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Investigating the Impact of Irrigation Water Quality on Secondary Metabolites and Chemical Profile of *Mentha piperita* Essential Oil: Analytical Profiling, Characterization, and Potential Pharmacological Applications

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Abstract: This study examines the impact of irrigation water quality on the synthesis of secondary metabolites and the chemical composition of *Mentha piperita* essential oil (MPEO). Three essential oils from *Mentha piperita* plants, irrigated with different water sources commonly used for mint irrigation in Morocco's Oriental region, were analyzed. The water sources were characterized based on various parameters, such as nitrites, nitrates, orthophosphates, chemical oxygen demand (COD), biological oxygen demand (BOD5), pH, and electrical conductivity. The essential oils were extracted using hydrodistillation, and their chemical composition was determined using gas chromatography coupled with mass spectrometry (GC/MS), revealing notable variations among the compositions of the three essential oils. In this study, in silico tests using the Prediction of Activity Spectra for Substances (PASS) algorithm; the absorption, distribution, metabolism, and excretion (ADME) model; and Pro-Tox II were conducted to evaluate the drug-likeness, pharmacokinetic properties, expected safety profile upon ingestion, and potential pharmacological activity of the identified compounds in MPEO. The antioxidant activity of the MPEOs was assessed through a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the total antioxidant activity (TAC) method. Additionally, the antimicrobial effectiveness of the essential oils was tested against four bacterial strains (*Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and four fungal strains (*Candida glabrata*, *Rhodotorula glutinis*, *Penicillium digitatum*, *Aspergillus niger*), demonstrating moderate to strong activities against the tested strains. This study concludes that regulating irrigation water quality can enhance the production of specific metabolites, making them potentially valuable as antioxidants and antimicrobial agents.

Keywords: secondary metabolites; phytochemical composition; *Mentha piperita*; hydrodistillation; water sources; antioxidant activity; antimicrobial activity; in silico

1. Introduction

Morocco is endowed with abundant floral resources, harboring a rich diversity of plant species in its vascular flora. The country's diverse topography, encompassing the Atlas Mountains, Mediterranean and Atlantic coasts, and the Sahara Desert, provides a multitude of ecosystems conducive to the thriving and proliferation of a wide range of plants. The vascular flora of Morocco comprises a total of 3913 species and 1298 subspecies (including 426 type subspecies), distributed across 155 families and 981 genera [1]. Mint, a perennial plant, is extensively cultivated in Morocco and remains accessible throughout the year, although its availability noticeably diminishes during the winter season. The total production of mint in Morocco is estimated at 70,000 tons per year [2].

Mentha piperita, commonly known as peppermint, is a perennial herbaceous plant belonging to the Lamiaceae family [3]. This specific botanical species was chosen as the focal point of investigation due to its prominent status as one of the foremost essential oil crops globally [4]. Peppermint has gained widespread acclaim for its extensive utilization in traditional medicine, attributed to its manifold advantageous properties for human well-being. Notably, it is highly esteemed for its capacity to alleviate diverse digestive ailments, encompassing stomachaches, nausea, and bloating. Additionally, the captivating aromatic characteristics of peppermint contribute to its popularity as a favored choice for imparting flavors to a diverse range of consumable products, including food, beverages, confectionery items, and even oral care commodities [5]. The versatile applications of peppermint across numerous domains underscore its significance and the substantial demand it garners in both medicinal and culinary contexts.

Mentha piperita is a plant well known for its wealth of secondary metabolites. The secondary metabolites present in *Mentha piperita* are responsible for its aroma, characteristic taste, and medicinal properties. They are of great interest in pharmaceutical research, as many drugs are derived from natural compounds found in plants. These metabolites may have useful medicinal properties, such as antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [6,7]. The increasing resistance of pathogens to synthetic chemicals has prompted numerous nations to devise strategies to address this issue, with the utilization of secondary metabolites derived from plants playing a prominent role in these endeavors [8,9]. Consequently, by effectively regulating the quality of irrigation water, it becomes conceivable to augment the production of these metabolites and harness their potential as potent antioxidants, antibacterial agents, and antifungal compounds [10].

