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Variation of Phenolic Contents, Antioxidant and Antibacterial Activities of Tunisian Olive Mill Wastewater Varieties among Various Industrial Procedures and Solvent Extractions

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Abstract. This study highlights the critical influence of industrial olive oil extraction processes and olive variety on the physicochemical and bioactive properties of Tunisian olive mill wastewater. Key parameters assessed including pH (5.23 to 6.30), water content (83.04% to 98.43%), solid content (5.19% to 30.35%), and lipid content (1.52% to 2.80%). Furthermore, the phenolic content varied substantially, ranging from 0.061 to 2.802 g GAE/L (gram gallic acid equivalent per liter), depending on extraction techniques such as maceration with ethanol or methanol/chloroform (1:1) and liquid–liquid extraction with ethyl acetate; these findings reveal how industrial processes and olive variety dictate the phenolic recovery. The industrial impact extends to antioxidant properties, with IC_{50} values ranging between 0.053 and 0.393 mg ET/mL (milligram Trolox equivalent per liter) using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid and 1.32–73.16 mg AAE/mL (milligram ascorbic acid equivalent per liter) using 2,2-diphenyl-1-picrylhydrazyl. Additionally, methanol/chloroform extracts exhibited significant antimicrobial activity, effectively inhibiting microbial growth highlighting the importance of the extracting solvent. By illuminating the significant variations introduced by industrial processes and olive variety, this study underscores their central role in optimizing phenolic compound recovery to enhance olive oil production efficiency while mitigating the ecological impact of wastewater management.

Tunisia, one of the world's top olive oil producers, cultivates 1.8 million hectares of olive oil with 86 million olive trees. The National Gene Bank of Tunisia conserves over 200 olive varieties from the country's germplasm (Debbabi et al. 2022). In the north, the main varieties are 'Chetoui', 'Sayali', and 'Gerboui', while 'Chemlali' and 'Oueslati' dominate the central–southern areas, alongside 'Zalmati', 'Zarrazi', and 'Tounsi' (Debbabi et al. 2022).

Olive oil extraction involves three main stages: crushing, malaxation, and separation. Initially, olives undergo cleaning, quality control (weight, acidity, fat yield), and storage. Milling follows, using traditional or modern techniques with heating to enhance oil extraction. The phase separation employs techniques such as pressing (discontinuous) or centrifugation (continuous), with the latter using decanters to process large quantities efficiently. The three-phase process includes hot water in addition to separate insoluble solids, oil, and aqueous phases through density differences (Mohammadnejad et al. 2021).

Olive oil extraction yields 20% oil and 80% byproducts, comprising 30% pomace (solid waste) and 50% olive mill wastewater (OMW) (Alkhalidi et al. 2023). During the 2- to 3-month production period, 10 to 30 million m^3 of OMW is generated (Fattoum et al. 2023). OMW is characterized by a red-black color, a foul smell, a pH of 4.7 to 5.15, and high conductivity (11 to 13 mS/cm) (Zaier et al. 2017). Uncontrolled disposal poses a significant environmental issue due to its high content of phenolic compounds (0.98% to 10.7%), contributing to high chemical oxygen demand COD (37 to 318 g/L) and biochemical oxygen demand (15 to 135 g/L) values (Tundis et al. 2021).

Interestingly, only 2% of the phenolic content from milled olives is found in the oil phase, while 98% remains in the pomace and OMW (Fattoum et al. 2023). These phenolic compounds are

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natural antioxidants with various health benefits, including cancer prevention, cardioprotection, and neuroprotection, as evidenced by numerous studies (El-Abbassi et al. 2012). Due to its high phenolic content, OMW has gained attention as a valuable antioxidant source for the food, cosmetic, and pharmaceutical industries (El-Abbassi et al. 2012).

Extensive research has explored OMW phenolic recovery using diverse extraction methods like adsorption, membrane separation, maceration, liquid–liquid extraction, and supercritical fluid extraction, with each technique requiring optimization for efficiency (Yangui and Abderrabba 2018). The phenolic profile and concentration vary based on factors such as olive variety, maturity, climatic conditions, harvest period, and the oil extraction process (Roig et al. 2006; Tundis et al. 2021).

This paper comprehensively assesses Tunisian OMW, examining the impact of industrial olive oil extraction processes on its characteristics and highlighting the significance of industrial practices on the phenolic content and bioactive properties of OMW extracts.

Materials and Methods

Plant material

The samples analyzed in this study originated from 9 olive oil extraction units situated across various regions of Tunisia (Northeast, Northwest, Middle, and South). Among these units, two use traditional discontinuous olive oil extraction processes (Rappanelli and Jha), while the remaining ones use continuous three-phase processes (Pieralisi, Flottweg, Alphalaval, Barracane, Polat, Naoura, and Amenduni). The samples were also sourced from five different olive varieties: 'Chetoui', 'Chemleli', 'Koroneiki', 'Pitchoulini', and 'Chaibi'.

