Maceration and Liquid-liquid extractions of phenolic compounds and antioxidants from Algerian olive oil mill wastewater

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Abstract

Purpose

Olive oil mill wastewater (OMW) is a major waste stream from the olive oil industry. It is highly polluted due to phenolic compounds. The present study focused on the physicochemical properties of OMW as well as the quantitative and qualitative effects of two methods of extraction (maceration and liquid-liquid extraction) of phenolic compounds.

Methods

Liquid-liquid extraction and maceration methods were used for the extraction of phenolics. Spectrophotometry and High-Performance Liquid Chromatography-Electrospray Ionization–Mass Spectrometry (HPLC-ESI-MS) were adopted to quantify the phytochemical contents and the phenolic compounds. The antioxidant potentials were evaluated using three assays DPPH, ABTS + and FRAP radical scavenging activities.

Results

The findings showed that the OMW was an acidic effluent (pH = 5.05) loaded with mineral and organic matter expressed in terms of a high value of electrical conductivity (EC = 13.51 mS/cm). Indeed, the OMW showed high dry matter, chemical oxygen demand (COD) and biological oxygen demand (BOD_5). The extract obtained by the maceration method showed the highest yields of total polyphenol, flavonoid, and tannins contents than the liquid-liquid extraction method. The LC-MS results revealed the presence of 16 phenolic compounds in the extract obtained by the maceration method and only 12 phenolic compounds were found in the extract obtained by the second method. Quinic acid was identified as the most abundant compound. Moreover, the macerated extracts possessed the highest antioxidant potential as evidenced by their strong DPPH, ABTS and FRAP radical scavenging activities compared to the liquid-liquid extracts.

Conclusions

The maceration methods seemed to be the most effective method for extracting phenolic compounds from OMW than liquid-liquid extraction. The OMW constitute a rich source of natural phenolic compounds that could be used as a potential source of natural antioxidants.

Introduction

The olive oil industry is an important sector of the economy, concentrated mainly in the Mediterranean countries (Alique et al. 2020), including Algeria. In addition to the solid wastes that are called pomace, it

generates very large amounts of liquid discharges and wastewater in olive oil presses. It consists of the water found in the fruit (olive) and the water added during olive oil extraction processes named olive oil mill wastewater (OMW) (Zakia et al. 2021). This industry is increasing annually with the cumulative demand for olive oil consumption, due to its very beneficial effect on health with its therapeutic, diet, and nutritional properties. Consequently, this industrial sector leads to an increase in solid and liquid wastes production that are a source of environmental pollution, especially in Mediterranean countries. The olive oil mill wastewaters are high conductivity and dark acidic liquids that contain interesting compounds including sugars, proteins, dietary fibres and phenolics (Gueboudji et al. 2021a, Haddad et al. 2017, Rocha et al. 2022). Moreover, this great soil damage is due to the slow degradation of these phenolic compounds, which are the reason for their dark color (Gueboudji et al. 2021c). This situation is exacerbated by the seasonality of olive oil production and the large quantities of vegetable water produced (Koutrotsios &Zervakis 2014).

Phenolic compounds are products of the secondary metabolism of plants, widely distributed to many phenolic groups and includes about 9 000 different known structures (Wan et al. 2021), ranging from simple phenolic molecules with low molecular weight like phenolic acids for high-polymerized compounds such as tannins. The phenolics possessed potential beneficial effects on health such as the prevention of ROS (reactive oxygen species)-related diseases such as aging, cancer, and chronic diseases (Khan et al. 2020). Phenolic compounds have several biological properties and are used in many industrial fields because of their antioxidant activity including the pharmaceutical, food, and cosmetic industries (Gueboudji et al. 2021a, Soberón et al. 2019).

