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Chemical analysis of olive pomace using HPLC-PDA and its antioxidant and antimicrobial activities In vitro

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Abstract

This study will work to benefit from plant waste in the field of food science, such as Olive pomace, which is an industrial byproduct resulting from the olive oil production process.

Objective: I show that the utilization of plant waste resulting from olive oil extraction namely olive pomace. This is significant to reduce environmental waste and benefit from it in producing extracts rich in phenolic and antioxidant substances that have antimicrobial properties Therefore, it is possible to use the extracts to fortify food and also act as an antimicrobial substance.

Methods: Various techniques have been used for estimation such as hot air oven, muffle furnace, soxhlet extractor, kjeldahl, spectrophotometer, HPLC and incubator.

Study area: Egypt, which ranks ninth in global olive production. Olive oil production waste in 2017 was 63,000 tons, and in 2022, it reached 98,000 tons. Globally, waste was 2,881,500 tons annually. Because the amount of waste increases annually, it must be utilized to reduce its risks to the environment and public health of humans.

Keywords Olive pomace; HPLC; antioxidant; Polyphenol; Antimicrobial activities

1. Introduction

The olive tree belongs to the family Oleaceae specie Olea europaea L. thriving primarily in Mediterranean climates. The olive tree serves diverse purposes, extending beyond its well-documented culinary importance in producing olives and olive oil to encompass medicinal applications and the provision of wood and traditional remedies (L, I, G, & C, 2020). Due to its economic and cultural importance, the cultivation of olive trees has exerted various impacts on the environment, prompting conservation efforts aimed at mitigating negative effects on biodiversity and natural ecosystems (W G. M., 2017).

Of all the edible oils, olive oil contains the largest amount of monounsaturated fat more than 70% (I, H, & Z, 2015). Olive oil is extracted by crushing olives to create pomace, which is subsequently homogenized and pressed to release the oil, olive oil is extracted. First to be extracted is premium extra virgin olive oil, which is made using centrifugation and only water. Olive pomace (OP), a solid by-product of olive oil extraction, is made up of pulp, stone, skin, and olive kernel

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fragments. The pulp accounts for about 70 – 90 %, the stone for 9-27%, and the seed for about 2-3% of the total olive weight. Removing the by-products from oil mills solves the environmental issues brought on by the manufacture of olive oil (P & Mª, 2006).

The cultivated areas and productivity of olive trees in Egypt, according to the (Ministry of Agriculture and Land Reclamation, 2010), 2005 to 2010 increased in the late seventies to more than one hundred thousand acres in the late nineties. Therefore, the amount of OP waste increases annually and can be used for environmental conservation and polyphenol compounds extraction because plants are a major source of phenolic compounds, which are synthesized as secondary metabolites during normal development in response to stress conditions, such as wounding and UV radiation (W & H, 2003).

Ninety percent of the total phenolic chemicals found in olive products are found in their free form, but OP's polyphenol quantities and compositions vary depending on the agroclimatic, fruit, variety, condition maturity, extraction technique, and fruit preservation, So in recent years, advancements in olive tree cultivation and oil production have integrated modern technologies and sustainable practices, resulting in improved efficiency and quality (M. H., I, K, M, A, & T, 2010).

OP is an important source of bioactive compounds with proven health advantages as it contains a significant amount of hydroxytyrosol, caffeic acid, oleuropein, rutin, tyrosol, vanillic acid, and p-coumaric (R, F, A, L, & M, 2016), and triterpenic acids, which have been associated with various biological activities, including antitumor, antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and cardioprotective effects (E, C, & M, 2018).

Interest in useing olive oil residues, particularly the ones obtained from the two-phase olive oil extraction system, as an inexpensive source of natural antioxidant phenolic compounds, at concentrations higher than in virgin olive oil (L, et al., 2001).

OP comprising 78-83% of the olive weight and containing 2.5-3.2% fat, is characterized by high moisture content (54-62%) and a variety of natural and inorganic compounds, including a range of phenolic compounds calcium, potassium, magnesium, sugars, and metals (A, S, M, M, & A, 2018).