The importance of irrigation water quality in relation to plant metabolism and the biosynthesis of secondary metabolites should not be underestimated. Water utilized for irrigation purposes can contain impurities such as excessive salts, heavy metals, or contaminants from industrial or agricultural sources, which can detrimentally affect plant growth and biochemical processes. These contaminants have the potential to disrupt cellular functions, impede nutrient absorption, and induce oxidative stress within plant tissues. Consequently, the synthesis of secondary metabolites, which often serve as defense compounds against pathogens and environmental stressors, may be compromised.

The principal aim of this research endeavor is to assess the influence of irrigation water quality on peppermint cultivation in the eastern region of Morocco, focusing on three commonly employed types of irrigation water. Specifically, we will investigate the effects of water quality on the chemical components of peppermint essential oil and explore its antioxidant, antibacterial, and antifungal properties. Through the execution of this study, we aim to gain valuable insights into the intricate relationship between irrigation water quality and the overall quality and bioactivities of MPEO.

2. Materials and Methods

2.1. Mentha Piperita Culture

Commercially sourced *Mentha piperita* seeds were obtained from Aromatiche and subsequently sown in a designated potting soil, namely, HAWITA-Flor, which possesses a clearly defined composition comprising 230 mg/L of nitrogen, 300 mg/L of phosphate

(P_2O_5), and 320 mg/L of potassium oxide (K_2O). The planting process followed the guidelines provided by the manufacturer, using plastic pots 150 cm long and 50 cm wide for cultivation. To maintain uniform growing conditions, the pots were placed in an identical location with the same conditions of humidity, regular monitoring, altitude, and temperature (average temperature between 6 °C and 18 °C in the city of Oujda during the four months of cultivation) with irrigation three times a week with an amount of three liters per pot and without undergoing fertilization or pesticide application. After a cultivation period of 120 days, the plants were harvested.

2.2. Irrigation Waters

2.2.1. Sampling

In this study, water samples of three distinct types were obtained and subjected to analysis at the Eastern Center for Water and Environmental Sciences and Technology (COSTEE) in Oujda, Morocco. The water samples were procured from three specific sources: the outlet of a wastewater treatment plant situated in Oujda city, a well-located farm in Bouchtat (Oujda, Morocco), and the Oued Elhey River in Jerada, Morocco. These three water sources are commonly employed for the irrigation of peppermint crops in the Eastern region of Morocco.

2.2.2. Physicochemical Analyses of Irrigation Water

i. Nitrates Assay

This assay for nitrates was performed following the protocol outlined in the ISO 7890-3:1988 standard [11], employing a spectrometric method utilizing sulfosalicylic acid. In the presence of sodium salicylate, nitrates react to form sodium paranitrosalicylate, which exhibits a yellow coloration and can be quantitatively analyzed spectrometrically at a wavelength of 415 nm. To quantify the concentration of nitrates in the water samples, a calibration curve was generated by preparing a series of known concentrations ranging from 0 to 5 mg/L. These concentrations were derived from a stock solution of nitrate nitrogen with a concentration of 5 mg/L.

ii. Orthophosphate Assay

The assay for orthophosphates followed the protocol described in the AFNOR NF EN ISO 6878 (April 2005) standard [12]. The colorimetric method with ammonium molybdate was employed to determine the concentration of orthophosphates. In an acidic environment and in the presence of ammonium molybdate, orthophosphates react to form a phosphomolybdic complex. This complex, when reduced by ascorbic acid, develops a blue color with maximum absorption at 700 nm. The intensity of the color is directly proportional to the quantity of phosphates present in the sample. In order to measure the concentration of orthophosphates in the water samples, a calibration curve was established utilizing a range of known concentrations spanning from 0.013 to 0.5 mg/L.