Some factors would influence the phenolic compound's composition of OMW such as the olive varieties, climatic conditions, olive oil extraction process and ripening degree of the fruit (Prazeres et al. 2021). However, the selective recovery of phenolic substances from OMW represents a valid approach for the reduction of their environmental toxicity and an opportunity to obtain high added-value molecules (De Bruno et al. 2018). Many recovery studies of OMW polyphenols have been carried out on a small scale and various techniques are used individually or in combination (Martins et al. 2021). These techniques mainly include solvent extraction, adsorption, membrane separation, supercritical fluid extractions, ultrasound treatment, and chromatographic processes (Soberón et al. 2019). Phenol recovery processes generally involve a condensation step before performing the sequential extraction steps with organic solvents such as methanol, ethanol, or hydro-alcoholic solutions. These processes aim to recover either a particular phenol in pure form or a mixture of phenol in the form of a crude product (Alonso-Riaño et al. 2020).

This study aimed to make a comparison between two methods of extraction of phenolic compounds from OMW and to evaluate the most suitable method for the recovery of these phenolic compounds, to maximize the phenolic yield and reduce the quantity of solvents used in extraction.

Experimental Work

Material

The olive oil mill wastewaters were taken from a modern olive oil cold extraction unit, located at Baghai-Khenchela (eastern Algeria) and were obtained after olive oil extraction from Zlitni olives variety in January 2020.

Physicochemical characteristics of OMW

Electrical conductivity (EC) and pH of OMW were directly measured using a conductivity meter and pH meter. The dry matter (DM) was determined after sample drying at 105°C. Fatty matter (FM) was determined by the chloroform/methanol method as described by (Aissam 2003). Chemical oxygen demand (COD) and biological oxygen demand (BOD_5) were evaluated, respectively by the potassium dichromate and the respirometric methods (Rodier et al. 1984).

Polyphenol extraction

Liquid-liquid extraction

The liquid-liquid extraction in ethyl acetate was done according to the method described by (De Marco et al. 2007). A volume of 20 mL of OMW were acidified at pH = 2 with a few drops of HCl and mixed with 30 mL of hexane. The solution was mixed vigorously and centrifuged at 3000 t/min for 5 min. After that, 20 mL of ethyl acetate were added, and the homogenate was shaken vigorously for 15 min and then centrifuged at 3200 t/min for 10 min in (4 ^{o} C). The phases were separated, and the extraction was repeated four times. The obtained five phases of ethyl acetate were collected and combined and the dissolved water was removed with sodium sulfate anhydrous, and the solvent was evaporated under vacuum in a rotary evaporator at 40^{o}C. The dry residues were dissolved in methanol and stored at -18 $^{\circ}$C. The extraction process was carried out in triplicate.

Extraction by maceration

OMW powder (1 g of) were mixed with 10 mL of pure methanol. Then, the mixture was vortexed for 15 min, and let macerate at 4 °C in the dark overnight and filtered through filter paper. The macerate was then collected and was added to 10 mL of methanol for a second time, the mixture was vortexed for 15 min and left to macerate for 1 hour. The two filtrates were combined and filtered through cellulose paper containing sodium sulfate. The solvent was evaporated at 40°C in a rotating evaporator under a vacuum. The dry residue was stored in 6 mL of methanol at -18 $^{\circ}$C. The extraction was performed in triplicate.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of each extract was determined following the Folin–Ciocalteu method (Müller et al. 2010). The TPC of extracts was estimated according to the calibration curve prepared using Gallic acid ($y = 0.0048 x + 0.0027$, $R^2 = 0.9982$). The results were expressed as grams of Gallic acid equivalents per 100 grams of dry matter of initial OMW (g GAE/100 g DM).

Determination of total flavonoid content (TFC)

The quantification of the total flavonoid content (TFC) of each extract was performed using the method described previously (Topçu et al. 2007). The TFC was calculated following the calibration curve prepared using quercetin ($y = 0.0034 x + 0.0311$, $R^2 = 0.9991$). The results were expressed as gram quercetin equivalents per 100 grams of dry matter of initial OMW (g QE/100g DM).