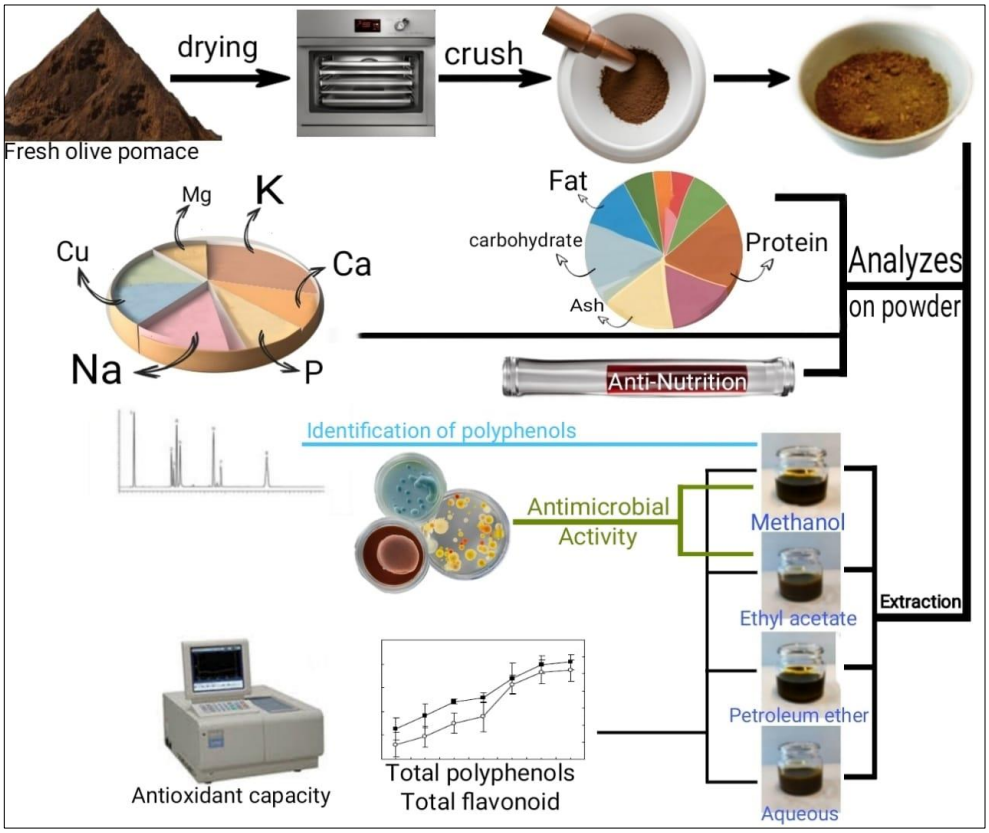

Figure 1 This scheme succinctly summarizes the procedures employed and the samples collected during this research

2. Materials and methods

2.1. Collection of raw materials

OP samples (30 kg) were obtained as by-products from species of Olea europaea (Olea europaea L., cv. Kalamata) from El-Sheikh Zuweid City, Sinai Governorate (31° 12'43'' N, 34° 6' 38'' E), Egypt. They were stored under refrigeration until preparation started.

2.2. Preparation of OP

OP samples were transported directly in paper bags to the laboratory food and science technology department, faculty of agriculture, Kafrelsheikh university. after being dried for 24 hours at 50°C in an electric air oven The material had been crushed into a fine powder to fit through a 60 mesh screen sieve. Until it was needed again, the resultant powder was kept in sealed glass jars at 4°C.

2.3. Proximate chemical composition

Chemical analysis was carried out on OP according to the method described by A.O.A.C. were followed.

Determine the moisture using an air oven at 105°C for drying to a constant weight. Crude protein was calculated from total nitrogen analysis using the Micro-Kjeldahl technique, and the result was multiplied by 6.25 (which is the factor value). Petroleum ether (boiling in the range 40–60°C) was used as the solvent in a soxhlet device to evaluate ether extract. Ash content was determined by pre-heating the samples and then ashing them in a muffle furnace at 550˚C to achieve a constant weight. crude fiber content was determined after digesting a known weight of a fat-free sample in refluxing 1.25% (w/v) sulfuric acid and 1.25% (w/v) sodium hydroxide total available carbohydrates were calculated by difference (A.O.A.C., 2010).

2.4. Minerals content of olive pomace

One milliliter of a strong hydrochloric acid solution was used to dissolve each individual ashed dried OP sample, and the volume was then adjusted to one hundred milliliters using distilled water (A, M, L, G, & R, 1982). (P H. R., 1972) reported that potassium (K) and sodium (Na) were measured with a flame photometer. Atomic absorption was used to assess the levels of calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) (A, M, L, G, & R, 1982).

2.5. Determination of the anti-nutritional factors of olive pomace

2.5.1. Determination of phytic acid

The (W & H, 1983) technique was applied to calculate the raw OP's phytic acid the amount. 50 milliliters of 0.2 NHCl were used to extract one gram of OP sample, and 0.5 milliliters (ml) of the extract were added to a test tube that had a glass stopper on it. Subsequently adding 1 milliliter of ammonium ferric sulfate solution, the tube was completely sealed with the stopper, heated for 30 minutes in boiling water, and then chilled for 15 minutes in ice. At ambient temperature, 2 milliliters of 2,2-bipyridine solution (1mg/1ml thioglycolic acid) was added to the test tube. After a minute, the wavelength of absorption was detected at 519 nm against distilled water. Phytate phosphorus salt in the range of 1–50 mg was used to create a calibration curve from which the phytic acid concentration was determined.