iii. Chemical Oxygen Demand (COD) Assay

The measurement of COD was conducted in accordance with the AFNOR NF T90-101 (February 2001) standard [13]. This assay involves the oxidation of oxidizable substances present in the sample by heating it for two hours in an acidic medium, using a known quantity of potassium dichromate as an oxidizing agent. Silver sulfate acts as an oxidation catalyst, while mercury (II) sulfate complexes chloride ions. During this process, the oxygen consumption by the sample leads to a color change, and the absorbance resulting from the reduction of potassium dichromate to chromium (III) oxide is directly proportional to the amount of oxidizable matter in the sample. In the colorimetric measurement, the quantity of chromium (III) oxide produced is determined using a spectrophotometer at two wavelengths: 420 nm (range: 15 to 150 mg/L) and 600 nm (range: 150 to 1500 mg/L).

iv. Biochemical Oxygen Demand (BOD5) Assay

The measurement of BOD5 was performed using the pressure change method, as outlined in the AFNOR NF EN 1899-2 (May 1998) standard [14]. This method relies on the observation of pressure changes resulting from the conversion of oxygen to carbon dioxide by microorganisms. To remove carbon dioxide and facilitate accurate pressure measurements, soda tablets are utilized, which convert carbon dioxide to sodium carbonate. This process leads to a decrease in pressure, which is detected and recorded using OxiTops. To ensure efficient exchange of oxygen between the gas phase and the sample liquid, continuous magnetic stirring is employed. The sample bottles are filled according to the specified BOD measurement range and subsequently sealed. The organic substances within the samples undergo decomposition by microorganisms, which consume oxygen during a 5-day incubation period at a controlled temperature of 20 ± 1 °C.

v. Nitrites Assay

The assay for nitrites was conducted following the protocol outlined in the AFNOR NF EN 26777 standard (May 1993) [15], specifically, the method employing molecular absorption spectrometry (classification index T90-013). In an acidic medium, the presence of N-(1-naphthyl) ethylenediamine dichloride initiates a diazotization reaction with 4-aminobenzenesulfonamide in the presence of nitrites. This reaction results in the formation of a pink complex, which can be quantified at a wavelength of 543 nm by spectrophotometry. To determine the concentration of nitrites in the samples, a calibration curve was established using a series of known concentrations ranging from 0, 0.02, 0.05, 0.1, 0.15, and 0.2 mg/L. This calibration curve was prepared using a daughter standard solution at a concentration of 1 mg/L, allowing for accurate quantification of nitrite levels in the samples.

2.3. Plant Material

Following a growth cycle of 120 days, the aerial parts of *Mentha piperita* plants were harvested. The leaves were carefully collected and subjected to a natural shade-drying process carried out during the spring season at an average temperature of 8 and 22 °C. This drying method, as described by [12], was employed to optimize the plant material yield. The dried leaves were subsequently utilized for the extraction of essential oil.

2.4. Essential Oil Extraction

The process of extracting MPEO from the dried and crushed leaves of *Mentha piperita* was carried out utilizing the commonly employed technique of hydrodistillation, as described in the existing scientific literature [16]. The extraction unit used was a modified Clevenger unit. In this process, 100 g of the dried and crushed *Mentha piperita* leaves were combined with 1000 mL of water, which was introduced into a 2 L flask. The apparatus was securely sealed and heated at a temperature of 100 °C. As the temperature increased, the volatile components of the plant material vaporized and subsequently condensed in a condenser, resulting in the formation of liquid essential oil. The obtained essential oil was carefully collected and stored in a tightly sealed container. To preserve its quality, the container was stored in a dark environment at a temperature of 4 °C.

