenging activity (RSA) for acetone extract after 15 min, 30 min and 60 min with DPPH· are presented in Table 4. The radical scavenging activity of the three studied peels showed low values, it was 4.4%, 8%, 10.7% and 20.4% for pomace acetone extract.

Flavonoids and tannins that found in the studied extracts are phenolics wherein the plant phenolicmixes are a noteworthy gathering of aggravates that go about as cell reinforcements or radical scavengers. DPPH· provides data about the reactivity of the tested compounds with DPPH·. When the odd electron becomes paired off with a radical scavenger, the absorption reduces and DPPH· solution is . The degree of reduction in the absorbance is an indicative of the antiradical potential of the compounds or the extract (Ramadan *et al.,* 2003).

Table 2.Phenolic compounds in olive pomace acetone extract (g/100 gdried extract)

Type of phenolic compound	Identified compound	Retention time(min)	Conc. (g 100 g DW)
	Glycosides compounds		
1	Narengin	12.487	0.30248
2	Rutin	–	ND
3	Quercitrin	13.563	0.02234
	A glycone compounds		
4	Catechin	8.524	0.02815
5	Epicatechin	9.646	0.01607
6	Ellagic acid	13.169	0.05666
7	Coumarin	14.404	0.00213
8	Quercetin	15.160	0.00804
9	Narenginin	15.468	0.00797
10	Kaempferol	15.668	0.00819
11	Luteolin	15.744	0.05468
12	Hisperitin	15.827	0.00137
13	Apigenin	16.835	0.01237
14	7-Hydroxyflavon	17.805	0.06571
	Natural organic compounds		
15	Syringic acid	3.515	0.63698
16	Gallic acid	6.920	0.00138
17	Pyrogallol	7.024	0.03552
18	4-Aminobenzoic acid	8.157	0.00258
19	Protocatechuic acid	8.323	0.04408
20	Chlorogenic acid	9.087	0.14328
21	Catechol	9.376	0.01975
22	Caffeine	9.801	0.01024
23	P. OH.benzoic acid	9.855	0.00866
24	Caffeic acid	10.206	0.00557
25	Vanillic acid	10.327	0.01554
26	P-Coumaric acid	11.760	0.024
27	Ferulic acid	12.024	0.03437
28	Iso-ferolic acid	12.407	0.00997
29	E-Vanillic acid	12.864	0.34969
30	Rosmarinic acid	12.965	0.00881
31	o-Coumaric acid	13.637	0.00521
32	Benzoic acid	13.767	0.12362
33	Salicylic acid	14.184	0.03059
34	3,4,5Methoxycinnamic acid	14.379	0.00359
35	Cinnamic acid	15.726	0.00824
Total			2.10783

Table 3. Phenolic compounds inolive pomace methanol extract (g/100 g dried extract)

Type of phenolic compound	Identified compound	Retention time (min)	Conc. (g 100 g DW)
	Glycosides compounds		
1	Narengin	12.487	0.13918
2	Rutin	12.535	0.4229
3	Quercitrin	13.563	0.03661
	A glycone compounds		
4	Catechin	8.524	0.13984
5	Epicatechin	9.646	0.06403
6	Ellagic acid	13.169	0.17147
7	Coumarin	14.404	0.01011
8	Quercetin	15.160	0.01988
9	Narenginin	15.468	0.01669
10	Kaempferol	15.668	0.01568
11	Luteolin	15.744	0.20339
12	Hisperitin	15.827	0.00183
13	Apigenin	16.835	0.01037
14	7- Hydroxyflavon	17.805	0.00442
	Natural organic compounds		
15	Syringic acid	3.515	4.08239
16	Gallic acid	6.920	0.01774
17	Pyrogallol	7.024	0.64372
18	4-Aminobenzoic acid	8.157	0.05067
19	Protocatechuic acid	8.323	0.06095
20	Chlorogenic acid	9.087	0.3175
21	Catechol	9.376	0.07391
22	Caffeine	9.801	0.03159
23	P. OH-benzoic acid	9.855	0.04522
24	Caffeic acid	10.206	0.06126
25	Vanillic acid	10.327	0.03497
26	*P*-Coumaric acid	11.760	0.04763
27	Ferulic acid	12.024	0.02508
28	Iso-ferolic acid	12.407	0.01772
29	E-Vanillic acid	12.864	0.51396
30	Rosmarinic acid	12.965	0.01646
31	*o*-Coumaric acid	13.637	0.03853
32	Benzoic acid	13.767	0.19928
33	Salicylic acid	14.184	0.13118
34	3,4,5Methoxycinnamic acid	14.379	0.01846
35	Cinnamic acid	15.726	0.02261
Total			7.70723