2.5.2. Determination of glucosinolate:

The glucosinolate content, an anti-nutritional factor in OP, was set up following the procedure outlined by (J, L, & G, 1965) 250 milliliters of distilled water were mixed with ten grams of air-dried OP. The homogeneous mixture was hydrolyzed for one hour at 54°C and then boiled for 2 hours, with the volume kept constant by the addition of water. Following the solution's filtration, 50 milliliters of hot water were used to slurry the pomace three times, and the total volume was adjusted to 600 ml. The barium sulfate was precipitated with an excess of 5% barium chloride solution, digested for a few hours or overnight in a steam room, and transferred to ashless filter paper (J, L, & G, 1965). After ashing, the precipitate's weight was determined, as reported by (Abd-ElGhany & F,T, 2006).

$$\text{Thioglucoside (\%)} = \frac{(\text{M wt thioglucoside})\ (\text{wt of } BaSO_4) \times 100}{(\text{M wt of } BaSO_4)\ (\text{sample wt})} \times 100 \quad \text{(Eq.1)}$$

$$\text{Destruction rate (\%)} = \frac{\text{Raw sample wt} - \text{treated sample wt}}{\text{Raw sample wt}} \times 100 \quad \text{(Eq.2)}$$

2.6. Determination of total polyphenols content (TPC)

The total phenolic content (TPC) in OP extracts was estimated according to (M S. , P, M, A, M, & Z, 2005) with the Folin-Ciocalteu reagent (A.O.A.S., 1990). Specifically, 0.2 milliliters of the OP extracts and 1 milliliter of diluted Folin-Ciocalteu reagent were mixed (prepared at a 1:10 ratio with distilled water). 0.8 mL of 7.5% sodium carbonate was added after the reaction had been running for 8 minutes, and the mixture was then allowed to stand for a further 30 minutes. The phenolic compounds were then quantified at 765 nm using a UV-visible spectrophotometer (UV 160). A calibration curve was produced using gallic acid as a standard, permitting the expression of the total phenolic content per gram of dry weight in milligrams of gallic acid equivalent (GAE).

2.7. Determination of total flavonoid content (TFC)

The TFC of OP was estimated calorimetrically according to (L, W, S, & P, 2002). Approximately 1 mL of OP extracts was mixed with 0.1 mL of 2.8 mL of distilled water, 1M (CH_3COOK) potassium acetate, and 0.1 mL of 10% ($AlCl_3$) aluminum chloride. Following an incubation period of 40 minutes at room temperature, by using distilled water as a blank, the absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer. To quantify flavonoids, quercetin was used as a standard, and a standard curve with a range of 0 to 50 mg/L was created. based on dry weight, the TFC concentration was given as milligrams of quercetin equivalent (QE) per gram (L, W, S, & P, 2002).

2.8. Identification of polyphenols and flavonoids of olive pomace using HPLC- PDA

HPLC (High-performance liquid chromatography) was used for the identification and quantification of polyphenols and flavonoids for the extract. The solvent flow rate was maintained at 1 mL/min, and the separation was performed at 35°C. Five microliters of the extract were injected into a column (250 mm×4.6 mm inner diameter, Agilent, USA) at room temperature. The solvent system utilized a gradient of A (8% w/v CH_3 COOH) and B (acetonitrile). The separation was achieved with the following gradients: at 0 min, 5% of solvent A and 95% of solvent B; at 5 min, 25% of solvent A and 75% of solvent B; at 10 min, 45% of solvent A and 55% of solvent B; at 15 min, 65% of solvent A and 35% of solvent B; at 20 min, 85% of solvent A and 15% of solvent B; and from 25 to 30 min, 99% of solvent A and 1% of solvent B. The UV-VIS detector was set at a wavelength of 280 nm for flavonoids and 330 nm for polyphenols. The assays were conducted using the Agilent Technologies 1200 HPLC series, which was equipped with an Agilent 1200 Series quaternary pump, vacuum degasser, and Agilent UV-VIS detector. Identification was carried out by comparing the retention time of the analyte with that of a reference standard, which was prepared separately for each compound. The results were expressed as milligrams per gram on a dry weight basis (mg/g ddp), and using the reference standards' calibration curves, the identified compounds were quantified (K, M, & A, 2018).

2.9. Antioxidant assays

2.9.1. DPPH radical scavenging activity

OP extracts's antioxidant activity was determined by applying the method described by (W, M, & C, 1995), which evaluates the ability of a compound to donate hydrogen atoms based on the stable DPPH (1,1-diphenyl-2-picryl-hydrazil) radical's scavenging activity. Specifically, one milliliter of the extract was mixed with 0.1 milliliter of DPPH at a 200 ppm concentration. A control was prepared by adding 1 mL of the same solvent to 0.1 mL of DPPH. Each tube's contents were well mixed, and the tubes were allowed to sit at room temperature for 30 minutes in the dark. The absorbance was then determined using a spectrophotometer (T80+UV/VIS) at 517 nm. The antioxidant activity was calculated using the appropriate equation.

$$\text{Radical scavenging activity \%} = 1 - A_{\text{sample}}/A_{\text{control}} \times 100 \quad \text{(Eq.3)}$$

Where: A= absorbance at 517 nm.

2.9.2. Hydrogen peroxide scavenging activity

The extraction's capacity to scavenge hydrogen peroxide was assessed using the following (N, et al., 2017) technique. After 10 minutes, a UV-2000 spectrophotometer was used to evaluate the reaction mixture's absorbance at 230 nm in

comparison to a blank solution containing the phosphate buffer without hydrogen peroxide. The H_2O_2 inhibition percentage was computed using Eq. (3).

