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PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITIES OF OLIVE MILL WASTEWATER

Fatiha Abdellah[1'2,*], Khaled Hamden[2], Tayeb Silarbi[1'2], Laid Boukraa[1], Si Mohamed Hammoudi[1]and Rachida Benaraba[1]

1-Laboratory of Research on Local Animal Products at Ibn Khaldoun University, Algeria.

2- Bio-resource laboratory: Integrative biology and valorization biolival-Monastir- Tunisia

Abstract

Olive Mill Wastewater (OMWW) is a significant environmental challenge due to its high organic matter content, particularly 1phenolic compounds, which can inhibit microorganism growth and cause pollution in surface water, soil, and ecosystems. Despite its pollution, OMWW is also a rich source of natural antioxidants, including polyphenols, which have various potential health benefits. This study focuses on the in vitro evaluation of the antibacterial and antioxidant activity of OMWW collected from traditional oil mills in Fez, Morocco, and Tizi Ouzou, Algeria. Physicochemical characterization revealed variations in pH, electrical conductivity, dry matter content, water content, ash content, organic materials, fat, suspended matter, polyphenols, and flavonoids between the two sources. Moroccan OMWW exhibited higher polyphenol content, known for its antioxidant properties. Bacteriological analyses indicated the absence of total aerobic mesophilic flora, total and fecal coliform, and *Staphylococcus aureus* in the studied OMWW, suggesting its potential antimicrobial properties attributed to its acidity and the presence of antimicrobial substances such as phenolic compounds. Moroccan and Algerian OMWW exhibited antibacterial effects against various bacterial strains, with minimum inhibitory concentrations (MIC) ranging from 3% to 14%. The antioxidant activity of OMWW was evaluated using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) tests, with Moroccan OMWW demonstrating slightly stronger antiradical and reducing power activities. This research highlights the promising potential of OMWW as a natural antimicrobial and antioxidant agent, potentially serving as a valuable source of bioactive compounds with potential therapeutic applications.

Keywords: Olive mill wastewater, Antioxidant activity, Antibacterial activity, Polyphenols.

Introduction

The olive oil industry, primarily in Mediterranean countries, significantly contributes to global agricultural production, accounting for 95% of the world's olive oil production. However, it also generates significant waste, known as Olive Mill Wastewater (OMWW). OMWW, which consists of 83-94% water, 4-16% organic compounds, and 0.4-2.5% mineral salts, includes 2-15% phenolic compounds, is discharged into rivers and sewage systems annually, causing severe surface water pollution. Despite its pollution, OMWW offers a unique silver lining: it is a rich source of natural antioxidants, particularly polyphenols, known for their beneficial biological properties. Researchers have explored innovative methods for treating and valorizing OMWW to mitigate pollution and harness its polyphenolic potential for applications in food production, cosmetics, pharmacology, and other sectors. This study aims to evaluate the antibacterial and antioxidant activities of OMWW in vitro, aiming to mitigate its environmental impact and reveal its value as a source of natural antimicrobial and antioxidant agents with various applications. By analyzing the physicochemical properties, bacteriological composition, and bioactive potential of OMWW, this study aims to promote responsible utilization and reduce its ecological footprint.
Approximately 95% of the world's production of olive oil is held by the Mediterranean countries, making it a significant industry (Tsiouplas et al., 2002). Because this industry primarily produces olive oil, it creates a significant amount of solid and liquid waste (olive mill wastewater), estimated to be around 3 million m3/year. (Zenjari et al., 2006). Depending on the production method used, the amount of Olive Mill Wastewater (OMWW) produced may reach values greater than 1 l / kg of olive (Martinez-Garcia et al., 2006).OMWW's composition varies greatly and is influenced by the type of olive used how ripe the fruit is, and whether it is extracted using a press or a centrifuge (Lopez & Ramos-Cormenzana, 1996). The typical weight composition of OMWW is 0.4-2.5% mineral salts, 4-16% organic chemicals, and 83-94% water.Phenolic molecules make up between 15 to 25% of the organic fraction. The rejection of OMWW in rivers and sewage systems without any treatment causes serious pollution of surface water, fauna, flora, and microflora of rivers and soil. It is reported that one cubic meter of OMWW causes similar pollution caused by 1500 people a day. The harmful effect of OMWW is mostly caused by their high organic matter content, especially phenolic compounds with very high polluting activity and inhibit the growth of microorganisms, especially bacteria that reduce natural biological degradation (Belaid, 2002). Even though OMWW has a high pollution profile, it's regarded as a great source of naturally occurring antioxidants like polyphenols. The biological characteristics of these polyphenols include antibacterial, antioxidant, hypolipidemic, cholesterol-lowering, and anti-carcinogenic effects (Mulinacci et al., 2001). This consideration led many researchers to find treatments and valorization of the OMWW. Firstly, to limit their pollution and solve a major environmental problem, and secondly, to develop their polyphenols for future applications in food production, the cosmetics industry, and pharmacology. The olive oil sector is increasingly adopting eco-friendly practices to address environmental challenges.