2.5. Qualitative and Semi-Quantitative Analysis of MPEO

The qualitative and semi-quantitative analysis of MPEO was conducted using a gas chromatograph coupled with a mass spectrometer. Specifically, a GC Shimadzu system, in conjunction with an MS QP2010, was employed to facilitate identifying and separating the compounds present in the essential oil. To separate the compounds, a capillary column (BPX25) coated with a 95% dimethylpolysiloxane diphenyl phase was employed. The column dimensions were 30 m in length, 0.25 mm in internal diameter, and had a 0.25 µm film thickness. As the carrier gas, pure helium with a 99.99% purity was used at a constant flow rate of 3 milliliters per minute. The experimental conditions for the analysis were as follows: the temperatures of the injection, ion source, and interface were maintained at 250 °C. The column oven was programmed to initially hold at 50 °C for 1 min, then increase to 250 °C at a rate of 10 °C per minute, and, finally, hold at the final temperature

for 1 min. The sample components were subjected to electron ionization (EI) mode at 70 eV for ionization. The mass range studied was from 40 to 300 m/z. To prepare the essential oil samples for analysis, each oil was introduced into the chamber at a volume of 1 L, diluted with a suitable solvent. Subsequently, 1 µL of the prepared essential oil was injected into the system using the split mode, with a split ratio of 90:1. Three evaluations were performed for each sample to ensure accuracy and reproducibility of the results. The identification of compounds in the essential oil involved comparing their retention times and mass spectrum fragmentation patterns with established standards and references available in databases such as NIST. The data were gathered and analyzed using Laboratory Solutions software (v2.5). This analytical approach allowed for both qualitative identification of the compounds present in MPEO and semi-quantitative analysis, providing valuable insights into the composition of the essential oil.

2.6. PASS, ADME, and the Prediction of the Toxicity Analysis (Pro-Tox II)

In the present investigation, the pharmacological activity of the principal chemical components found in *Mentha piperita* essential oils (MPEO) was evaluated using the Pharmacological Assessment of Structure Similarity (PASS) method [17]. Molecular structures were first converted into SMILES (Simplified Molecular Input Line Entry System) format using ChemDraw, then subjected to analysis using the PASS online application, which provided predictions of their probable activity (Pa) and probable inactivity (Pi) [18,19]. In addition, to evaluate the physicochemical properties, drug similarity, and pharmacokinetic characteristics of the identified compounds, we employed the SwissADME (http://www.swissadme.ch/ accessed on 19 April 2023) and pkCSM (http://biosig.unimelb.edu.au/pkcsm/ accessed on 19 April 2023) web servers [20–22]. To investigate the toxicity levels and obtain insightful data on various toxicological parameters, such as LD50 and toxicity class, we utilized Protox II (https://tox-new.charite.de/protox_II/, accessed on 19 April 2023), a valuable tool specifically designed for this purpose [23]. By employing these methodologies and analytical tools, we have acquired compelling findings regarding the potential therapeutic applications and potential side effects related to the main chemical components identified in MPEO.

2.7. Tests of the Antioxidant Activity

2.7.1. DPPH Radical Scavenging Assay

The assessment of antioxidant activity in *Mentha piperita* essential oils (MPEOs) was conducted using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the established protocols outlined by Kandsi et al. (2022) and Zrouri et al. (2022) in references [24,25]. To prepare the DPPH solution, 2 mg of DPPH was dissolved in 100 mL of methanol. Subsequently, various concentrations of MPEOs, ranging from 5 to 500 µg/mL, were prepared. Each concentration was added to 2.5 mL of the methanol solution containing DPPH, resulting in a final volume of 3 mL. After the mixing of the components, the mixture was left to incubate at room temperature for a duration of 30 min. Subsequently, solution's absorbance was quantified at a wavelength of 515 nm, with reference to a blank sample. The percentage of DPPH radical scavenging activity was then determined using the following formula:

$$\textbf{Radical Scavenging Activity}(\%) = \left[\left(\frac{\mathbf{A_{blank}} - \mathbf{A_{sample}}}{\mathbf{A_{blank}}}\right)\right] \times \mathbf{100}$$

where $\mathbf{A_{blank}}$ represents the absorbance of the control reaction (all reagents present except for the extract) and $\mathbf{A_{sample}}$ represents the absorbance of the extract at different concentrations. The determination of the IC_{50} value was achieved through the construction of a graph correlating the percentage inhibition with the concentrations of the extract. As a reference, ascorbic acid was employed as the positive control in this experiment.