2.10. Antimicrobial Activity

According to (S, T, J, N, & R, 2015) method, the agar well diffusion test was used to evaluate the antibacterial activity. Five pathogenic microbial species were tested, including the yeast strain (*Candida albicans* EMCC105), the Gram-positive bacteria (*Bacillus cereus* ATCC10876 and *Staphylococcus aureus* ATCC25923), the Gram-negative bacteria (*E.coli* ATCC25922 and *Klebsila pneumonia* ATCC700603). For twenty-four hours, the yeast strains and bacteria were cultivated in nutritional broth at 37 °C. A cork borer (0.5 cm) was used to produce wells after 100 microliters of the inoculum (1× 〖10〗 ^8 CFU/ml) had been dispersed on agar media in Petri plates. Subsequently, each well received 100 µl of the tested substance. Following a 24-hour incubation of the plates at 37 °C, the diameter of the region surrounding each well -including the well itself- was measured in millimeters to calculate the zone of inhibition. Measurements were taken in triplicate, and the mean values were recorded (S, T, J, N, & R, 2015).

3. Results and discussion

3.1. Proximate chemical composition

Table 1 Proximate chemical composition of olive pomace (on dry weight basis)

Chemical composition	Proportion %
Moisture	4.23
Dry matter	95.77
Crude protein	8.23
Ether extract	3.96
Ash content	7.17
Crude fiber	36.89
Total carbohydrates	80.64
Available Carbohydrates	43.75
Lignin	6.10
Cellulose	11.28

All values given are means of three determinations.

The data in Table 1 indicate that the moisture, ether extract (crude fat), ash, and crude protein contents in olive pomace amounted to 4.23%, 8.23%, 3.96%, and 7.17%, respectively. The percentage of total carbohydrates was 80.64, of which 43.75 were available carbohydrates, 6.10 were lignin, and 11.28 were cellulose.

These variations can be ascribed to variations in origins and varieties. The present data and review of the literature showed that olive pomace contained 18.48 to 37.23% crude fiber. All of this was a response to the use of OP in human diets as a source or substitute for fiber. Similar results were reported by (H M. M., 2007).

3.2. Mineral elements content olive pomace (on a dry weight basis):

Minerals are important in the body as they are involved in the formation of teeth and bones, constitute part of enzymes, are essential components of tissues and body fluids, and play a role in nerve function (E T. , 2022). It produces various hormones, transmits nerve impulses, and can also regulate the normal heartbeat (Gharibzahedi & Jafari, 2017). People's needs for mineral elements vary according to gender, age, and physiological condition (E T. , 2022). Most of the trace elements (Cu, Fe, Mn, Mg, and Zn) play a vital role as a structural part of many enzymes. Macro elements compared to trace elements have more important functions in nerve cells (signal transmission). Although trace elements have major roles in the formation of red blood cells, regulation of glucose levels, and protection through activation of antioxidant enzymes, major minerals such as Ca and K have high potential for controlling blood pressure. Minerals that are also involved in the cerebral system such as (Cr) and (Mn) and the immune system such as Zn (Gharibzahedi & Jafari, 2017). Also, iron, iodine, and zinc deficiency, along with vitamin A deficiency, can lead to a mortality rate of about 20% among

children under 5 years of age (P & M, 2009). Therefore, potential strategies to reduce mineral deficiency should be presented to avoid harmful diseases/disorders associated with their deficiency (S, S, S, & S, 2014).

Table 2 Mineral elements content of olive pomace (mg/g on a dry weight basis)

Minerals	Proportion (mg/g)
Potassium (K)	1415.5
Sodium (Na)	219.9
Calcium (Ca)	123.1
Phosphorus (P)	90.1
Magnesium (Mg)	81.2
Copper (Cu)	22.3
Iron (Fe)	19.2
Manganese (Mn)	10.1
Zink (Zn)	4.2

The data contained in Table 2 makes it abundantly evident that OP is thought to be a rich source of minerals as the analysis shows that the main element in OP is potassium (1415.5 mg/g), followed by sodium (219.9 mg/g), Calcium (123.1 mg/g), Phosphorus (90.1 mg/g) was found in low quantity for olive pomace compared to other major elements, Magnesium (81.2 mg/g), Copper (22.3 mg/g), Iron is a good source in OP (19.2mg/g), Manganese (10.1 mg/g) and Zink (4.2 mg/g).

Humans obtain their mineral needs through food; thus, foods can be fortified with olive pomace.

3.3. Anti-nutritional factor of olive pomace (phytic acid and total glucosinolate content)

At physiological pH, the phytate molecule has a negative charge and is known to bind important divalent cations, including zinc, iron, calcium, and magnesium. As a result of this binding, these minerals become insoluble complexes, making them inaccessible for absorption and use. It has been found that phytic acid significantly reduces the bioavailability of minerals, hence impacting the nutritional value of seeds and legumes [(H, F, C, & C, 2002); (A, A, F, S, A, & H, 2007)].

Table 3 Anti-nutrition content of olive pomace on phytic acid and total glucosinolates as hydrocyanic acid (HCN mg/g)

Components	Proportion (mg/g)
Phytic acid (%)	0.90
Total glucosinolates (mg/g)	5.58

According to the analysis shown in Table 3, phytic acid content was 0.90% in OP. Generally, OP contains less phytic acid than many other foods. For example, raw peanuts and raw sesame seeds contain 2.63% and 6.5%, respectively (E E. H., 2011), and raw kabuli chickpea seeds contain 2.34% (23.4 mg/g) (Arafa, M, M, & A, 2015).