The olive oil industry is now recognizing the hidden treasures of organic wastewater (OMWW), which can be harnessed to reduce its ecological footprint while gaining valuable bioactive compounds. This shift aligns with the global movement towards a circular economy, where waste is viewed as a potential resource. This study explores the multifaceted potential of OMWW, focusing on its physicochemical composition, bacteriological characteristics, and bioactive potential. The research aims to contribute to the evolving narrative of waste management and sustainable resource utilization in the olive oil industry, focusing on its antibacterial and antioxidant activities.The dual objective of reducing pollution while harnessing the beneficial properties of OMWW underscores the commitment to creating a more sustainable and responsible future for the olive oil industry and beyond. The study offers insights into the unexplored potential of OMWW, highlighting its role in the emerging paradigm of a circular economy where waste is transformed into valuable resources in keeping with the broader vision of a more sustainable and environmentally responsible world. The objective of this study is the *in vitro* evaluation of the antibacterial and antioxidant activity of olive mill wastewater, to use them as natural antimicrobial and antioxidant agents. This study aims to evaluate the potential applications of Olive Mill Wastewater (OMWW) by assessing its antibacterial and antioxidant activities. The physicochemical properties of OMWW collected from two regions, Fez, Morocco, and Tizi Ouzou, Algeria, are assessed. The antibacterial activity is investigated by determining the Minimum Inhibitory Concentration (MIC) against various bacterial strains. The antioxidant capacity is assessed using DPPH and FRAP tests, comparing IC_{50} values and reducing power with standard antioxidants. The study compares OMWW sources to identify potential variations in effectiveness. The chemical composition of OMWW is explored to identify specific compounds responsible for its antibacterial and antioxidant properties. The environmental impact of OMWW is acknowledged, as it may reduce pollution in surface water, soil, and ecosystems. Therapeutic applications of OMWW-derived compounds are highlighted, particularly in food production, cosmetics, pharmacology, and health-related industries. Further scientific understanding is sought to understand the mechanisms of action and chemical composition of OMWW, paving the way for future research and applications. This study contributes to environmental conservation and developing natural antimicrobial and antioxidant agents for industrial and therapeutic purposes.

Materials and Methods

Biological Material

The conventional oil mills situated in two distinct places provided the olive mill wastewaters utilized in this investigation: Fez Morocco Region (Latitude: 34°01′59″ North Longitude: 5°00′01″ West) producing the Picholine Marocaine olive variety and Tizi Ouzou Algeria region (Latitude: 36°42′42″ North Longitude: 4°02′45″ Est) processing Chemlal olive variety. The collections were carried out during the winter harvest period. The samples were introduced into polypropylene cans and, upon arrival at the laboratory, stored in the dark at four °C to preserve their original biological and physicochemical characteristics.

Physicochemical Characterization of Olive Mill Waste Water

The investigated Olive Mill Wastewater was characterized using a variety of physicochemical methods. A pH meter with an OHAUS mark was used to measure the pH. The total dry matter was calculated following a 24-hour drying process at 105 °C. Following mineralization at 550 °C for five hours, the inorganic substance was identified (Iboukhoufel, 2014). The weight difference between the dry matter obtained by evaporation at 105 °C and the ash residue from the calcinations at 550 °C was used to calculate the volatile matter, which was given in grams per litre. The electrical conductivity was measured using a conductivity meter mark PHYWE (Ouabou, 2014). The method of chloroform/methanol described by Aissam (2003) was used to determine the fat content of OMWW.