Determination of total tannin content (TTC)

The quantification of the total tannin content (TTC) of each extract was performed by the method described previously (Hagerman 2002). The TTC was estimated according to the calibration curve prepared using catechin ($y = 0.0037 x + 0.0681$, $R^2 = 0.9979$). The results were expressed in grams of catechin equivalent per 100 grams of dry matter of initial OMW (g CE/100g DM).

Liquid chromatography-mass spectrometry (LC-MS) analysis of phenolic compounds

The identification of phenolic compounds in OMW extracts was determined by LC-MS according to the methodology described by Mahmoudi et al. (Mahmoudi et al. 2020, Mahmoudi et al. 2021a, Mahmoudi et al. 2021b). The analysis of phenolic compounds was performed on a Shimadzu UFLC XR system (Kyoto, Japan), equipped with a SIL-20AXR auto-sampler, a CTO-20 AC column oven, a LC-20ADXR binary pump and a quadrupole 2020 detector system. This instrument was equipped with an Inertsil ODS-4 C18 3 µm column (L150×3.0 mm i.d.). The column temperature was set at 40 °C and the injection volume was 20 µl with a flow rate of 0.5 mL/min. Water 95% + MeOh 5% + Acetic acid 0.2% and CAN 50% + H2O 50% + Acetic acid 0.2% were used as mobile phases A and B, respectively. The analysis was performed using a linear gradient programmed as follows: 0,01-14 min, from 10% to 20% B; 14-27 min, 0 from 20% to 55% B; 27-37 min, from 55% to 100% B ; 37-45 min, 100% B ; 45-50 min 10% B. Dissolving line temperature was 275°C, nebulizing gas flow 1,50 L/min, the drying gas was set at 15,00 L/min and temperature of Heat block was 450°C. LC-ESI (-) MS mass spectra [M-H] - were acquired using Lab Solutions software. The identification and the quantification of obtained pics were determined by comparison with the relative retention times and UV spectra with those of standard phenolic compounds as detailed in (Mahmoudi et al. 2021b).

Antioxidant assays

DPPH free radical-scavenging activity

The antioxidant activity of different extractions was evaluated using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (Mahmoudi et al. 2021b). The results were given as 50% inhibition concentration (IC_{50}) and compared with the antioxidant standards (BHT, Ascorbic acid and Trolox). To assess this activity, 0.5 mL of extract at different concentrations was mixed with 0.5 mL of a solution of DPPH (0.2 mM in methanol). After vigorous shaking, the mixture was left to stand at room temperature for 30 min and the absorbance was read at 517 nm

$ABTS^{+}$ free radical scavenging activity

The ABTS scavenging activity was determined as described (Arnao et al. 2001). The ABTS solution was prepared by mixing ABTS (7 mM) with potassium persulfate and the mixture was incubated in the dark before use. The prepared solution was diluted with methanol to have an absorbance of 0.7 ± 0.02. After adding 25 µL of Trolox extract or standard to 2 mL of the diluted ABTS solution, the absorbance at 734 nm was measured for 5 min. The results were given as 50% inhibition concentration (IC_{50}) and compared with the antioxidant standards (BHT, Ascorbic acid and Trolox).

FRAP ferric reducing antioxidant power

The FRAP activity was evaluated according to (Kocak et al. 2016). A volume of 2 mL of FRAP reagent was added to 0.3 mL of the extract samples in a 10 mL volumetric flask, adjusted to a final volume of 10 mL with ultrapure water. The obtained solution was allowed to stand at room temperature for 5 min and then centrifuged for 10 min at 10 000 rpm to remove any kind of solid matter. Absorbance was measured at 593 nm and the results were given as 50% inhibition concentration (IC_{50}) and compared with the antioxidant standards (BHT, Ascorbic acid and Trolox).

Statistical analysis

Data obtained were presented as mean ± standard deviation of three dependent determinations. Significant differences between means of total phenolic, total flavonoids, tannins contents, LC-MS analysis and antioxidant activity results were determined by analysis of variance (ANOVA) and Duncan's multiple ranges. Differences considered significant at $p < 0.05$. Statistical analyses were performed using XLSTAT software (www.xlstat.com).