Table 4. Scavenging activity of olive pomace extracts against DPPH· free radicals

Time (min)	% inhibition		
	Acetone extract	Methanol extract	BHT (Standard)
Zero	4.4 ±0.31	17± 0.3	71.55d± 0.27
30	8± 0.29	79±0.29	81.99c± 0.79
60	10.7± 0.31	80.7± 0.33	89.86b± 0.36
120	20.4± 0.31	83± 0.32	94.40a± 0.36
LSD at 0.05	0.47	0.86	0.47

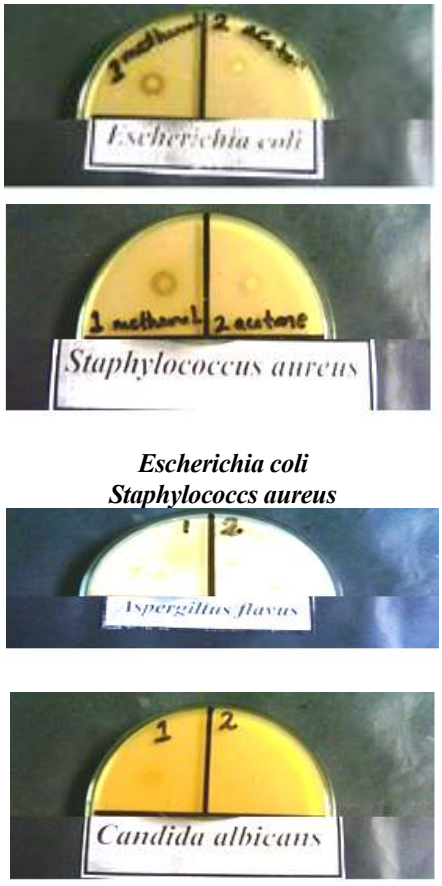
LSD: the least significant difference

Antimicrobial activity of olive pomace extracts

Acetone and methanolic 70% extracts of pomace weretested for antibacterial activity (Table 5). The result showed thatpomace extracts had the same anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli*. There was no activity against *Candida albicans* and *Aspergillus flavus*(Figure1). Olive pomace could be considered as a good antibacterial agent against both gram-positive and gram-negative bacteria to replace the synthetic medicines in thetreatment of diseases caused by these bacteria.

Table 5. Inhibition zone diameter (mm) of acetone and methanol extracts of olive pomace against tested bacteria and fungi on Mueller-Hinton agar mediumusing disc diffusion method

Sample		Inhibition zone diameter (mm/mg sample)			
		Escherichia coli (G-)	*Staphylococcus aureus* (G+)	*Aspergillus flavus* (fungus)	*Candida albicans* (fungus)
Control (DMSO)		0.0	0.0	0.0	0.0
Standard agent	Ampicillin Antibacterial agent	22	18	…	…
	Amphotericin B Antifungal agent	…	…	17	19
Acetone extract		10	10	0.0	0.0
Methanol 70% extract		14	15	0.0	0.0