Determination of Total Phenolic Content (TPC)

The OMWW's total phenolic content (TPC) was ascertained using the technique outlined by Beretta et al. (2005). After mixing 500 µL of Folin-Ciocalteu reagent (10%) with 500 µL of OMWW at various concentrations (10 to 50 mg/mL) for five minutes, 1500 µL of 10% Na2CO3 solution was added. Each mixture was given a 30-minute dark incubation period at room temperature before having its absorbance measured at 765 nm. Every determination was made three times.

Determination of Total Flavonoid Content (TFC)

A colorimetric method was used to determine the amount of flavonoids present. This method is based on the development of a complex between the aluminum ion and the carbonyl and hydroxyl groups of flavonoids, which results in the production of a yellow color. One millilitre (1 mL) of a 2% aluminum chloride solution was combined with one millilitre (1 mL) of OMWW solutions at various concentrations (10 to 50 mg/mL). The absorbance of the reaction mixture was measured at 430 nm against a blank of distilled water after 30 minutes of dark incubation. Every determination was made three times.

Bacteriological Characterization of Olive Mill Waste Water

Bacteriological analyses of OMWW have worn the enumeration of the total aerobic mesophilic flora (TAMF) total and fecal coliform (TC and FC) and *Staphylococcus aureus*.

Evaluation of The Biological Activities

The study evaluates the biological activities of Olive Mill Wastewater (OMWW) to understand its potential applications in agriculture, pharmaceuticals, and food industries. It assesses its antibacterial and antioxidant activities, highlighting its promising aspects.

Bacterial Strains and Inoculums Standardization

The university hospital Mustapha Pasha of Algiers (Algeria) kindly contributed the following microorganisms: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* OXA R ATCC 43300, *Staphylococcus aureus* OXA S ATCC 25923, *Staphylococcus aureus* ATCC 33862. The strains were kept alive for the experiment by subculturing in a particular medium; five colonies from 24-hour cultures were used to create the inoculum suspensions. The colonies were shaken for 15 seconds while suspended in 5 millilitres sterile saline (0.9% NaCl). Using sterile saline, the density was brought down to the turbidity of a 0.5 McFarland Standard, or 1–5 x 10^8cfu/mL.

Determination of the Minimum Inhibitory Concentration (MIC)

Using the agar incorporation technique, the MIC values of the tested Olive Mill Wastewater were ascertained against the tested strains of bacteria. Increasing concentrations of the OMWW were added to a Mueller-Hinton medium until a final volume of 5 mL was reached. After gently shaking the mixture and pouring it onto plates, each microbial strain was inoculated with 0.5 McFarland of the standard inoculum, and the plates were then incubated for 24 hours at 37°C.The OMWW concentration at which the investigated bacterial strains could not grow was used to calculate the minimum inhibitory concentration or MIC.

Evaluation of The Antioxidant Effect of Olive Mill Waste Water

Test of Ferric Reduction Antioxidant Power (FRAP test)

The ferric reducing antioxidant power of the tested OMWW was determined according to the method of Oyaizu, 1986. One mililitre of OMWW at different concentrations (1-10 mg/ml) and the standards Vitamin C and gallic acid (2 to100 µg/ml) as positive control were applied to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 min. Then 2.5 ml trichloro acetic acid (TCA at 10%) was added to the mixture and it was centrifuged at 3000 rpm for 10 min. Then 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (1%). The absorbance was measured at 700 nm after allowing the solution to stand for 30 min at room temperature. The reducing potential of OMWW and standards (gallic acid, vitamin C) is expressed by values of 50% effective concentrations (EC_{50}) that correspond to the concentration of sample necessary to give an absorbance equal to 0.5 at 700 nm.