To maintain their original characteristics, the samples obtained were directly centrifuged and stored in clean 1.5-L bottles. Subsequently, they were carefully preserved at −4 °C to safeguard their freshness and integrity throughout the analysis process.

Transitioning from the general overview of collected samples, the discussion will now focus on a detailed analysis of each individual sample, including Chem S Pier from the variety 'Chemleli' in the South and from continuous three-phase processes: Pieralisi; Chet M Flw from the variety 'Chetoui' in the Middle and from continuous three-phase processes: Flottweg; Koro N Al from the variety 'Koroneiki' in the Northeast and from continuous three-phase processes: Alphalaval; Chet N Al from the variety 'Chetoui' in the Northeast and from continuous three-phase processes: Alphalaval; Koro N Bar from the variety 'Koroneiki' in the Northeast and from continuous three-phase processes: Barracane; Chet N Bar from the variety 'Chetoui' in the Northeast and from continuous three-phase processes: Barracane; Chem N Al from the variety 'Chemleli' in the Northeast and from continuous three-phase processes: Alphalaval; Pit N Al from the variety 'Pitchoulini' in the Northeast and from continuous three-phase processes: Alphalaval; Chem N Rap from the variety 'Chemleli' in the South and from a discontinuous traditional process: Rappanelli; Chet M Jha from the variety 'Chetoui' in the Middle and from a discontinuous traditional process: Jha; Chet M Polat from the variety 'Chetoui' in the Middle and from continuous three-phase processes: Polat; Chet M Naoura from the variety 'Chetoui' in the Middle and from continuous three-phase processes: Naoura; Chaibi N Al from the variety 'Chetoui' in the Northwest and from continuous three-phase processes: Alphalaval; and Chaibi N Am from the variety 'Chetoui' in the Northwest and from a continuous three phase processes: Amenduni.

Chemicals

The following chemicals were used: ethanol, methanol, chloroform, cyclohexane, ethyl acetate, Na_2SO_4 anhydrous, Folin-Ciaocalteu reagent, gallic acid (standard), sodium carbonate, Trolox (standard), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Physiochemical characterization of the OMW

pH. The pH meter type HANNA was used to measure the hydrogen potential (pH). The instrument was precalibrated with buffer solutions at pH 4 and 7 (Zaier et al. 2017).

Water and solid content. The water content was determined by drying 10 g of OMW at 103 °C for 24 h. The differences in OMW and moisture content determine the residual solid residue after water removal (Gortzi et al. 2008).

Total solid in suspension content. Total suspended solids are the undissolved solids. To obtain them, centrifuging the fresh OMW at 179 g_n for 20 min is required. The residue is then dried overnight at 105 °C (Bouknana et al. 2014).

Lipid content. To determine the lipid content, 10 g of OMW and 15 mL of hexane were mixed three times. The mixture was agitated vigorously in a 100-mL separating funnel and centrifuged for 5 min at 101 g_n before being combined. The final process is the drying of the supernatant through evaporation using a rotating evaporator at 79 °C until a single oily phase is obtained (Gortzi et al. 2008).

Phenolic compound extraction

Maceration extraction. For maceration, 10 mg of dried OMW was mixed with 10 mL of the solvent. The various samples were macerated for ∼7 h before being centrifuged at 179 g_n for 10 min, and finally the supernatants obtained were preserved at 4 °C. The solvents used are methanol, ethanol, and solvent mixture (chloroform/methanol 1:1).

Liquid–liquid extraction. Acidifying the OMW with chlorohydric acid until pH <2 and removing the fat with hexane is advisable before starting the extraction process to precipitate proteins, release bio-phenols, and increase the solubility of phenolic compounds in the organic solvent (De Marco et al. 2007). The liquid–liquid extraction was carried out following the method described by De Marco et al. (2007). In a separating funnel, 10 mL of acidified and delipidated OMW was mixed with 20 mL of ethyl acetate and shaken for 15 min. This extraction was repeated four times. The ethyl acetate phases containing the phenolic compounds were collected, and anhydrous Na_2SO_4 was added to remove any water. The Na_2SO_4 residue was filtered out, and the solvent was evaporated under vacuum using a rotary evaporator at 40 °C. The final residue was stored in 6 mL of methanol at −1 °C.

Total phenol content determination

The total phenol content of the extracts was determined calorimetrically using Folin–Ciocalteu reagent, following the method described by Škerget et al. (2005). A 100-μL sample of the extract was mixed with 1 mL of Folin–Ciocalteu reagent. After 5 min, 800 μL of sodium carbonate (Na_2CO_3, 75 g/L) was added. The mixture was incubated for 30 min in the dark, and the absorbance was measured at 760 nm. The control and standard procedures were identical, with gallic acid used as the

standard. The results are expressed as grams of gallic acid equivalent (GAE) per liter of OMW.

Antioxidant activity determination

ABTS. The antioxidant activity of phenolic extracts and their fractions was evaluated using the method described by Ghazouani et al. (2016). This assay aims to scavenge the ABTS radical cation, which has a characteristic absorption at 734 nm, converting it into a colorless product. The discoloration caused by the compound is compared with that caused by Trolox.