Escherichia coli
Staphylococcs aureus

***Aspergillusflavus* (fungus)**
***Candida albicans* (fungus)**

Figure 1. Inhibition effect (mm) of olive pomace acetone and methanol extracts against fungi and bacteria on Muelle-Hintonagarmedium using disc diffusion method

Cytotoxic effect of pomacemethanolic extract on the human cell line (HCT116, HePG2, MCF7, T47D and HFB4)

The results showed that olive pomace methanol extract exhibited a strong cytotoxic impact (Tables 6-8, Figures 2 and 3). Olive pomace was found to possess very potent inhibitory traits against hepatocellular carcinoma cell line (HePG2), breast carcinoma cells lines (MCF7-T47D) and colon carcinoma cell line (HCT116) cell lines. Methanol extract reachedto 91.3 %, 100%, 100 % and 51.2% against HCT119, HePG2, MCF7 and T47D, respectively. IC_{50}ofthis extract against HCT119, HePG2, MCF7 and T47Dcell line equals 46.5, 90.4, 24.4 and 50.6 µL/mL, respectively. This effect of olive pomace extract may be due to the high amount of phenolic compounds .These compounds work on the signal genes to increase the levels of enzymes involved in the detoxification and anti-carcinogenic actions. In addition, other compounds in OP have a great role on some cancer cell and the effect of anticancer in the present extract may be due to these compound. According to properties of natural polyphenols such as phenolic acids and flavonoids, several polyphenols such as chlorogenic acid, caffeic acid, gallic acid, p-coumaric acid, kaemferol, and naringenin were detected in the urine, indicating their absorption and bioavailability. Marilena *et al.* (2004) studied the ant proliferative action of caffeic corrosive, syringic corrosive, sinapic corrosive, protocatechuic corrosive, ferulic corrosive and 3,4-dihydroxyphenylacetic corrosive on the human breast cancer T47D cell line, at fixations pretty much like those normal from ordinary utilization of sustenances. Their outcomes demonstrate that phenolic acids deliver development hindrance of disease cells, *in vitro*, showing an extra defensive e on hormone-subordinate breast tumors. *In vitro* study of olive pomacemethanolic extract showed no activity on the normal cell line (human dermal fibroblast cell line,HFB4).

Table 6. Cytotoxicity effect of olive pomace methanolextracton HCT116 (Colon cell line)

Sample	LC_{50} (µg/mL)	LC_{90} (µg/mL)	% Inhibition
Olive pomace	46.5	83.5	91.3% at 100ppm
DMSO	--------	--------	1% at 100ppm
Negative control	--------	--------	0 %

Table 7. Cytotoxicity effect of olive pomace methanol extracton HePG2 (Human hepatocellular carcinoma cell line)

Sample	LC_{50} (µg/mL)	LC_{90} (µg/mL)	% Inhibition
Olive pomace	9.1	23.1	100% at 100ppm
DMSO	--------	--------	1% at 100ppm
Negative control	--------	--------	0 %

Table 8.Cytotoxicity effect of olive pomace methanol extracton MCF7 (Human Caucasian breastadenocarcinoma)

Sample	LC_{50} (µg/mL)	LC_{90} (µg/mL)	% Inhibition
Olive pomace	24.4	45.8	100% at 100ppm
DMSO	--------	--------	3% at 100ppm
Negative control	--------	--------	0 %

LC50: Lethal concentration of the sample which causes the death of 50% of cells in 48 h

LC90: Lethal concentration of the sample which causes the death of 90% of cells in 48 h