Test of *DPPH • (2,2-diphenyl-1-picryl-hydrazyl)* scavenging activity

The scavenging activity of OMWW for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by Tien, et la (2005) with some modifications; 1.5mL of various dilutions of OMWW were mixed with 1.5 mL of a 0.2mM ethanolic DPPH solution. After an incubation period of 30 min at 25 C°, the absorbance was determined at 517 nm, the wavelength of maximum absorbance of DPPH was recorded as a (sample). A blank experiment was also carried out applying the same procedure to a solution without the test material (OMWW) and the absorbance was recorded as A (blank). The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \textbf{ inhibition} = \mathbf{100} \times \frac{\mathbf{A\ (blank) - A\ (sample)}}{\mathbf{A\ (blank)}}$$

Antiradical activity of OMWW was expressed as inhibition concentration (IC_{50}), defined as the concentration of the tested material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid, gallic acid were used as a standard. All measurements were performed in triplicate.

Statistical Analysis

Every experiment was conducted thrice or more, and the mean ± standard deviation was used to show the findings. ANOVA was used to assess the statistical significance, and the Tukey test was then used for multiple comparisons, taking into account differences that were statistically significant at $P < 0.05$.

Result and Discussion

Physicochemical Characterization of Olive Mill Waste Water

Table 1 shows the result of the physicochemical characterization of the studied olive mill wastewaters.

Table 1: Physicochemical characterization of the studied OMWW

Parameters	**Content**	
	Moroccan OMWW	Algerian OMWW
pH	4.29±0.01^{a}	5±0.01^{b}
Conductivity (ms/cm)	25.3± 0.1^{c}	49.24 ±0.095^{d}
dry matter (%)	5.03±0.02^{e}	9.38±0.38^{f}

Water content(%)	94.98±0.015^{g}	90.61±0.01^{h}
Ash content (%)	22.62±0.02^{i}	21.44±0.44^{i}
Organic materials (%)	76.68±0.02^{j}	78.55±0.005^{k}
Fat (g/l)	2.75±0.05^{l}	2.37±0.025^{m}
Suspended matter (g/l)	4.97 ±0.025^{n}	21.61± 0.011^{O}
Poly phenols (g/l)	.57±0.18^{p}	7.24±0.11^{q}
Flavonoid (g/l)	0.012±0.0014^{r}	2.38±0.25^{s}

The values for each trait followed by different letters indicate significant differences by ANOVA post hoc LSD Tukey test $p < 0.05$.

pH

The results showed that all of the OMWW under study were acidic, with the Moroccan OMWW having the highest acidity (pH = 4.29) compared to the Algerian OMWW (pH = 5). The organic acids (fatty acids, phenolic acids, etc.) cause this acidity. These findings fall within the range (4.5 to 6) documented in the literature. The longer the OMWW is stored, the more acidic it becomes. The processes of self-oxidation and polymerization, which convert phenolic alcohols into phenolic acids, explain this (Hamdi, 1991).

Electrical Conductivity

Electrical conductivity is closely related to the concentration of dissolved substances and their nature. In the literature, the conductivity of the OMWW ranged between 18 and 50 ms/cm (Di Serio et al., 2008), which corroborates with our findings (25.3 ms/cm for the Moroccan OMWW and 49.24 ms/cm for Algerian OMWW). Our results are superior to those obtained by Esmail et al. (2014), reported that the values of the electrical conductivity of their OMWW samples ranged from 17.40 to 19.09 ms/cm. These differences may be due to the content of salts present in each OMWW.

Dry matter (DM)

The dry matter (DM) content of the OMWW recorded in our study was 5.04% for the Moroccan OMWW and 9.38% for the Algerian OMWW. The Algerian OMWW has the most important content. However, these changes in the DM could be explained by the difference in the vegetative stage and the olive harvest season and variety.

Water content

The high moisture content of the OMWW (94.97% for the Moroccan OMWW and 90.62%. for the Algerian OMWW) can be explained by the existing water in the olives and the water added during the olive oil extraction process (Procida & Ceccon, 2006).

Organic Material

The content of the OMWW in organic matter shown in Table 1 indicates the organic nature of the studied OMWW (76.66% for Moroccan OMWW and 78.55% for Algerian OMWW). These results are similar to that of Hacchicha et al. (2008). Our findings are lower than those reported by Leulmi (2011) and Aissam (2003), who obtained values of 98.01% and 90%, respectively. The differences observed may be due to climatic conditions, the methods of olive oil extraction, and the olive's storage time before maturation.