According to the analysis shown in Table 3, the anti-nutritional factor in OP contains 5.58 mg/g of total glucosinolates (as hydrocynic acid).

3.4. Total polyphenols and total flavonoids contents

Various solvents (methanol 70%, ethyl acetate 70%, petroleum ether 95%, and water) were used to extract the phenolic compounds from olive pomace by soaking for 48 hours. Additionally, distilled water was used for the same procedure for 30 minutes at 50°C.

According to the analysis shown in Table 4, the type of solvent had a significant impact on extraction yield; Petroleum ether 95% and ethyl acetate 70% were found to be less effective than methanol 70% and aqueous. Methanol 70% was best as the extraction yield solvent.

Table 4 Total polyphenols contents (TPC) and total flavonoid contents (TFC) of olive pomace extracts

Solvent type	Total polyphenols contents (TPC) (mg GAE/g)	Total flavonoids contents (TFC) (mg QE/g)
Methanol 70%	8.48	28.05
Ethyl acetate 70%	5.02	24.17
Petroleum ether 95%	4.80	22.68
Aqueous	6.68	25.98

mg GAE/g= mg of sample expressed as mg gallic acid equivalent, using a calibration curve, to report the total polyphenol content.

mg QE/g= mg sample of mg quercetin equivalents using an action curve have reported the total flavonoid content.

3.5. Determination of phenolic and flavonoid compounds (mg/g dried defatted pomace) using HPLC- PDA of OP methanol extract

The HPLC analysis revealed the intricate chromatographic profile of the olive pomace extract, providing crucial insights into its composition.

The phenolic compounds isolated from olive pomace are an extraordinary source of bioactive substances with numerous health benefits. in table 5 this rich combination includes oleuropein (14.89 %) followed by pyrogallol (14.57 %), benzoic acid (13.97 %), 3-hydroxytyrosol (12.34 %), hesperidin (5.98 %), 4-hydroxybenzoic acid (4.55 %), catechol (4.39 %), naringin (4.17 %), salicylic acid (3.18 %), hesperetin (3.16 %), ellagic acid (3.13 %), protocatechuic acid (1.64 %), vanillic acid (1.54 %), ρ-coumaric acid (1.25 %), epicatechin (1.22 %), ferulic acid (1.12 %), caffeine (1.09 %), gallic acid (1.02 %), 4-aminobenzoic acid (1 %), quercetin (0.96 %), catechin (0.78 %), syringic acid (0.77 %), caffeic acid (0.65 %), rutin (0.62 %), naringenin (0.52 %), coumarin (0.45 %), rosmarinic acid (0.43 %), apigenin (0.16 %), rhamnetin (0.15 %), kaempferol (0.15 %) and chlorogenic acid (0.15 %).

Table 5 Phenolic and flavonoid compounds (mg/g OP) using HPLC- PDA of OP methanol extract

Phenolic group	Compounds	Formula	Amount (mg/100g)	(%)
Secoiridoids	Oleuropein	$C_{25}H_{32}O_{13}$	10.032	14.89
Phenyl alcohols	3-Hydroxytyrosol	$C_8H_{10}O_3$	8.314	12.34
Phenolic acids/Hydroxybenzoic acid derivatives	Gallic acid	$C_7H_6O_5$	0.684	1.02
	Salicylic acid	HOC_6H_4COOH	2.140	3.18
	4-Hydroxybenzoic acid	$C_7H_6O_3$	3.063	4.55
	Benzoic Acid	C_6H_5COOH	9.413	13.97
	Vanillic acid	$C_8H_8O_4$	1.039	1.54
	Syringic acid	$C_9H_{10}O_5$	0.521	0.77
	Protocatechuic acid	$C_7H_6O_4$	1.102	1.64
	Ellagic acid	$C_{14}H_6O_8$	2.108	3.13
	Pyrogallol	$C_6H_6O_3$	9.813	14.57
	Catechol	$C_6H_6O_2$	2.957	4.39

	4-Aminobenzoic acid	$C_7H_6NO_2$ ($H_2NC_6H_4CO_2H$)	0.674	1
Phenolic acids/ Hydroxycinnamic acid derivatives	ρ-coumaric acid	$C_9H_8O_3$	0.843	1.25
	Caffeic acid	$C_9H_8O_4$	0.435	0.65
	Ferulic acid	$C_{10}H_{10}O_4$	0.753	1.12
	Chlorogenic acid	$C_{16}H_{18}O_9$	0.104	0.15
	Rosmarinic acid	$C_{18}H_{16}O_8$	0.291	0.43
Flavonoids/ Flavones	Apigenin	$C_{15}H_{10}O_5$	0.109	0.16
	Rutin	$C_{27}H_{30}O_{16}$	0.418	0.62
	Rhamnetin	$C_{16}H_{12}O_7$	0.099	0.15
Flavonoids/ Flavonols	Quercetin	$C_{15}H_{10}O_7$	0.647	0.96
	Kaempferol	$C_{15}H_{10}O_6$	0.104	0.15
Flavonoids/ Flavanones	Naringenin	$C_{15}H_{12}O_5$	0.351	0.52
	Hesperidin	$C_{28}H_{34}O_{15}$	4.032	5.98
	Naringin	$C_{27}H_{32}O_{14}$	2.810	4.17
	Hesperetin	$C_{16}H_{14}O_6$	2.131	3.16
Flavonoids/ Flavan-3-ol	Catechin	$C_{15}H_{14}O_6$	0.524	0.78
	Epicatechin	$C_{15}H_{14}O_6$	0.821	1.22
Coumarins	Coumarin	$C_9H_6O_2$	0.305	0.45
Methylxanthine	Caffeine	$C_8H_{10}N_4O_2$	0.732	1.09
Total			67.369	100