The extracts were diluted, to obtain optimal results, and 100 μL of each diluted extract was mixed with 900 μL of ABTS solution. Absorbance was measured after 6 min of incubation at 734 nm. The ABTS method is favored for routine laboratory work due to its stability and simplicity, although its indirect nature limits its scope.

DPPH. The DPPH test is a simple and rapid method that requires only a ultraviolet-visible absorption spectrometer. The radical scavenging ability of the DPPH radical was evaluated using the procedure described by Brand-Williams et al. (1995). For each extract concentration, 20 μL of the sample was added to 980 μL of the DPPH solution. After vortexing, the tubes were placed in the dark at room temperature for 2 h. Absorbance was then measured at 517 nm using a spectrophotometer. Ascorbic acid, a standard antioxidant, was used as a positive control, and its absorbance was measured under the same conditions as the test samples.

Antimicrobial activity

An initial evaluation was conducted of the antibacterial activity of phenolic extracts of five samples: Chem S Pier using ethyl acetate, Chet N Al using ethyl acetate, Pit N Al using ethyl acetate, Chet M Naoura using ethyl acetate, and Koro N Bar using methanol/chloroform (1:1) using the protocol described by Omrani et al. (2021). The samples were screened for the following activity using a 6.0-mm-diameter paper-disc method. It was determined by a disc diffusion method on Mueller–Hinton agar and then measure the inhibition zone. The antibacterial study was focused on standard bacterial strains of *Enterococcus faecalis* (ATCC29212), *Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27859), and *Escherichia coli* (ATCC 25922). The controls used are control 1: methanol and control 2: methanol/chloroform (1:1).

Statistical analysis

All analyses were conducted in triplicate, and the results are expressed as mean values ± standard deviation. The one-way and multivariate analysis of variance followed by Duncan's multiple range tests was used to compare means. At $P < 0.05$, the differences between individual mean values were deemed to be significant. Statistical analysis was performed by using the statistical program package STATISTICA (Statsoft, Tulsa, OK, USA). In addition, correlation coefficients were calculated to determine the relation between total polyphenols, and the inhibition percentage and the IC_{50} test.

Results and Discussion

Physiochemical characterization of olive mill wastewater

Physicochemical characterization was investigated, and all results are gathered in Table 1. Olive mill wastewaters are very well known for their acidic aspect. In fact, the pH of the samples varies between 5.23 (Chaibi N Al) and 6.91 (Chet M Flw) with an average of 5.54. These results are in the same range with what has been found in the bibliography: 5.1 to 5.2 (El-Abbassi et al. 2012), 5.7 by Rousidou et al. (2010), 5.1 to 7.5 (Amaral et al. 2008), and 4.59 (Dammak et al. 2016). The high value recorded in the sample Chet M jha can be explained by the fact that some units add potassium nitrate to neutralize the OMW before dumping it to protect the soil and the groundwater.

Furthermore, water is the primary component of OMW; the samples investigated in this study showed a range of water content between 83.04% and 98.43%. As a result, the solid content is within the range of 1.57% to 16.96%. The findings are consistent with the existing literature, as Foti et al. (2021) reported water content ranging from 80% to 96% (Foti et al. 2021), and other authors (Gueboudji et al. 2023; Mohammadnejad et al. 2021; Rahmanian et al. 2014) reported values between 83% to 94%. In addition, the study underlines the significance of comprehending the water/solid ratio in natural waste for effective waste management plans.

The suspended total solid content fluctuates notably, ranging from 5.19% in the case of Chem N Al to 30.35% in Chet M Flw. It is noteworthy that the results reported are higher than those reported by Fattoum et al. (2023), who reported a suspended solid content of 0.83% ± 0.1% for stored OMW. Other resources, such as the work of El-Abbassi et al. (2012), have reported 48 to 87 g/L of total solids in suspension, and Zaier et al. (2017) reported 9.43 to 21.36 g/L.

The lipid content in the samples varies between 1.52% (Chet M Polat) and 2.80% (Chet M jha) with an average of 2.42; these results closely match the expected range of 1.0% to 1.5% (Rahmanian et al. 2014) and 1.0% to 23% (Foti et al. 2021). Thus, the slight difference in the lipid content results in Table 2 across different samples could be attributed to the olive varietal difference.

The observed variations in the results among the samples are significant, indicating that the physicochemical characteristics are likely influenced by industrial extraction methods and olive varieties. This suggests the need for further investigation to confirm this influence and better understand their combined impact on optimizing olive oil production.

Phenolic compound extraction

The polyphenol content extracted via maceration and liquid–liquid extraction methods is detailed in Table 3. Analysis of the overall results revealed significant variability and richness in the phenolic compounds of the studied samples, with phenolic contents ranging from 0.06 to 2.80 g GAE/L of OMW. This variability is primarily attributed to multiple factors, including the industrial extraction process, olive variety, and the phenolic extraction method and solvent used.