Results And Discussion

Physicochemical criteria

The physicochemical properties of the studied OMW were presented in Table 1. The OMW obtained from the Zlitni variety were acidic effluents (pH = 5.05) loaded with mineral and organic matter expressed in terms of a high value of electrical conductivity (EC = 13.51 mS/cm). Indeed, the OMW showed high dry matter, chemical oxygen demand (COD) and biological oxygen demand (BOD_5) and were found to be, respectively, 110.67, 208 g/L and 75 g/L. However, the level of the fatty matter was low (0.99%). Results of physicochemical criteria of OMW were in accordance with those obtained in the literature. OMW was an acidic liquid, with pH values varying from 3 to 5 and with an electrical conductivity value of 16.79 mS/cm. Generally, it composed of water (83–94%), organic matter (4–16%), lipids (1 to 14%), COD (40–220 g/L), BOD_5 (35–110 g/L) (Alique et al. 2020, Değirmenbaşı &Takaç 2018). The quality and quantity of OMW varied according to different factors such as production process type, olives verities, use of pesticides and fertilizers, ripening stage, climatic conditions, and geographic area (El-Abbassi et al. 2017).

Table 1
Physicochemical criteria of the studied Olive Mill Wastewater (OMW)

Parameters	Value
pH	5.05 ± 0.05
EC: electrical conductivity (mS/cm)	13.51 ± 0.07
FM: fatty matter (%)	0.99 ± 0.10
DM: dry matter (g/L)	110.67 ± 6.03
COD: chemical oxygen demand (g/L)	208.00 ± 10.00
BOD_5: biological oxygen demand (g/L)	75.00 ± 4.36

Total Phenolic, Flavonoids And Tannins Contents

The total polyphenol, flavonoid and tannin contents extracted by the two methods were presented in Fig. 1. Statistical analysis showed significant differences between means of total polyphenols, total flavonoids and tannins contents extracted with two methods. The maceration was more efficient than the liquid-liquid extraction method to obtain high total polyphenol (22.97 versus 6.47 g GAE/100g DM), total flavonoid (2.34 versus 1.1 g QE /100g DM) and tannin contents (2.47 versus 0.847 g CE /100g DM). When compared to the liquid-liquid extraction technique, these contents increased by 255.02, 112.73, and 191.62 per cent, respectively. OMW was characterized by the richness in phenolic compounds. It has been noted that the TPC of OMW has an amount of 788.96 ± 1.41 mg /100mL found by (Romeo et al. 2020) and varying from 0.5 to 24 g/L (Değirmenbaşı &Takaç 2018). The results that we obtained are found in this range.

Identification And Quantification Of Phenolic Compounds By Lc-ms Analysis

The content of phenolic compounds obtained by LC-MS was shown in Table 2. Thirty-one (31) compounds were identified under the analytical conditions, of which 16 compounds were found in extracts obtained through the maceration and only 12 compounds were found in the extract obtained by liquid-liquid extraction.

Table 2
LC-ESI-MS analysis of phenolic compounds from of OMW Zlitni variety obtained by two extraction methods