Ash

Our OMWW samples showed similar mineral contents of 22.62% for the Moroccan OMWW and 21.44% for the Algerian OMWW. These results agree with the 21.42% mineral content found by Hachicha et al. (2008). However, they are relatively high compared to those obtained by Iboukhoulef (2014) and Sifoun (2008), reported a value of 1.11g / l of minerals. This difference can be explained by the variety and degree of maturity of olives. Moreover, the method of extraction of olive oil is found to be a factor influencing the mineral content of OMWW (Aissam, 2003).

Suspended Matters

The content of non-dissolved material in the studied OMWW is 4.95 g / l for the Moroccan OMWW and 21.6 g/l for the Algerian OMWW. This is mainly due to the non-retained particles of the solid-liquid separation step of olive oil.

Fat

The studied OMWW has become viscous due to the presence of the oily fraction. Our OMWW showed similar fatty content in the order of 2.7g / l for the Moroccan OMWW and 2.4 g/l for the Algerian OMWW. These results are higher than that obtained by Yahiaoui (2012), who found 0.9 g / l of fat and inferior to the 9 g / l of fat reported by Homari et al. (2013). The change in the geographical area can explain the difference between our results and those of previous studies, the variety and ripeness of the olives, the duration of storage of olives prior to crushing, and the olive oil extraction system.

Polyphenol Content

The studied OMWW have 18a phenolic content of 8.57 g / L for the Moroccan OMWW and 7.24 g/L for the Algerian OMWW. This content is much higher than that obtained by yahiaoui (2012) (1.2g / L), Moussaoui et al. (2010) (266mg / L), and Leger (1999), who found a phenolic content in the range between 40 to 700 mg / L. On the other hand, our results are lower than that obtained by Aissam (2003) (9.7g / L). The polyphenol profile of the OMWW may vary under the influence of various factors, including the olive variety, their ripeness, climate, temperature, and the extraction process.

Flavonoid Content

According to our results, it appears that 19the Algerian OMWW has shown a high flavonoid content (2.38g/L) compared to the Moroccan OMWW (0.012g/L).Following the results of the quantitative characterization, the studied OMWW is rich in polyphenols and flavonoids, presenting a promising natural source of beneficial bioactive compounds for human health.

Bacteriological Characterization of The Studied OMWW

The bacteriological characterization of the studied OMWW is represented in Table 2. The studied OMWW showed the total absence of all the investigated germs, including total aerobic mesophilic flora, total and fecal coliform (TC and FC), and *Staphylococcus aureus*. This may be related to the physicochemical characteristics of the OMWW, including acidity and the presence of antimicrobial substances such as phenolic compounds, tannins, and fatty acids known for inhibiting the growth of microorganisms (Aissam, 2003).

Table 2: Bacteriological characterization of the studied OMWW

Bacterial strains	**Result**	
	Moroccan OMWW	**Algerian OMWW**
Total Aerobic Mesophilic Flora (TAMF)	**Absence**	**Absence**
Staphylococcus aureus	**Absence**	**Absence**
Total coliform (TC)	**Absence**	**Absence**
Fecal coliform (FC)	**Absence**	**Absence**

Results of the Antibacterial Activity of OMWW

The results of the antibacterial activity of the studied OMWW against the tested bacterial strains are presented in Table 3.

Table 3: Result of the Antibacterial Activity of OMWW

Bacterial strains	MIC Value	
	Moroccan OMWW	Algerian OMWW
Staphylococcus aureus OXA R ATCC 43300	5%	3%
Staphylococcus aureus OXA S ATCC 25923	5%	5%
Staphylococcus aureus ATCC 33862	5%	7%
Bacillus cereus ATCC 10876	4%	10%
Escherichia coli ATCC 25922	5%	14%
Pseudomonas aeruginosa ATCC 27853	5%	10%