2.7.2. Total Antioxidant Capacity

The total antioxidant capacity (TAC) of the MPEOs was assessed using the phosphomolybdenum method outlined by Elbouzidi et al. [26]. In this approach, a combination of 0.1 mL of the standard solution or extract and 0.3 mL of the reagent solution (comprising 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was incubated at 95 °C for 90 min. Following the incubation period, the mixture was cooled to room temperature, and the absorbance was measured at 695 nm. To quantify the total antioxidant capacity, a standard curve was generated employing various concentrations of ascorbic acid standards. The outcomes were expressed in terms of ascorbic acid equivalents (AAs). To ensure accuracy and reliability, all experiments were conducted in triplicate.

2.8. Antimicrobial Activity Tests of MPEOs

2.8.1. Antibacterial Activity

i. Bacterial Strains and Growth Conditions

The antimicrobial activity of MPEOs was evaluated against four bacterial strains obtained from the Laboratory of Microbiology and Biotechnology at the Faculty of Sciences in Oujda, Morocco. The strains tested included *Micrococcus luteus* (LB 14110) and *Staphylococcus aureus* (ATCC 6538™), both Gram-positive bacteria, as well as *Pseudomonas aeruginosa* (ATCC 15442™) and *Escherichia coli* (ATCC 10536™), which are Gram-negative bacteria. Before testing essential oil activity, bacterial strains were grown in Mueller Hinton medium and incubated at 37 °C for 24 h to ensure optimal growth. The bacterial concentration was then measured and adjusted to 10^6 cells/mL using a UV-visible spectrophotometer at 620 nm, providing a consistent starting concentration for the antimicrobial assays.

ii. Disc Diffusion Method

The disc diffusion method, also known as the Kirby–Bauer method, was employed in this study to determine the antibacterial activity of MPEOs against the aforementioned bacterial strains [27]. The procedure involved inoculating a standardized bacterial suspension onto Petri dishes containing Mueller–Hinton agar medium. Subsequently, filter paper discs with a diameter of 6 mm (Whatman No. 1) were impregnated with 15 µL of each essential oil and placed on the agar surface. The Petri dishes were then incubated at 37 °C for 48 h. Following incubation, the plates were refrigerated at 4 °C for 2 h to facilitate the diffusion of the active compounds. The resulting zone of inhibition around the discs was measured to assess the antibacterial activity of the essential oils. All experiments were conducted in triplicate to ensure the reliability of the results.

iii. Minimum Inhibitory Concentration (MIC)

To evaluate the antibacterial efficacy of MPEOs, the minimum inhibitory concentration (MIC) was determined according to the method described in reference [28]. The MIC values were evaluated using the microdilution method in 96-well microplates. The concentrations of the essential oil samples ranged from 8% to 0.0015%. A bacterial inoculum, adjusted to a concentration of 10^6 cells/mL, was included in each well of the microplate, while gentamicin served as a positive control. Subsequently, the microplates were incubated at 37 °C for 24 h to facilitate bacterial growth. After incubation, 15 µL of a 0.015% resazurin solution was added to the microplate wells, followed by an additional 2 h incubation at 37 °C. The presence of live bacteria was determined by observing the transformation of blue resazurin into pink resorufin [29]. The MIC for each tested MPEO sample was recorded as the minimum concentration at which no resazurin transformation occurred. All experiments were performed in triplicate to ensure the reliability of the results. The obtained data were subjected to statistical analysis to evaluate the significance of differences between the essential oil samples.

iv. Minimum Bactericidal Concentration (MBC)