The samples studied were collected from both traditional extraction processes and modern three-phase processes. As shown in Table 3, traditional press machines (e.g., Jha and Rappanelli) consistently produced OMW with lower phenolic levels. For instance, Chet M Jha yielded phenolic concentrations ranging from 0.06 to 0.42 g GAE/L of OMW, while Chem S Rap produced 0.45 to 0.77 g GAE/L. In contrast, three-phase machines (e.g., Pieralisi, Alphalaval, Flottweg, and Amenduni) exhibited higher concentrations, ranging from 0.15 g GAE/L for Chaibi N Al to 2.80 g GAE/L for Pit N Al. This difference is primarily due to the use of mixing water in three-phase processes, which

Table 1. Physicochemical characterization.

Machine	Variety	Sample	pH	Water content	Solid content	Total solid in suspension	Lipid content
Pieralisi	Chemleli	Chem S Pier	5.55	83.04 ± 4.95	16.96 ± 4.95	26.41 ± 3.18	2.61 ± 0.17
Flottweg	Chetoui	Chet M Flw	6.3	93.11 ± 0.07	6.89 ± 0.07	30.35 ± 0.55	2.47 ± 0.16
Alphalaval	Chetoui	Chet N Al	5.36	95.42 ± 1.74	4.58 ± 1.74	13.22 ± 7.09	2.58 ± 0.16
Alphalaval	Koroneiki	Koro N Al	5.33	94.30 ± 4.45	5.7 ± 4.45	6.205 ± 0.57	2.50 ± 0.09
Barracane	Chetoui	Chet N Bar	5.42	92.97 ± 0.46	7.03 ± 0.46	10.12 ± 0.23	2.70 ± 0.17
Barracane	Koroneiki	Koro N Bar	5.33	91.43 ± 0.13	8.57 ± 0.13	10.065 ± 0.45	2.41 ± 0.27
Alphalaval	Chemleli	Chem N Al	5.31	88.82 ± 3.34	11.18 ± 3.34	5.19 ± 0.06	2.41 ± 0.21
Alphalaval	Pitchoulini	Pit N Al	5.41	93.10 ± 0.07	6.90 ± 0.07	5.39 ± 0.66	2.40 ± 0.11
Rappanelli	Chemleli	Chem N Rap	5.48	92.13 ± 0.31	7.87 ± 0.31	5.69 ± 2.09	2.67 ± 0.07
Jha	Chetoui	Chet M jha	6.91	98.06 ± 0.36	1.94 ± 0.36	6.38 ± 4.32	2.80 ± 0.05
Polat	Chetoui	Chet M Polat	5.53	92.48 ± 0.07	7.52 ± 0.07	14.07 ± 1.82	1.52 ± 0.01
Naoura	Chetoui	Chet M Naoura	5.3	91.17 ± 0.27	8.83 ± 0.27	20.01 ± 0.59	1.89 ± 0.03
Alphalaval	Chaibi	Chaibi N Al	5.23	98.43 ± 0.12	1.57 ± 0.12	5.84 ± 0.09	2.34 ± 0.05
Amenduni	Chaibi	Chaibi N Am	5.06	93.52 ± 0.20	6.48 ± 0.20	14.16 ± 0.62	2.53 ± 0.08

enhances polyphenol extraction, leading to significantly higher phenolic levels compared with traditional presses.

The 'Pitchoulini' variety exhibited the highest phenolic content, with 0.90 g GAE/L for maceration extraction with ethanol, 2.80 g GAE/L for maceration extraction with methanol/chloroform, and 2.48 g GAE/L for liquid–liquid extraction with ethyl acetate. Conversely, the 'Chaibi' variety showed the lowest concentrations, with 0.59 g GAE/L for ethanol maceration, 0.31 g GAE/L for liquid–liquid extraction with ethyl acetate, and lower values for methanol/chloroform extraction. These findings underscore the significant impact of olive variety on phenolic content, confirming that varietal differences influence phenolic compound concentrations.

The extraction method and solvent also markedly affect phenolic content. For example, liquid–liquid extraction with ethyl acetate yielded high values in some samples, such as Chem S Pier (2.13 g GAE/L), Koro N Al (2.33 g GAE/L), and Chet N Bar (1.98 g GAE/L). In other cases, methanol/chloroform extractions resulted in higher concentrations, such as Pit N Al (2.80 g GAE/L) and Chet M Flw (1.12 g GAE/L). This variability can be attributed to differences in solvent polarity, solubility, and chemical affinity for specific phenolic classes (Fattoum et al. 2023).

These results align with previous studies, including that of Sar and Akbas (2023), which reported phenolic contents ranging from 2.16 to 4.03 g GAE/L in OMW from three-phase processes. Similarly, De Marco et al. (2007) found phenolic compound concentrations between 1.1 and 2.5 g tyrosol/L of OMW using three-phase extraction. Conversely, Gueboudji et al. (2023) reported even higher concentrations, ranging from 6.47 to 22.97 g GAE/100 g dry matter, in OMW extracted using modern processes.