Based on the gathered data, we discovered that the investigated OMWW exhibited antibacterial activity against every tested strain, with the most sensitive species being *Staphylococcus aureus* OXA R ATCC 43300, with a MIC value of 3%. We discovered that Escherichia coli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, two Gram-negative bacteria, are especially susceptible to the effects of Moroccan OMWW. In contrast to Moroccan OMWW, Algerian OMWW exhibits a greater inhibitory effect against *Staphylococcus aureus* OXA R ATCC 43300. The studied OMWW showed a similar antibacterial activity against *Staphylococcus aureus* OXA S ATCC 25923. The antibacterial effect of the OMWW is mainly related to the action of the monomeric phenols and brown pigments or catecholmelaninique (Hamdi & Ellouz, 1993). These compounds act on bacteria by denaturing cell proteins and altering membranes (Ranalli, 1991). In particular, oleuropein, the major compound of OMWW was, exhibited an antibacterial activity against a wide variety of Gram-positive and Gram-negative bacteria involved in human pathologies (Bisignano et al., 1999).Oleuropein is inserted between the membrane phospholipids and causes destabilization of the cytoplasmic membrane, which may result in cellular components leakage (phosphate, calcium) that are involved in maintaining the balance of energy reserves and intracellular pH. Moreover, oleuropein demonstrated antiviral activity by inactivating the virus or inhibiting replication without affecting the host cell (Micol et al., 2005). Numerous studies have demonstrated the capacity of hydroxytyrosol and other phenolic compounds present in OMWW to suppress the growth of various fungi and bacteria, including those harmful to humans. Hydroxytyrosol was also active against foodborne pathogens such as Listeria monocytogenes, S. aureus, Salmonella enterica, and Yersinia spp (Foti et al., 2021). Al yamani et al. (2020) reported that the phenolic extracts of Moroccan OMWW have an antibacterial effect against Escherichia coli and Listeria innocua. It has also been reported that OMWW has an antibacterial effect against pathogenic bacteria, including Pseudomonas syringae and Corynebacterium michiganens (Benlemlihe & ghanem, 2012). The results of a study by Senani and Moulti-Mati (2011) showed that the polyphenols of OMWW exert a strong 22antifungal activity against Aspergillus flavus and Aspergillus parasiticus. Serra et al. (2008) showed that natural OMWW extracts exhibited a higher antimicrobial activity than the three individual biophenols (quercetin, hydroxytyrosol, and oleuropein), suggesting a synergic effect among molecules. In addition to its phenolic compounds, OMWW is rich in long-chain fatty acids, especially oleic and linoleic acids, characterized by their antimicrobial activity. These fatty acids also act through a barrier effect, limiting the accessibility of microorganisms to dietary fiber by minimizing contact between the enzymes and wall polysaccharides (Zuhainis Wan et al., 2008).

Results of The Antioxidant Activity

Antioxidant Activity Evaluated by The DPPH Test

The results of the antioxidant effect evaluated by the DPPH test are depicted in Figure 1. The OMWW showed a very important 23antiradical activity with IC_{50} values of 0.93 ± 0.015 mg / mL for the Moroccan OMWW and 1.042±0.01 mg / mL for the Algerian OMWW. These values are significantly ($p<0.05$) lower than those of the standard antioxidants, vitamin C, and gallic acid, which exhibit IC_{50} values in the order of 0.0072±0.00015 mg/mL and 0.0044±0.00014 mg/mL, respectively. The Moroccan OMWW has the best scavenging activity, probably due to its high polyphenols content (8.57 g/l) compared to Algerian OMWW (7.24 g/l). El Moudden et al. (2022) reported that the phenolic extract of the Moroccan OMWW exhibited an important antiradical activity, evaluated by the DPPH and ABTS essay, higher than that of the standard Trolox. El yamani et al. (2020) showed that the phenolic extract of their studied OMWW had an antiradical activity against the DPPH free radical.