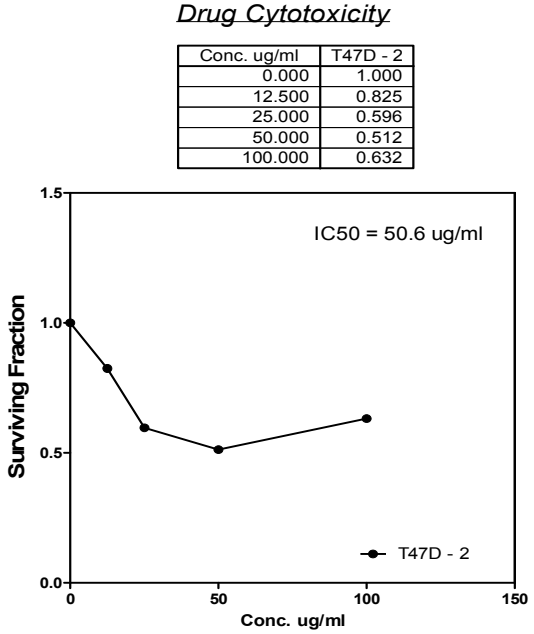


Figure2. Cytotoxicity effect of olive pomace methanol extract onbreast carcinoma cell line (T47D)

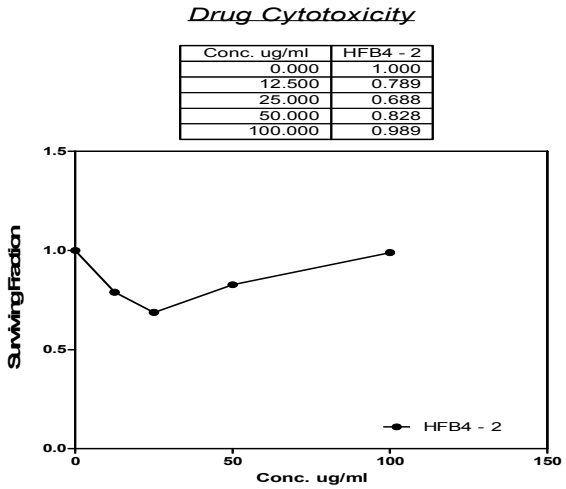


Figure 3. Cytotoxicity effect of olive pomace methanol extract on the normal cell line (human dermal fibroblast cell line (HFB4)

CONCLUSION

Acetone and/or methanol extracts of olive pomace could be considered as a good antibacterial factor against both gram-negative and gram-positive bacteria to supplant the synthetic medicines in the treatment of diseases caused by these microscopic organisms. In addition, OP extract possess a potent inhibitory effect against hepatocellular carcinoma (HePG2) cell line, breast carcinoma (MCF7 and T47D) cells lines and colon carcinoma (HCT116) cell line.

REFERENCES

Amer, M. M. A., M. M. Wasif, A. M. Abo-Aytta and F. A. A.Gabr (1994). Chemical and Biological evaluation of Juniperusphoenicea as a hypoglycaemic agent. ZagazigJ. Agric. Res.21 (4) 1077-1091.

AOAC (2005). Determination of Moisture, Ash, Protein and Fat. Official Methods of Analysis, 18th Ed. AOAC International.

AOAS (1990). In Official methods and recommended practices of the American Oil Chemists' Society (4th ed.). Champaign: American Oil Chemists' Society.

Balbaa, S. I., Hilal, S.H. and Zaki, A.Y. (1976): "Medicinal PlantConstituents", Dar EL-Shaab, Printing House, Cairo, 2 nd Ed.,٢٤٤ .

Bauer, A.W., W.M. Kirby, C. Sherris and M. Turck (1996). Antibiotic susceptibility testing by a standardized single disk method, Ame. J. Clin. Pathol., 45: 493 – 496.

Bisignano, G., Tomaino, A., Cascio, RL, Crisafi, G., Uccella, N. and Saija A.(1999). On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. J PharmaceutPharmacol 51:971 – 974.

Capasso, R., Cristinzio, G., Evidente, A. and Scognamiglio, F. (1992). Isolation, Spectroscopy and Selective Phytotoxic Effects of Polyphenols from Vegetable Waste-Waters. Phytochemistry, 31(12): 4125-4128.