Phenolic compounds	formula	Retention time	[M-H] - m/z	Liquid – liquid extraction (ppm)	Extraction by maceration (ppm)
Quinic acid	$C_7H_{12}O_6$	2.043	191	4.80 ± 4.5^b	35.0 ± 2.6^a
Catechin (+)	$C_{15}H_{14}O_6$	12.45	289	0.03 ± 0.3^a	0.3 ± 0.6^a
Caffeic acid	$C_9H_8O_4$	16.167	179	N.D.	2.44 ± 2.12
Rutin	$C_{27}H_{30}O_{16}$	25.283	609	0.02 ± 0.04^b	0.20 ± 0.10^a
Hyperoside (quercetin-3-*O*-galactoside)	$C_{21}H_{20}O_{12}$	25.494	463	0.04 ± 0.07^b	0.17 ± 0.02^a
Luteolin-7-*O*-glucoside	$C_{21}H_{20}O_{11}$	25.867	447	N.D.	0.10 ± 0.00
Naringin	$C_{27}H_{32}O_{14}$	27.413	579	0.02 ± 0.04^b	0.10 ± 0.00^a
4,5-di-O-caffeoyquinic acid	$C_{25}H_{24}O_{12}$	28.017	515	N.D.	0.20 ± 0.00
Quercetrin (quercetin-3-*O*-rhamonosid	$C_{21}H_{20}O_{11}$	28.35	447	0.04 ± 0.06^b	0.30 ± 0.10^a
Apegenin-7-*O*-glucoside	$C_{15}H_{10}O_5$	28.061	431	N.D.	0.70 ± 0.00
Salviolinic acid	$C_{36}H_{30}O_{16}$	29.394	717	0.10 ± 0.10^b	0.40 ± 0.00^a
Kampherol	$C_{15}H_{10}O_6$	33.292	285	1.60 ± 1.00^b	3.0 ± 0.20^a
Quercetin	$C_{15}H_{10}O_7$	33.35	301	0.50 ± 0.70^a	0.3 ± 0.10^b
Naringenin	$C_{15}H_{12}O_5$	35.317	271	0.40 ± 0.20^a	0.5 ± 0.70^a
Apegenin	$C_{15}H_{10}O_5$	35.872	269	0.42 ± 0.18^b	0.7 ± 0.00^a
Cirsiliol	$C_{17}H_{14}O_7$	36.97	329	2.30 ± 2.96^a	1.3 ± 0.10^b
Total phenols content		-	-	9.836 ± 0.323	45.112 ± 0.213

Each value is the mean ± Standard deviation (SD); N.D.: not determined. Means in the same line with different letters differ significantly ($p < 0.05$);

Phenolic compounds identified were quinic acid, catechin (+), caffeic acid, rutin, hyperoside (quercetin-3-*O*-galactoside), luteolin-7-*O*-glucoside, naringin, 4,5-di-*O*-caffeoyquinic acid, quercetrin, apegenin-7-*O*-glucoside, salviolinic acid, kampherol, quercetin, naringenin, apegenin and cirsiliol. Four phenolic acids were identified in the macerated extract and were caffeic acid, luteolin-7-*O*-glucoside, 4,5-di-*O*-

caffeoyquinic acid and apigenin 7-*O*-glucoside. The results indicated that the maceration method gives higher values than the liquid-liquid extraction method in quinic acid, rutin, hyperoside, luteolin-7glucoside, 4,5-di-*O*- caffeoyquinic acid and salviolinic acid. The two methods give the same values in terms of catechin (+), caffeic acid, naringin, quercetrin (quercetin-3-*O*-rhamonosid), apegenin-7-*O*-glucoside, salviolinic acid, kaempferol, quercetin, naringenin, apegenin and cirsiliol. The highest level of phenolic compounds was recorded in the macerated extract with an average value of 45.112 ppm, compared with extracts obtained by the liquid-liquid method (9.836 ppm). The quinic acid was the major phenolic compound with an average value of 4.82 in extracts obtained by liquid-liquid followed, in decreasing order by cirsiliol (2.3 ppm), kaempferol (1.6 ppm), quercetin (0.5 ppm), apigenin (0.42 ppm), naringenin (0.4 ppm), catechin (+) (0.3 ppm), salviolinic acid (0.1 ppm), hyperoside (quercetin-3-*O*-galactoside (0.04 ppm), quercetrin (quercetin-3-*O*-rhamonosid) (0.04 ppm), rutin (0.02 ppm) and naringin (0.02 ppm). For the maceration method, quinic acid was also the major phenolic compound with an average value of (35.1 ppm) and followed by kaempferol (3 ppm), caffeic acid (2.44 ppm), cirsiliol (1.3 ppm), apegenin (0.7 ppm), apegenin-7-*O*-glucoside (0.7 ppm), naringenin (0.5 ppm), salviolinic acid (0.4 ppm), quercetrin (quercetin-3-o-rhamonosid (0.3 ppm), quercetin (0.3 ppm), Rutin (0.2 ppm), 4,5-di-*O*-caffeoyquinic acid (0.2 ppm), hyperoside (quercetin-3-*O*-galactoside (0.17 ppm), luteolin-7-*O*- glucoside (0.1 ppm) and naringin (0.1 ppm).