Hydroxytyrosol was the major compound found in the extract, which is also considered the most powerful and one of the most powerful antioxidants in the olive tree. This is interesting since this compound is one of the antioxidants of olive oil that confers protection of blood lipids from oxidative stress (EU, 2013).

As a novel food ingredient, hydroxytyrosol has recently been declared safe by the European Food Safety Authority under proposed uses and use levels, i.e., in fish and vegetable oils up to 215 mg/kg and margarine up to 175 mg/kg (T, et al., 2017).

3.6. Antioxidant capacity

Table 6 presents the absorbance data at 700 nm for each sample, reflecting color development at three different concentrations (5, 10, and 20 mg/ml).

With a range of 0.72 to 1.43 for methanolic extract at 5 and 20 mg/ml, respectively, OP had the highest reducing power. The water extract followed, with concentrations ranging from 0.56 to 1.24 at 5 and 20 mg/ml, respectively.

The reducing power of OP, which were 0.50 and 1.24 at concentrations of 5 and 20 mg/ml, respectively, for petroleum ether extract. The reducing power of the ethyl acetate extract was 0.36 and 0.85 at concentrations of 5 and 20 mg/ml, respectively.

Table 6 Antioxidant capacity of olive pomace extracts determined by reducing power FRAP and DPPH+ cation radical

Solvent type	Antioxidant Methods	Concentration (mg/ml)		
		5 (mg/ml)	10 (mg/ml)	20 (mg/ml)
Methanol 70%	FRAP	0.72	0.98	1.43
	DPPH	18.98	27.88	34.93
Ethyl acetate 70%	FRAP	0.36	0.52	0.85
	DPPH	14.32	20.84	27.92
Petroleum ether 95%	FRAP	0.50	0.86	1.38
	DPPH	12.83	18.63	26.34
Aqueous	FRAP	0.56	0.89	1.24
	DPPH	16.21	22.71	29.43

FRAP = The ferric-reducing ability of plasma as an indicator of antioxidant strength.

DPPH= 2,2-diphenyl-1-picryl hydrazyl.

High levels of reducing power indicate the presence of substances capable of donating electrons to free radicals, allowing them to react and transform into more stable compounds (D & A, 2007).

The ability of OP methanol, ethyl acetate, petroleum ether, and aqueous extracts to scavenge radicals was evaluated using the stable DPPH radical. One commonly used metric to assess antioxidant activity is the concentration of an antioxidant required to reduce the starting DPPH concentration by 50% (IC_{50}) (M, A, & C, 1998). The lower EC_{50} value suggested higher levels of antioxidant activity. The ability of The studied extracts to scavenge DPPH radicals enabled researchers to evaluate their antioxidant activity. The hydrogen-donating properties of DPPH are responsible for their antioxidant scavenging actions (M, P, & A, 2010).

Table 6 clearly shows that the water and methanol extracts of OP exhibited the highest scavenging effect (IC_{50}), with inhibition percentages ranging from 18.98% to 34.93% and 16.21% to 29.43%, respectively, at concentrations of 5 and 20 mg/ml. This was followed by petroleum ether and ethyl acetate extracts, with inhibition percentages ranging from 12.83% to 26.34% and 14.32% to 27.92%, respectively, at concentrations of 5 and 20 mg/ml.

3.7. Antimicrobial Activity

Table 7 shows the impact of extracts at varying concentrations on five pathogenic microbial species: yeast strain (*Candida albicans* EMCC105), the Gram-positive bacteria (*Bacillus cereus* ATCC10876 and *Staphylococcus aureus* ATCC25923), the Gram-negative bacteria (*E.coli* ATCC25922 and *Klebsiella pneumonia* ATCC700603). For yeast and Gram-negative bacteria, the two extracts displayed almost identical inhibition zones; however, for Gram-positive bacteria, the olive pomace extract extracted with methanol 70% displayed an inhibition zone that was larger than the extract extracted with ethyl acetate 70%. Phenolic compounds are responsible for the antibacterial properties of plant extracts (M, et al., 2020). These compounds are also the main constituent of OP extracts (O, et al., 2019), and Borjan found that compounds found in OP can inhibit bacteria and germs (D, M, Ž, & M, 2020).