To evaluate the bactericidal effect of MPEO, the minimum bactericidal concentration (MBC) was determined. For this purpose, a 3 µL sample from the negative control wells

was transferred to Mueller–Hinton Agar (M-H) growth medium and incubated at 37 °C for an additional 24 h. The objective of this step was to assess whether any surviving bacteria from the negative control were capable of multiplying under favorable conditions. If no bacterial growth was observed in the M-H agar medium, the corresponding concentration was recorded as the MBC for each tested MPEO sample. All experiments were performed in triplicate to ensure the reliability of the results. The obtained data underwent statistical analysis to assess the significance of differences between the essential oil samples.

2.8.2. Antifungal Activity

i. Fungal Strains and Disc Diffusion Method

To evaluate the antifungal activity of MPEO, two yeast species, *Candida glabrata* and *Rhodotorula glutinis*, along with two mold species, *Penicillium digitatum* and *Aspergillus niger*, were employed. Cultures of *Aspergillus niger* and *Penicillium digitatum* were grown on BIOKAR's PDA medium (potato dextrose agar) at a temperature of 25 °C for a duration of seven days. Following incubation, the spore concentration was accurately adjusted to 2×10^6 spores/mL using a hemocytometer (Thoma cell). *Candida glabrata* and *Rhodotorula glutinis* were cultivated on yeast peptone dextrose (YPD) medium at 25 °C for 48 h, and the cell concentration for each yeast strain was adjusted to 10^6 cells/mL. The antifungal activity of MPEO against these strains was assessed using the disk diffusion method, which was described in the preceding section and is recommended in the *Manual on Antimicrobial Susceptibility Testing* [30].

ii. Minimum Inhibitory Concentration (MIC)

The MIC was determined following the methodology described earlier. The MPEO was diluted using 0.15% agar PCB as the diluent. The MIC assay was performed using the same 96-well microplate method, employing a concentration range of 8% to 0.0015%, as previously mentioned. The fungal suspension used in the assay had an approximate concentration of 2×10^8 spores/mL. Incubation of the microplates was conducted at 25 °C for 48 h for yeasts and 72 h for molds. Following the incubation period, 15 µL of resazurin was added to the wells to facilitate the detection of growth.

iii. Minimum Fungicidal Concentration (MFC)

The minimum fungicidal concentration (MFC) was determined by transferring 3 µL of the contents from wells that showed no visible growth onto yeast extract glucose (YEG) medium. Subsequently, the plates were incubated at 25 °C for 48 h for yeasts and 72 h for molds. The MFC is defined as the lowest concentration at which no visible fungal growth is observed on the YEG medium.

2.9. Statistical Analysis

The results obtained from the experiments were presented as mean values ± standard deviation, which were calculated based on triplicate measurements. All statistical analyses were conducted using the IBM Statistical Package for Social Sciences (IBM SPSS version 23). The data were analyzed using analysis of variance (ANOVA) with a one-factor design, utilizing the general linear model procedure. Post hoc tests, specifically the smallest significant difference test, were performed to compare the means at a significance level of 5%.

3. Results

3.1. Physico-Chemical Analysis of Irrigation Waters

pH, an essential parameter for plant growth, plays a pivotal role in nutrient absorption as it directly impacts the availability of nutrients in the environment. Additionally, pH serves as an important indicator of water quality, influencing the content of essential oil in plants, which can be affected by water salinity and acidity [10]. Among the vital elements for plant development, phosphorus and nitrogen are crucial for processes such as

root system establishment, photosynthesis, and reproductive functions [31,32]. Chemical oxygen demand (COD) is a measure of water's ability to consume oxygen during the breakdown of organic matter and oxidation of inorganic chemicals [33]. Higher COD values indicate lower dissolved oxygen content, potentially leading to oxygen-deficiency-induced stress in plants [34]. Elevated levels of biochemical oxygen demand can suggest the presence of organic matter or pollutants in the water, which can have detrimental effects on plant health [35].