Results showed that quinic acid was the major compound in the extracts obtained by the two methods of extraction. Several researchers were identified phenolic compounds by HPLC after liquid-liquid extraction and several compounds were characterized such as gallic acid, hydroxytyrosol-4-β-glucoside, hydroxytyrosol, tyrosol, caffeic acid, p-coumaric acid and oleuropein aglycone (El-Abbassi et al. 2012). Moreover, Romeo et al. (Romeo et al. 2020) identified ten compounds including chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, verbascoside, luteolin and apigenin. Some phenolic compounds frequently prevalent in OMW, such as gallic acid, p-coumaric acid, were not detected in our extracts. These compounds are easily oxidizable and their transformation was possible (Belaid et al. 2002). The presence of caffeic acid, luteolin-7-*O*-glucoside, 4, 5-di-O-caffeoyquinic acid, apigenin-7-*O*-glucoside only in the extracts obtained by the maceration method would be explained by their oxidization and therefore their rapid possible transformation.

It was noted that the difference in the quantity and quality of the phenolic compounds was due to the loss of a percentage of them that remains trapped in hexane and ethyl acetate phases during the liquid-liquid extraction. It was confirmed that not all phenolic compounds were extracted by ethyl acetate, especially the phenolic compounds of high molecular weight as tannins (El-Abbassi et al. 2017). In addition, Freeze-drying was recommended to preserve the phenolic fraction of the olive oil mill wastewaters from any variation. According to Turkmen et al. (Turkmen et al. 2007), phenolic compounds were generally extracted using suitable solvents such as methanol, ethanol and acetol, N, N-dimethylformamide. Therefore, methanol was a polar solvent that allow the extraction of polyphenols. The maceration method was simple, high efficiency, and economical for polyphenols extraction. The efficiency of the method was mostly influenced by the solvent, the pH of the extraction medium that

determined the compound solubility, the temperature, the extraction steps, and the solvent volume, as well that the size and shape of the particles (Alonso-Riaño et al. 2020).

Antioxidant Potentials

The free radical scavenging activity determined by DPPH., ABTS, .and FRAP was widely used to estimate the antiradical/antioxidant capacity of phenolic compounds of OMW extracted with two methods and compared the data to many reference standards to obtain more useful and arguably essential results. ANOVA one away analysis revealed a significant difference of antioxidant potential depending on the extraction methods (Table 3). The results of DPPH radical scavenging activity showed that the macerated extracts exhibited the highest antioxidant activity evidenced by a low IC_{50} value (7.55 µg/mL) higher than that of BHT (11.11 µg/mL), ascorbic acid (12.28 µg/mL) and Trolox (16.12 µg/mL). Similarly, the analysis data of the ABTS assay showed that the extract obtained from the maceration extraction method give the best activity with (IC_{50}:6.08) lower than that of ascorbic acid and BHT (1.52 and 2.2 µg/mL) respectively, and higher than that of Trolox and the liquid-liquid extract (9.06 and 13.51 µg/mL). From the results of FRAP, extracts of the maceration extraction method were exhibited the highest antioxidant activity (3.12 µg/mL) than ascorbic acid (9.94 µg/mL), and extracts from liquid-liquid extraction (11.56), and much higher activity than Trolox (17.06 µg/mL) and BHT (20.05 µg/mL) (Table 4). This is supported by the findings of Romeo et al. (Romeo et al. 2020) who reported that the olive mill wastewater showed strong antiradical DPPH and ABTS scavenging activities (114.37 and 2569.19 mmol TE/100mL. The obtained results showed that the total polyphenol content was highly and positively correlated with the antioxidant capacity evaluated by the DPPH, ABTS and FRAP assays. According to De Marco et al. (De Marco et al. 2007), the phenolic compounds of OMW were characterized by a strong antioxidant potential. The *in vitro* antioxidant activity of natural extracts has received much more attention. These methods involved the presence of oxidizing species such as free radicals and metal complexes (Alam et al. 2013). Several studies have shown that the antioxidant activity depends on the concentration of total polyphenols, the antioxidant structures, as well as the reaction time (Abramovič et al. 2018, Gueboudji et al. 2021b, Leouifoudi et al. 2015). The antioxidant potential of the studied extracts would be explained by the load of the total and the type of phenolic compounds and by the assembly of three compounds found in high concentrations in the extracts tested that are quinic acid, kaempferol, and cirsiol. Therefore, the present results of the antioxidant activity of phenolic extracts were in accordance with their phenolic compounds composition. Indeed, the ability to reduce free radicals is largely influenced by the phenolic composition of the sample.