Furthermore, El-Abbassi et al. (2012) comparing semimodern (OMW1) and modern three-phase (OMW2) processes, found higher phenolic content in OMW1 (9.8 g/L) compared with OMW2 (6.1 g/L). This difference can be attributed to olive ripeness and farming practices. Additionally, our study's results exceeded those reported by Fattoum et al. (2023), which ranged from 0.15 ± 0.01 to 1.17 g GAE/L of OMW from stored samples, while our fresh samples demonstrated significantly higher phenolic content, emphasizing the role of sample freshness.

Table 2. Comparative overview of olive oil production's processes (Mohammadnejad et al. 2021).

Method	Input	output	Description	Machine
Press	• Olives (1 ton) • Washing water (0.1–0.12 m^3) • Energy (40–63 kWh)	• Oil (200 kg) • Solid waste (400 kg) • Wastewater (0.4–0.6 m^3)	• Less water (about 40%) • Less wastewater • Low capital cost • Concentrated/highly polluted wastewater	• Rappanelli • Jha • Polisenelli
Centrifugation				
Three-phase	• Olives (1 ton) • Washing water (0.1–0.12 m^3) • Decanter water (0.5–1 m^3) • Oil polishing water (0.01 m^3) • Energy (90–117 kWh)	• Oil (200 kg) • Olive pomace • (500–600 kg) • OMW (1–1.2 m^3)	• High water requirement (500 L/ton of olive paste) • High OMW	• Molinetto • Pieralisi • Alphalaval • TEM (Toscana Enologica Mori) • Ferraroni
Two-phase	• Olives (1 ton) • Washing water • (0.1–0.12 m^3) • Energy (>90 kWh)	• Oil (200 kg) • Semisolid pomace • (800–950 kg) • OMW (0.0851.1 m^3)	• Eco-friendly • Less OMW • Save water and energy (by 80% and 20%, respectively) • Cheaper than three-phase (25%) • Difficult treatment of semi-solid pomace	• Amenduni • Comet • Alphalaval • Pieralisi

OMW = olive mill wastewater.

Table 3. Total phenolic content through different extracting methods and solvents.

			Phenolic compound concn (g GAE/L of OMW m ± *SD*)		
			Maceration		Liquid–liquid
Machine	Variety	Sample	Ethanol	Methanol/chloroform (1:1)	Ethyl acetate
Pieralisi	Chemleli	Chem S Pier	0.88 ± 0.01	1.69 ± 0.04	2.13 ± 0.17
Flottweg	Chetoui	Chet M Flw	0.45 ± 0.04	1.12 ± 0.04	0.97 ± 0.10
Alphalaval	Chetoui	Chet N Al	0.84 ± 0.02	1.26 ± 0.16	1.12 ± 0.12
Alphalaval	Koroneiki	Koro N Al	0.87 ± 0.01	1.30 ± 0.17	2.33 ± 0.23
Barracane	Chetoui	Chet N Bar	0.87 ± 0.01	1.82 ± 0.10	1.98 ± 0.08
Barracane	Koroneiki	Koro N Bar	0.81 ± 0.04	1.0 ± 0.14	1.39 ± 0.15
Alphalaval	Chemleli	Chem N Al	0.86 ± 0	1.29 ± 0.14	1.70 ± 0.06
Alphalaval	Pitchoulini	Pit N Al	0.90 ± 0	2.80 ± 0.09	2.48 ± 0.13
Rapanelli	Chemleli	Chem N Rap	0.77 ± 0.04	0.45 ± 0.05	0.63 ± 0.13
Jha	Chetoui	Chet M jha	0.42 ± 0.04	0.06 ± 0.01	0.31 ± 0.03
Polat	Chetoui	Chet M Polat	0.35 ± 0.02	0.31 ± 0.04	0.78 ± 0.12
Naoura	Chetoui	Chet M Naoura	0.72 ± 0.14	0.64 ± 0.03	0.52 ± 0.04
Alphalaval	Chaibi	Chaibi N Al	0.59 ± 0.03	0.15 ± 0.01	0.31 ± 0.05
Amenduni	Chaibi	Chaibi N Am	0.90 ± 0	0.82 ± 0.03	0.57 ± 0.06

GAE = gallic acid equivalent; OMW = olive mill wastewater; *SD* = standard deviation.

In conclusion, the varieties 'Pitchoulini', 'Koroneiki', 'Chemlali', and 'Chetoui' consistently demonstrated the highest phenolic content. Regarding extraction methods, three-phase machines (e.g., Alphalaval, Pieralisi, and Barracane) that use substantial water during olive mixing produced OMW with the richest phenolic composition, highlighting the importance of both variety and process in determining phenolic content.

Antioxidant activity determination

OMW's availability in large quantities and high concentrations of phenolic compounds suggests that it could become a natural source of valuable and powerful antioxidants in the future (Sar and Akbas 2023). Our results show the antioxidant activity variations across the different samples (Table 4).