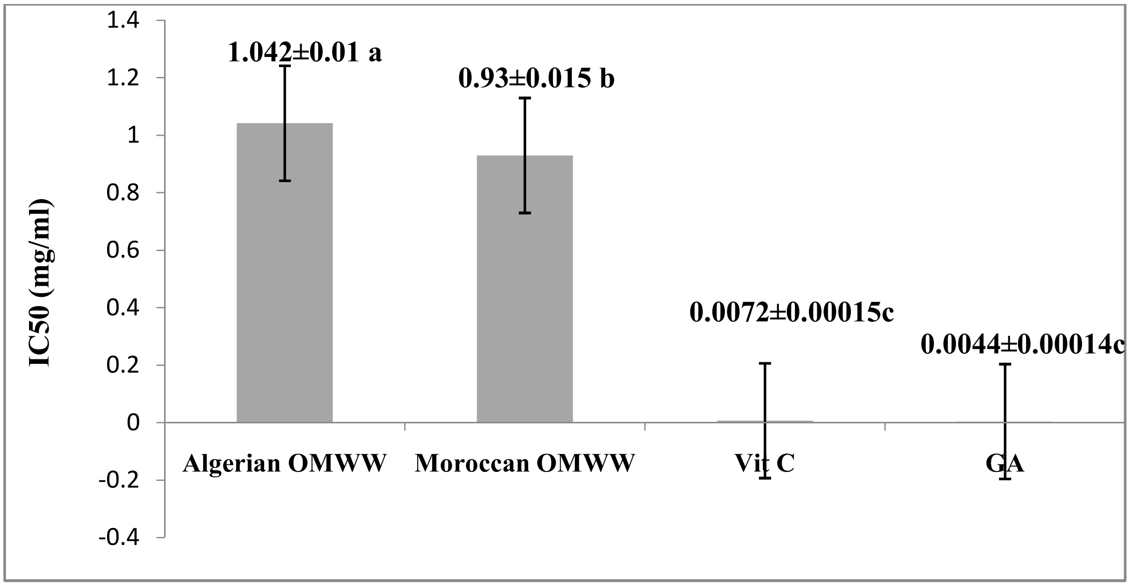

Figure 1: Concentrations responsible for 50% inhibition of DPPH • radical (IC50). (The values followed by different letters indicate significant differences by ANOVA post hoc LSD Tukey test $p < 0.05$)

The antiradical capacity of the OMWW could be attributed to some potentially active phenolic compounds such as oleuropein and Tyrosol. Pérez-Bonilla et al. (2006) have shown that oleuropein, Tyrosol, and hydroxytyrosol, three predominant olive polyphenols, possess antioxidant activity evaluated by the DPPH test. The antiradical activity exhibited by OMWW is probably due to a combined action of various endogenous antioxidants in this material, such as polyphenols and the fat, essentially the unsaturated fatty acid predominantly represented by oleic acid (omega-9) and the linoleic acid (omega 6) (Benlmlih Ghanam & 2012). Many studies have shown that the polyunsaturated fatty acid "Omega 3" can reduce oxidative stress and inflammation (Brasky et al., 2010; Mas et al., 2010). Kim et al. (2010) showed that the omega-3 fatty acids and omega-6 protect neurons against oxidative stress.

Antioxidant Activity Evaluated by The Test of Ferric Reducing Antioxidant Power (FRAP Test)

The reducing power results of the studied OMWW and the standard antioxidant gallic acid and vitamin C are shown in Figure 2.

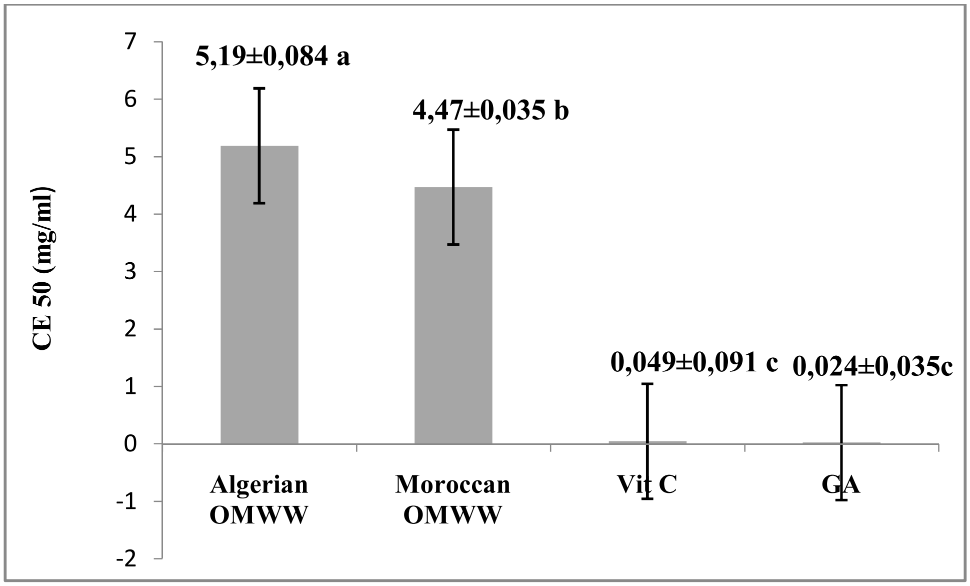

Figure 2: Reducing Power of OMWW, Vitamin C and gallic acid

(The values followed by different letters indicate significant differences by ANOVA post hoc LSD Tukey test p <0.05).