Table 3
The mean squares of the antioxidant activities

Source	DF	DPPH (IC_{50} µg/mL)	ABTS (IC_{50} µg/mL)	FRAP (IC_{50} µg/mL)
Effect of extraction Methods	4	58.9*	74.38*	129.89*
Error	10	0.6	0.36	0.63
* : significant effect				

Table 4
Antioxidant's activity of extracts by DPPH, ABTS and FRAP

	DPPH IC_{50}µg/mL	ABTS IC_{50}µg/mL	FRAP IC_{50}µg/mL
Extracts from the liquid-liquid extraction	18.93 ± 1.58^{a}	13.51 ± 0.02^{a}	11.56 ± 1.71^{c}
Extracts from the maceration	7.55 ± 0.49^{d}	6.08 ± 0.82^{c}	3.12 ± 0.3^{e}
BHT	11.11 ± 0.31^{cd}	2.2 ± 0.14^{d}	20.05 ± 0.19^{a}
Ascorbic acid	12.28 ± 0.34^{c}	1.52 ± 0.19^{e}	9.94 ± 0.19^{d}
Trolox	16.12 ± 0.3^{b}	9.06 ± 0.15^{b}	17.06 ± 0.2^{b}
Each value is the mean ± Standard deviation (SD). Means in the same column with different letters differ significantly ($p < 0.05$)			

Conclusion

The phytochemical contents and the antioxidant potentials of olive mill wastewater were deeply investigated. Phenolic compounds and antioxidant activities were highly and significantly dependent on the extraction method of polyphenols. A total of 16 phenolic compounds were mostly predominated by the quinic acid. Furthermore, DPPH·, ABTS·+ and FRAP antioxidant activities showed a significant variation between the two different methods and the maceration extraction gives the highest concentration of phenolic compounds and the highest free radical scavenging activities. The maceration extraction is a cheap, simple, and easy method. Finally, it should be noted that OMW constitutes a very complex and fragile matrix to handle because it presented a natural antioxidant that can be used in various industries such as food and pharmaceutical. The recovery of polyphenols offers the double opportunity to obtain biomolecules with high additive value on the one hand and to reduce the pollutant nature of OMW on the other hand, particularly present in the countries bordering the Mediterranean Sea.

Declarations

Ethics Approval

Not applicable.

Consent to Participate

All authors consent to participate in the works of the manuscript.

Consent to Publish

All authors consent to submit and publish the manuscript.

Availability of data and materials

Data and materials are available upon request.

Competing Interests

The authors declare no competing interests.