In general, we observed in ABTS an IC_{50} value ranged from 0.053 mg ET/mL to 0.393 mg ET/mL (milligramTrolox Equivalent per liter), while for DPPH, the IC_{50} values ranged from 1.32 mg ascorbic acid equivalent (AAE)/mL to 73.16 mg AAE/mL. Compared with the literature, our results are more significant than Gueboudji et al. (2023), who found IC_{50} equal to 6.08 ± 0.8 mg·L^{-1} for ABTS and 7.55 ± 0.49 mg·L^{-1} for DPPH. These results are also more significant than the results stated by Dali et al. (2023), such as 16.90 mg·L^{-1} for ABTS and 50.10 mg·L^{-1} for DPPH, as well as the results of stocked OMW stated by Fattoum et al. (2023), which were 15.75 mg·mL^{-1} for ABTS. The variation of the antioxidant activity is due to multiple factors including the industrial extracting process for instance, it is noticeable that the three-phase machines, such as Alphalaval, Pieralisi and Barracane, have important activity

Table 4. Antioxidant activity through different extracting methods and solvents using ABTS and DPPH.

			ABTS			DPPH		
			Maceration		Liquid–liquid	Maceration		Liquid–liquid
Machine	Variety	Sample	Ethanol	Methanol/chloroform (1:1)	Ethyl acetate	Ethanol (mg/mL)	Methanol/chloroform (1:1) (mg/mL)	Ethyl acetate (mg/mL)
Pieralisi	Chemleli	Chem S Pier	0.12 ± 0.063	0.12 ± 0.003	0.15 ± 0.029	12.65 ± 6.33	20.53 ± 1.63	18 ± 1.82
Flottweg	Chetoui	Chet M Flw	0.37 ± 0.006	0.37 ± 0.027	0.27 ± 0.077	61.97 ± 5.58	13.93 ± 3.14	21.42 ± 3.53
Alphalaval	Chetoui	Chet N Al	0.16 ± 0.006	0.11 ± 0.017	0.23 ± 0.012	3.49 ± 0.12	6.96 ± 0.28	2.78 ± 0.1
Alphalaval	Koroneiki	Koro N Al	0.04 ± 0.003	0.18 ± 0.022	0.06 ± 0.001	16.79 ± 4.07	18.31 ± 1.11	9.45 ± 0.87
Barracane	Chetoui	Chet N Bar	0.17 ± 0.009	0.16 ± 0.004	0.11 ± 0.004	7.36 ± 0.58	7.62 ± 1.18	2 ± 0.59
Barracane	Koroneiki	Koro N Bar	0.30 ± 0.041	0.28 ± 0.026	0.15 ± 0.020	3.61 ± 2.15	1.32 ± 0.33	3.68 ± 0.99
Alphalaval	Chemleli	Chem N Al	0.16 ± 0.015	0.19 ± 0.007	0.08 ± 0.046	1.45 ± 0.59	5.97 ± 0.356	17.81 ± 2.23
Alphalaval	Pitchoulini	Pit N Al	0.09 ± 0.008	0.05 ± 0.006	0.13 ± 0.010	11.23 ± 0.61	18.61 ± 0.74	25.18 ± 1.20
Rapanelli	Chemleli	Chem N Rap	0.30 ± 0.033	0.35 ± 0.024	0.27 ± 0.030	17.83 ± 6.49	14.72 ± 4.55	14.29 ± 3.93
Jha	Chetoui	Chet M jha	NA	NA	NA	NA	NA	NA
Polat	Chetoui	Chet M Polat	0.38 ± 0.025	0.38 ± 0.021	0.40 ± 0.017	2.67 ± 0.87	73.16 ± 27	2.42 ± 0.52
Naoura	Chetoui	Chet M Naoura	0.34 ± 0.030	0.32 ± 0.013	0.41 ± 0.012	24.07 ± 1.12	27.76 ± 1.08	20.71 ± 10.35
Alphalaval	Chaibi	Chaibi N Al	NA	NA	NA	NA	NA	NA
Amenduni	Chaibi	Chaibi N Am	0.14 ± 0.013	0.30 ± 0.018	0.29 ± 0.040	30.85 ± 18.89	16.41 ± 4.06	12.31 ± 2.93

ABTS = 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid); DPPH = 2,2-diphenyl-1-picrylhydrazyl; NA = not available.

Table 5. Inhibition zones (mm) of different extracts.

	Inhibition zones (mm)						
Source	Chem S Pier	Chet N Al	Pit N Al	Chet M Naoura	Koro N Bar	Control 1	Control 2
E. coli	6	12	17	13	25	25	20
P. aeruginosa	6	15	18	18	23	21	22
S. aureus	6	14	16	12	25	15	6
E. faecalis	6	15	15	15	20	12	7