The antioxidant activity of the studied OMWW evaluated by the reducing potential test revealed that they have an important 24reducing power with EC50 about 4.47 ± 0.035 mg / mL for the Moroccan OMWW and 5.19±0.084 mg/mL for the Algerian OMWW. These values are much lower than those of the standard antioxidants gallic acid and vitamin C, which have EC_{50} values of0.024±0.035 mg/mL and 0.049±0.091 mg/mL, respectively. The Moroccan OMWW exhibited the best-reducing power compared to the Algerian OMWW. This could be due to its richness in polyphenols (8.57g/l) compared to Algerian OMWW (7.24g/l). The reducing potential of the studied OMWW is due to the presence of molecules capable of donating electrons that can react with free radicals and convert them into stable products. Recently, it has been demonstrated that oleuropein, a major 25polyphenol present in OMWW, has a higher antioxidant activity (EC_{50} = 5.88 mg / mL) than BHT (EC_{50} = 8.667 mg / mL). At the same time, Tyrosol seems less active with 26an EC_{50} of 176.25 mg / mL. It has been widely proved that OMWW contains several natural phenolic compounds, especially ortho-diphenols. Among these natural products are caffeic acid and, more particularly, hydroxytyrosol and oleuropein, which have very high antioxidant powers. In a study done by Boudoukhana (2008), the antioxidant activity of hydroxytyrosol was found to be similar to that of thymol, carvacrol, 6-gingerol, and zingerone. The polyphenols of OMWW have an antioxidant effect on human intestinal epithelial cells (Manna et al., 1997) and a cytostatic effect on some tumor cells (Owen et al., 2000). Individually, compounds such as hydroxytyrosol and vanillic acid demonstrated strong antioxidant activities (Owen, 2000). They have also been associated with some beneficial effects in protecting against diseases. Hydroxytyrosol protects the fatty acids from oxidation and, therefore, reduces the deposition of LDL in the arterial walls (Visioli, 1998). In light of all the obtained results on the antioxidant activity of OMWW, it appears that they have important antioxidant activity, and this is due to their quantitative and qualitative compositions, especially polyphenols that give them a significant therapeutic potential for the fight against diseases related to oxidative stress.

Conclusion

This study explores the potential of Olive Mill Wastewater (OMWW), a byproduct of the olive oil industry, primarily concentrated in Mediterranean countries. OMWW is a rich source of phenolic compounds, contributing to its diverse biological activities. Its antibacterial and antioxidant properties were evaluated, revealing its applications in various fields and its potential as a sustainable resource. Physicochemical characterization of OMWW from different regions, specifically Morocco and Algeria, revealed variations in pH, dry matter, water content, ash content, organic materials, fat content, suspended matter, and the concentration of polyphenols and flavonoids. Factors such as olive variety, ripeness, climatic conditions, and extraction process can influence these differences. Bacteriological analyses showed the absence of pathogenic bacteria in OMWW, indicating its potential as an eco-friendly alternative for waste management and reducing environmental pollution. The presence of phenolic compounds, fatty acids, and other bioactive components disrupts bacterial cell membranes and proteins, demonstrating their antibacterial and antioxidant properties. OMWW's antioxidant activity was demonstrated through DPPH scavenging and FRAP tests, demonstrating its ability to neutralize free radicals and reduce oxidative stress. The presence of polyphenols, such as oleuropein and hydroxytyrosol, contributes to its potent antiradical activity and reducing potential. OMWW has demonstrated remarkable potential as a valuable resource, offering sustainable practices and transforming waste into valuable assets. Further research into the isolation and characterization of specific bioactive compounds within OMWW may reveal new opportunities for its utilization in various fields.

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collective efforts of these individuals have significantly advanced our understanding of Olive Mill Wastewater and its potential applications.

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