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Authors' Contributions

All authors contributed to the study conception and design. The first draft of the manuscript was written by Zakia Gueboudji and Maher Mahmoudi. The supervision of the work was performed by Kenza Kadi, Kamel Nagaz and Kamel Hessini. The laboratory experiments and data analysis were performed by Dalila Addad Hedia Hannachi: Leila Ben Yahya, and Belgacem Lachehib contribute to the laboratory experiments. All authors have read and approved the manuscript.

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Figures

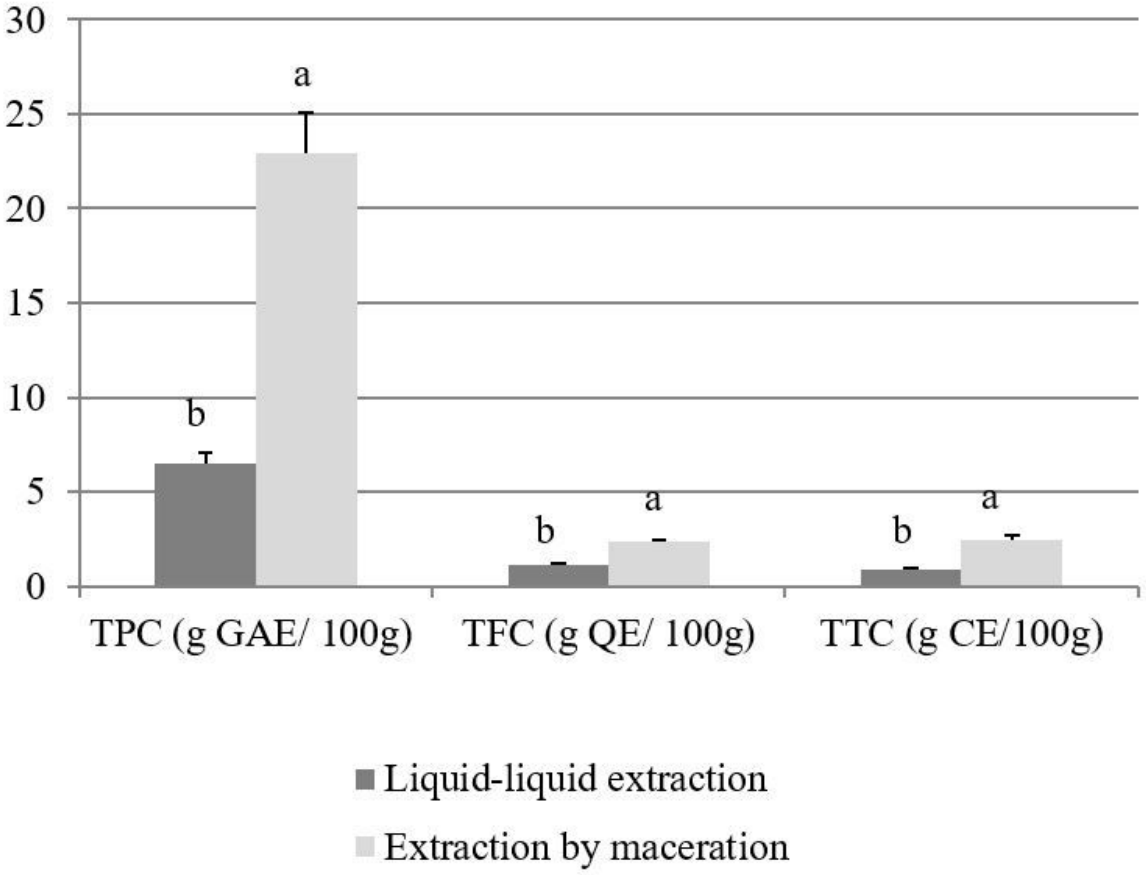

Fig.1

Figure 1

Total polyphenols, flavonoids, and tannins contents of Zlitni OMW extracts by two methods of extraction (TPC: total polyphenols content; TFC: total flavonoids content; TTC: total tanins content; GAE: gallic acid equivalent; QE: quercetin equivalent; catechin equivalent); for each content with different letters differ significantly (p <0.05).

Supplementary Files

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