Table 7 Inhibition zone diameter (mm) against *Escherichia coli*, *Klebsila pneumonia*, *Bacillus cereus*, *Candida albicans*, and *Staphylococcus aureus* at 0.025, 0.050, and 0.075 mg/ml of extracted OP concentrations

Pathogenic strain	Tested material	Inhibition zone diameter (mm)	Sample concentration (mg/ml)
Yeast			
Candida albicans EMCC105			
Methanol 70%	22	17	13
Ethyl acetate 70%	21	17	15
Gram-positive Bacteria			

Bacillus cereus ATCC10876			
Methanol 70%	22	20	17
Ethyl acetate 70%	17	14	12
Staphylococcus aureus ATCC25923			
Methanol 70%	21	17	15
Ethyl acetate 70%	19	12	ND
Gram-negative Bacteria			
E.coli ATCC25922			
Methanol 70%	19	12	ND
Ethyl acetate 70%	17	12	15
Klebsila pneumonia ATCC700603			
Methanol 70%	19	17	15
Ethyl acetate 70%	19	17	12

ND: Not Detected.

4. Conclusion

Olive pomace (OP), a by-product of the industrial process used to produce olive oil, is underutilized in Egypt despite being rich in vital nutrients. Due to its nutritional value, OP has the potential to enhance the nutritional quality of food products when used as a fortifying agent. This study focused on the olive species Olea europaea (Olea europaea L. cv. Kalamata) and examined both OP and its extracts - rich in polyphenols and exhibiting strong antioxidant activity- using various solvents. The proximate chemical composition revealed that OP is a rich source of crude protein and carbohydrates. It is evident from the data that OP is considered a significant source of minerals such as potassium, sodium, and calcium. The anti-nutritional content of OP was evaluated based on phytic acid and total glucosinolates (expressed as hydrocyanic acid), and the results were lower than those reported for many other foods foods like raw peanuts and raw sesame seeds.

Biological studies have demonstrated that the methanolic extract exhibits a stronger antibacterial effect against yeast and Gram-positive bacteria compared to the ethyl acetate extract, although both show comparable activity against Gram-negative bacteria.

These findings suggest that the extracts could be utilized to fortify food with minerals, phenolic compounds, and antioxidants, as well as to serve as effective antimicrobial agents.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Bibliography

[2] A, C., M, V., L, K., G, V., & R, C. (1982). Chemical analysis for plant and soils, Gent, Belgium: Laboratory of Analytical and Agrochemistry. Scientific Research, Article No. 42,80-284.

[3] A, K. W., A, Z., F, M., S, T., A, K. B., & H, S. (2007). Comparison of sprout quality characteristics of desi and kabuli type chickpea cultivars (Cicer arietinum L.). LWT-Food Sci. Technol., 40, 937-945.

[4] A, M. E., S, F. A., M, A. M., M, E. K., & A, M. T. (2018). Antioxidant and anticancer efficacy of therapeutic bioactive compounds from fermented olive waste. Grasas y Aceites, 69(3), 266.

[5] A.O.A.C. (2010). Association of Official Analytical Chemists. In Official Methods of Analysis (16 th ed.). Arligton, Virginia, USA.

[6] A.O.A.S. (1990). Official Methods and Recommended Practices of the American Oil Chemists' Society. In American Oil Chemists' Society. (4th ed.). Champaign, lllinois, USA.

[7] Abd-ElGhany, & F,T, F. (2006). Evaluation of Using Some Agro-Industrial by-Products in Growing Rabbit Nutrition. In M.Sc. Thesis. Fac. of Agric., Cairo Univ., Egypt.

[8] Arafa, S. G., M, K. E., M, A.-G. A., & A, G. K. (2015). Effect of some technological treatments on the chemical and nutritional properties of chickpea (Cicer arietinum L.) seeds. The International Journal of Science & Technoledge, 3, (6): 192-198.

[9] D, A. S., & A, U. (2007). Antioxidant properties of various solvent extracts of mulberry (Morus indica L.) leaves. Food Chemistry, 102, 1233–1240.

[10] D, B., M, L., Ž, K., & M, H. K. (2020). Microbiological and Antioxidant Activity of Phenolic Compounds in Olive Leaf Extract. Molecules, 25(24), 5946.

[11] E, E. H. (2011). Effect of Heat Treatments on Certain Antinutrients and in vitro Protein Digestibility of Peanut and Sesame Seeds. Food Sci. Technol. Res., 17 (1): 31 – 38.

[12] E, M., C, R., & M, B. (2018). Residual Olive Paste as a Source of Phenolic Compounds and Triterpenic Acids. Eur. J. Lipid Sci. Technol., 120, 1700368.

[13] (2022). Essential Minerals: Nutritional Requirements, Dietary Sources, and Deficiencies. In T. E, W. T, T. J. N, & B. A. G (Eds.), Nutrition Guide for Physicians and Related Healthcare Professions (pp. 365-376).

[14] EU, E. (2013). establishing a list of permitted health claims made on foods other than those referring to the reduction of disease risk and to children's development and health. Off. J. Eur. Union, 56, 43-45.

[15] Gharibzahedi, S. T., & Jafari, S. M. (2017). The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. Trends in Food Science & Technology, Volume 62, 119-132.