Civantos, L. (1999). Comparacio´ n entre sistemas de extraccio´ n, in Obtencio´n del Aceite de OlivaVirgen, Ed by Civantos L. Editorial Agr´ıcolaEspan˜ ola, Madrid, pp 189 –201.

Dubois, M. A., W. F. Geddes, and F. Smith (1960): The carbohydrates of the gramineas. A quantitive study of carbohydrate of wheat germ. Cereal Chem., 37: 557-561.

El-Menshawi, B.S., Fayad, W., Mahmoud, K.M., El-Hallouty, S.M. El-Manawaty, M., Olofsson, M.H., & Linder, S. (2010). Screening of natural products for therapeutic activity against solid tumors. Indian Journal of Experimental Biology, 48, 258-264.

Goupy, P., M. Hugues, P. Biovin and M.J. Amiot (1999). Antioxidant composition and activity of barley (*Hordeumvulgare* L.) and malt extracts of isolated phenolic compounds. J. Sci. Food Agric., 79:1625-1634.

Halliwell, B., Rafter, J. and Jenner, A. (2005). Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? American Journal of Clinical Nutrition, 81(Suppl.):268S–76S.

Jagessar, R.C. and Cox, M. (2010). Phytochemical Screening of The CHCl3 and CH3CH2OH Extract of Stems, Twigs, Roots and Barks of *Conocarpus erectus*. L. Inte. J. Acade. Res., 2(5): 36-45.

Lafka, T. I., Lazou, A. E., Sinanoglou, V. J. and Lazos, E.S. (2011). Phenolic and antioxidant potential of olive oil mill wastes. Food Chemistry 125(1): 92-98.

Liebowitz, L.D., H.R. Ashbee, E.G.V. Evans, Y. Chong, N. Mallatova, M. Zaidi, D. Gibbs and G.A.S. Group (2001). A two year global evaluation of the susceptibility of Candida species to fluconazole by disk diffusion. Diagn. Microbial. Infact. Dis., 4:27-33.

Marilena, K.; Vassilia-Ismini, A.; George, N.; Artemissia-Phoebe, N.; Anastassia N.; Anastassia, H.; Efstathia, B.; Elena, K.; George, B.; Dimitrios, B.; Achille, G. and Elias, C. (2004). Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. Breast Cancer Res., 6:R63-R74

Matar, M.J., L. Ostrosky-Zeichner, V.L. Paetzinck, J.R. Rodriguez, E. Chen and J.H. Rex (2003). Correlation between E-test, disc diffusion and microdilution methods for antifungal susceptibility testing of fluconazola and voriconazola. Antimicrob. Agent Chemother., 47: 1647 – 1651.

Mensor, L.L., Menezes, F.S., Leitao, G.G., Reis, A.S., dos Santos, T.C., Coube, C.S., (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method Phytother. Res., 15:127-130.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. J. Immunol. Methods 65:55–63.

Mulinacci N, Romani A, Galardi C, Pinelli P, Giaccherini C and Vincieri FF. (2001).Polyphenolic content in olive oil waste waters and related olive samples. J. Agric. Food Chem, 49:3509 –3514.

NCCLS (1993). Methods for Dilution Antimicrobial susceptibility Tests for Bacteria that Grow Aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standard, Villanova, Pa.

NCCLS (1997). Performance, antimicrobial susceptibility of Flavobacteria, 41.

NCCLS (2002). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Canidium-Forming Filamentous Fungi: proposed Standard M38-A. NCCLS, Wayne, PA, USA.

NCCLS (2003). Methods for Antifungal Disc Diffusion Suscepibility Testing of Yeast: Proposed Guideline M44-B. NCCLS, Wayne, PA, USA.

Obied, H. K., Allen, M. S., Bedgood, D. R., Jr., Prenzler, P. D. and Robards, K. (2005). Investigation of Australian olive mill waste for recovery of biophenols. Journal of Agricultural and Food Chemistry 53(26): 9911-9920.