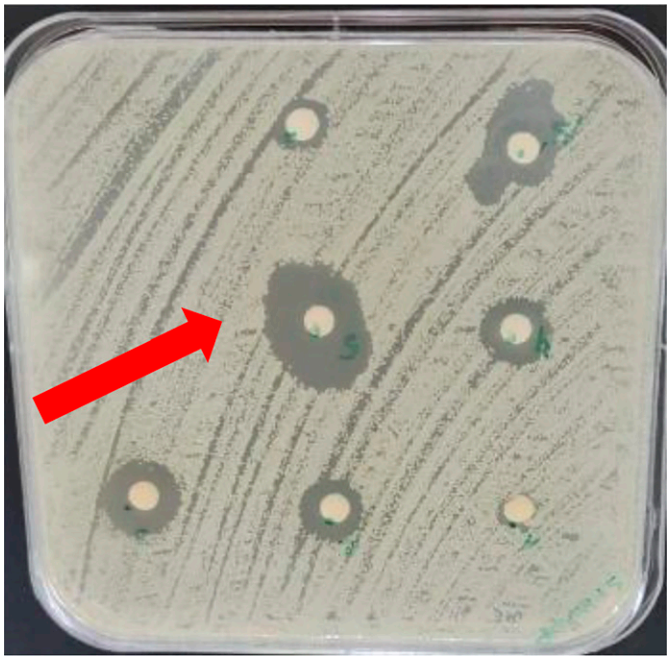

Fig. 1. *Staphylococcus aureus* – Koro N Bar activity.

that varies between 0.04 and 0.41 mg ET/mL for ABTS and 1.32 and 73.16 mg EAA/mL for DPPH. Conversely, extracts from traditional methods (Jha and Rappanelli) display minimal activity, 0.27 to 0.30 mg ET/mL for Chem N Rap using ABTS and 14.29 to 17.83 mg EAA/mL using DPPH to negligible activity for Chet M jha extracts. The influence of olive varieties and industrial process are highlighted by these results; the varieties 'Koroneiki', 'Pitchoulini', 'Chemleli', and 'Chetoui' seem to have the significantly ($P < 0.05$) highest antioxidant activities by ABTS and DPPH (Table 4), while the variety 'Chaibi' has no significant results.

When comparing the methods and solvents used in the extraction of phenolic compounds, it becomes evident that the liquid–liquid extraction method with ethyl acetate yields the most important results, followed by maceration using ethanol and finally maceration using methanol/chloroform. The explanation for the differences in antioxidant activity in the OMW samples from different varieties using different methods may be that activity could be dependent not only on the concentration of the phenolic compounds but also on the phenolic profiles, according to Fattoum et al. (2023) and Gueboudji et al. (2023).

Antibacterial activity

The antibacterial activity of five samples was evaluated (Table 5, Figs. 1–4). It is noticeable that all the extracts except Koro N Bar using methanol/chloroform do not possess any antibacterial activity (Figs. 1–4). Comparatively, Chem S Pier had an inhibition zone of 6 mm for all the tested microorganisms, followed by Chet N Al extract, which had inhibition zones of 12 mm against *E. coli*, 15 mm against *P. aeruginosa*, 14 mm against *S. aureus*, and 15 mm against

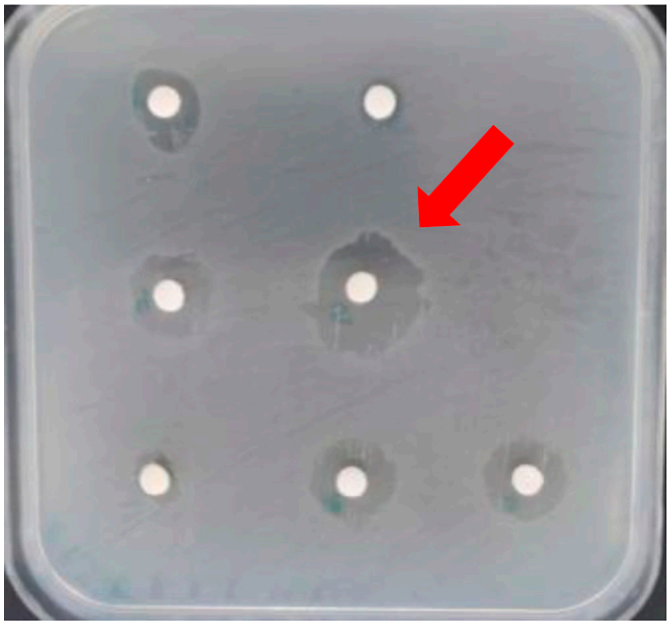

Fig. 2. *Enterococcus faecalis* – Koro N Bar activity.