[16] H, L. W., F, L., C, C., & C, R. (2002). Minerals and phytic acid interactions: is it a real problem for human nutrition Internat. J. of Food Sci. and Tech., 37, 727-739.

[17] H, M. M. (2007). The Possibility Of Using Irradiated Untraditional feed-stuffs in Rabbits Nutrition. PhD. Thesis, Animal Production Dept., Fac. Agric., Benha Univ., Egypt.

[18] I, L., H, H., & Z, A. (2015). Olive mill waste extracts: polyphenols content, antioxidant, and antimicrobial activities. Advances in Pharm. Sci., I.D 714138, 11.

[19] J, M. E., L, K. D., & G, M. G. (1965). Total glucosinolate method for Brassica species. J. Assoc. Offic. Anal. Chemists, 42:889-1596.

[20] K, M. E., M, S. G., & A, O. (2018). Ultrasound assisted extraction of polyphenols with high antioxidant activity from olive pomace (Olea europaea L.). Carpathian journal of food science and technology, 11(1), 193-202.

[21] L, C. A., I, L.-C. M., G, S., & C, A. A. (2020). The olive-tree leaves as a source of high-added value molecules: oleuropein. Stud Nat Prod Chem, 64:131–80.

[22] L, C. W., W, Y. J., S, H. C., & P, D. D. (2002). Antioxidant activity of sesame coat. Food Chem, 78, 347-354.

[23] L, L.-M., D, N., S, M., J-C, S., J, L., M, D., et al. (2001). Simple phenolic content in olive oil residues as a function of extraction systems. Food Chemistry, 75 501–507.

[24] M, B., P, H. K., & A, G. K. (2010). Antioxidant and free-radical-scavenging effects of fruits of Dregea volubilis. J Nat Sci Biol Med., 1(1): 29–34.

[25] M, S. C., A, L., & C, S. F. (1998). A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture, 76, 270–276.

[26] M, S., P, K., M, H., A, R.-H., M, S., & Z, K. (2005). Phenols, proanthocyanidins, flavones, and flavonols in some plant materials and their activities. Food Chem., 89: 191-198.

[27] M, T., E, K. B., C, Z., A, K., T, P., J, K., et al. (2020). Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. Antioxidants, 9(2), 165.

[28] M. H., A., I, A., K, E., M, A., A, A.-T., & T, R. (2010). Optimisation, characterisation, and quantification of phenolic compounds in olive cake. Food Chemistry, 23, 117-122.

[29] Ministry of Agriculture and Land Reclamation. (2010). Economic Affaris Sector/ Agricultural censuses. Retrieved from Ministry of Agriculture and Land Reclamation: https://www.agri.gov.eg/agri-counter/40

[30] N, A.-O., S, O., N, M., Y, I., D, H., C, S. M., et al. (2017). Bioactive metabolites involved in the antioxidant, anticancer, and anticalpain activities of Ficus carica L., Ceratonia siliqua L., and Quercus ilex L. extracts. Industrial Crops and Products, 95, 6-17.

[31] O, G., F, S., Y, M., J, G., L, K. B., M, M., et al. (2019). Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. Biotechnology Reports, 23, e00347.

[32] P, H. R. (1972). A Textbook of Soil Chemical Analysis. (J. Murray, Ed.) London, UK: Cambridge University press 1972.

[33] P, M. S., & Mª, M. V. (2006). Production of pomace olive oil. Grasas Y Aceites, 57 (1).

[34] P, W. J., & M, B. R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets--iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol., 182(1):49-84.

[35] R, C., F, M., A, F., L, I. M., & M, P. (2016). Extraction, benefits, and valorization of olive polyphenols. European Journal of Lipid Science and Technology, 118, 503-511.

[36] S, G. M., S, R., S, R. H., & S, J. M. (2014). Recent Developments on New Formulations Based on Nutrient-Dense Ingredients for the Production of Healthy-Functional Bread: A Review. Journal of Food Science and Technology, 51, 2896-2906.

[37] S, K., T, R. S., J, K. M., N, A. S., & R, M. (2015). In-vitro antibacterial, antifungal, antioxidant and functional properties of Bacillus amyloliquefaciens. Annals of clinical microbiology and antimicrobials, 14, 9.

[38] T, D., J, B. L., B, B., T, D., S, F.-T., M, H., et al. (2017). Safety of hydroxytyrosol as a novel food pursuant to Regulation (EC). European Food Safety Authority EFSA, 258/97. EFSA J. 2017,15, e04728, doi:10.2903/j.efsa.2017.4728.

[39] W, B.-W., M, C. E., & C, B. L. (1995). Use of a free radical method to evaluate antioxidant activity. LWT- Food Science and Technology, 28(1): 25-30.

[40] W, G. M. (2017). Effect of Irrigation Techniques and Quantities on Kalamata Olive Trees under North Sinai Conditions. J. Plant Production, Vol. 8(8): 821 - 828.

[41] W, H., & H, L. J. (1983). Sensitive method of rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric., 34, 1423- 1426.

[42] W, S., & H, S. (2003). Antioxidant activity of carotenoids. Mol Aspects Med, 24(6):345-51.