Ordon, J.D., M.A. Gomez and M.I. Vattuone (2006). Antioxidant activities of Sechiumedule (Jacq.) Swartz extracts. Food Chem., 97: 452-458.

Petti, S., & Scully, C. (2009). Polyphenols, oral health and disease: A review. Journal of Dentistry, 37(6), 413-423.

Pfaller, M.A., L. Burmeister, M.A. Bartlett and M.G. Rinaldi (1988). Multicenter evaluation of four methods of yeast inoculum preparation. J. Clin. Microbio.,26 : 1437-1441.

Ranalli, A., Lucera, L. and Contento, S. (2003). Antioxidizing potency of phenol compounds in olive oil mill wastewater. Journal of Agricultural and Food Chemistry 51(26): 7636-7641.

Romero C, Brenes M, Garcia P and Garrido A. (2002). Hydroxytyrosol 4-β-D-glucoside, an important phenolic compound in olive fruits and derived products. J Agric Food Chem., 50:3835 –3839.

Saviozzi, A., Riffaldi, R., Levi-Minzi, R., Scagnozzi, A. and Vanni, G. (1993). Decomposition of vegetation-water sludge in soil. Bioresource. Technol., 44:223 –228.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. Jul 4;82(13):1107-12.

Skerget, M., P. Kotnik, M. Hadolin, A. Rizner-Hras, M. Simonic and Z. Knez (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their activities. Food Chem., 89: 191-198.

Tadros, S.H. (1979).Pharmacognostical Study of EnterolobiumcyclocarpumGriseb. Growing in Egypt. Ph.D. Thesis,Faculty of pharmacy. CairoUniversity,

Thabrew, M.I., Hughes, R.D., & McFarlane, I.G. (1997): Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. Journal of Pharmacy and Pharmacology, 49, 1132-1135.

Trease, L. T. and W. C. Evans (1978): "Text Book of Pharmacognosy"Bailliere. Tindall and Cox, London, 11th Ed., 536.

الخصائص المضادة للأكسدة والميكروبات والسرطان لمخلفات الزيتون

خالد محمد محمد وهدان و طه فتحى طه

قسم الكيمياء الحيوية – كلية الزراعة – جامعة الزقازيق – الزقازيق – مصر

تم الحصول على مخلفات تصنيع الزيتون من معصرة زيت الصالحية بمحافظة الشرقية. في هذا البحث تم تقدير التركيب الكيميائي (الرطوبة ومجموع الليبيدات والبروتينات والكربوهيدرات والألياف والرماد) لمخلفات الزيتون. اظهر النتائج أيضا احتواء مستخلص الاسيتون والميثانول (٧٠%) لمخلفات الزيتون على الفلافونويد والتانينات والقلويدات. مستخلص الميثانول احتوى على الصابونين والسكريات المختزلة بينما لم يحتوي عليهم مستخلص الاسيتون. مستخلص الاسيتون احتوى على التربينات بينما لم يحتوي عليهم مستخلص الميثانول. أظهرت النتائج ان استخلاص المركبات الفيتوكيميائية يعتمد على قطبية المذيب المستخدم في عملية الاستخلاص. تم أيضا تقدير الفينولات والفلافونيدات الكلية. باستخدام الكروماتوجرافى السائل فائق الكفاءة تم التعرف على المركبات الفينولية في مستخلصات مخلفات الزيتون. تم أيضا تقدير الخصائص المضاد للأكسدة والمضادة للبكتريا (سالبة وموجبة لجرام) والمضادة للفطريات والمضادة للسرطان في مستخلصات مخلفات الزيتون. أظهرت النتائج ان مخلفات الزيتون لها تأثير فعال ومانع ضد

hepatocellular carcinoma (HePG2), breast carcinoma (MCF7 and T47D) and colon carcinoma (HCT116) cell lines