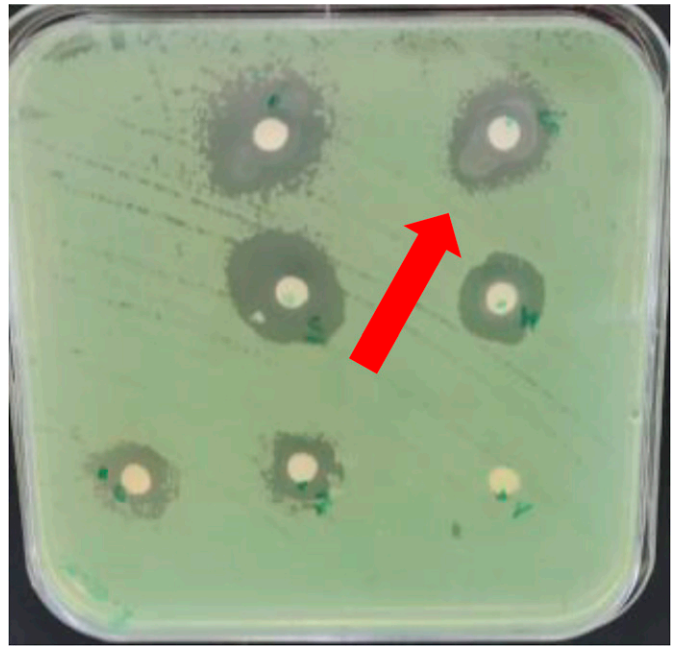

Fig. 3. *Pseudomonas aeruginosa* – Koro N Bar activity.

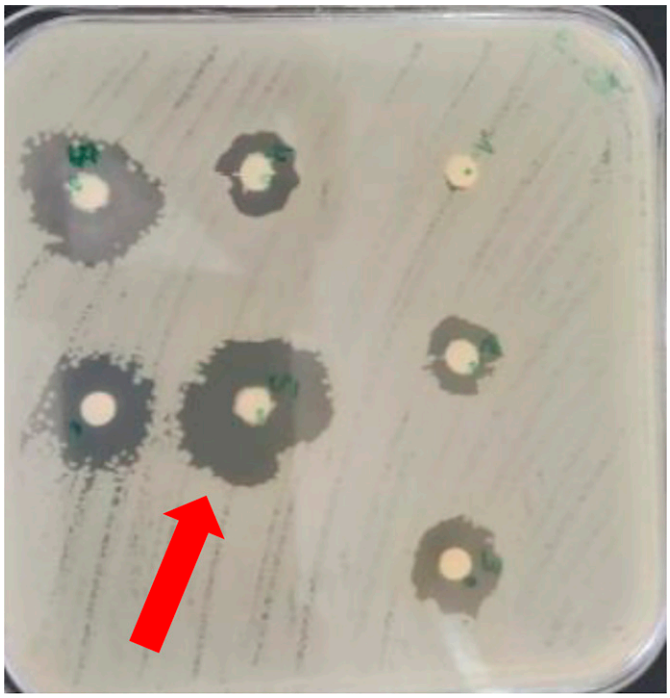

Fig. 4. *Escherichia coli* – Koro N Bar activity.

E. faecalis. Also, Pit N Al had inhibition zones of 17 mm against *E. coli*, 18 mm against *P. aeruginosa*, 16 mm against *S. aureus*, and 15 mm against *E. faecalis*, and Chet M Naoura is in the same range with inhibition zones of 13 mm against *E. coli*, 18 mm against *P. aeruginosa*, 12 mm against *S. aureus*, and 15 mm against *E. faecalis*. On the other hand, Koro N Bar extract showed a positive activity with inhibition zones of 25 mm against *E. coli*, 23 mm against *P. aeruginosa*, 25 mm against *S. aureus*, and 20 mm against *E. faecalis*. These results are related closely to its polyphenol content (1 g GAE/L of OM) and its antioxidant activities as measured by ABTS and DPPH (0.18 and 0.007, respectively).

These results note that the choice of extracting solvent plays a crucial factor in the extraction of phenolic compounds. Different solvents have varying affinities for phenolic compounds, which allows for the selective extraction of compounds with distinctive biological activity, including their effectiveness or absence as antibacterial agents (El Mannoubi 2021).

These results confirm those of Sar and Akbas (2023), which proved that phenolics extracted from OMW from a Turkish variety through a process of liquid–liquid extraction using methanol can have a significant antibacterial activity. Also, it was reported by Affes et al. (2021) that the phenolic compounds extracted from the bacteria of *Aeonium arboretum* have demonstrated promising antimicrobial activity.

Conclusions

This study provides a comprehensive analysis of the factors influencing Tunisian OMW, focusing on the olive oil extraction process and olive variety in relation to the physicochemical properties, phenolic content, and bioactive characteristics of the wastewater. The findings show significant variations in pH, water, solid, and lipid content, as well as phenolic compounds, which are influenced by both the extraction techniques and the olive varieties used. Furthermore, the study emphasizes that antioxidant activity is not solely dependent on total phenolic content but also on the specific types and profiles of phenolic compounds extracted. The industrial extraction process plays a pivotal role in shaping the composition of OMW, directly affecting its antioxidant properties. Additionally, the choice of extraction methods and solvents has a considerable impact on the antibacterial activity of OMW. These findings provide valuable insights for optimizing the recovery of phenolic compounds, which have gained significant attention for their potential therapeutic applications. These bioactive molecules could help mitigate oxidative stress, potentially reducing the risk of chronic diseases such as cardiovascular disorders, neurodegenerative conditions, and certain cancers. Moreover, this study contributes to enhancing the efficiency of olive oil production and addresses the environmental challenges associated with OMW management.

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