The results presented in Table 1 suggest that the pH levels for TWW, well water, and river water are 9.00 ± 0.03, 7.58 ± 0.04, and 7.5 ± 0.01, respectively, falling within the acceptable range of 6 to 9, as per Moroccan water quality standards for irrigation. Regarding electrical conductivity (EC), TWW records a value of 2.76 ± 0.10 mS/cm, well water measures 2.55 ± 0.05 mS/cm, and river water exhibits 1.78 ± 0.05 mS/cm. All three water sources remain below the limit value of 12 mS/cm. In terms of nitrate levels (NO_3^-), TWW shows 4.74 ± 0.04 mg/L, well water has 3.81 ± 0.03 mg/L, and river water registers the lowest value of 1.31 ± 0.05 mg/L. These values are below the specified limit of 30 mg/L. Nitrite levels (NO_2^-) are detected only in TWW (2.03 ± 0.04 mg/L) and well water (1.41 ± 0.01 mg/L), while river water has no detectable nitrites (0.11 ± 0.01 mg/L). Chemical oxygen demand (COD) is highest in TWW at 142.6 ± 2.80 mg/L O_2, significantly different from the lower values of 13.00 ± 1.3 mg/L O_2 for well water and 10 ± 0.01 mg/L O_2 for river water. The Moroccan water quality standard for COD in irrigation is set at 150 mg/L O_2. In terms of biochemical oxygen demand over five days (BOD_5), TWW exhibits the highest value of 77.5 ± 2.50 mg/L O_2, while well water records 4.00 ± 0.025 mg/L O_2, and river water has the lowest at 0.45 ± 0.05 mg/L O_2. Finally, orthophosphate (PO_4) levels are found to be very similar among the three water sources, with TWW at 0.017 ± 0.002 mg/L, well water at 0.013 ± 0.002 mg/L, and river water at 0.011 ± 0.002 mg/L. In conclusion, the results demonstrate variations in different parameters among the three water sources used for irrigation. These comparisons highlight significant distinctions among the water sources in terms of their quality, which could affect the chemical profile of the irrigated plants.

Table 1. Results of analysis performed on the three types of water used for irrigation.

Parameters	Water			Limit Values [2]
	TWW [1]	Well	River	
pH	9.00 ± 0.03 a	7.58 ± 0.04 b	7.5 ± 0.01 b	6–9
EC (mS/cm)	2.76 ± 0.10 a	2.55 ± 0.05 b	1.78 ± 0.05 c	12
Nitrates NO_3^- (mg/L)	4.74 ± 0.04 a	3.81 ± 0.03 b	1.31 ± 0.05 c	30
Nitrites NO_2^- (mg/L)	2.03 ± 0.04 a	1.41 ± 0.01 b	0.11 ± 0.01 c	-
(COD) [3] (mg/L O_2)	142.6 ± 2.80 a	13.00 ± 1.3 b	10 ± 0.01 b	150
(BOD5) [4] (mg/L O_2)	77.5 ± 2.50 a	4.00 ± 0.025 b	0.45 ± 0.05 c	-
Orthophosphate PO_4 (mg/L)	0.017 ± 0.002 a	0.013 ± 0.002 a	0.011 ± 0.002 a	-

All values in this table represent mean ± SD (n = 3). Distinct letters are used to denote significant differences (a, b, and c) within the same row ($p < 0.05$). [1]: water collected at the outlet of the wastewater treatment plant (Oujda, Morocco). [2]: Moroccan water quality standards for irrigation. [3]: chemical oxygen demand. [4]: biochemical oxygen demand over five days.

3.2. Yield of the Three Essential Oils

The hydrodistillation process of Mentha piperita leaves yielded essential oils ranging from 0.66% to 0.98%. These values align with the results documented by Scavroni et al. [36] and Baser et al. [37]. Notably, when comparing the essential oil yields among the three different plantations (Table 2), a significant variation is observed. The plants irrigated with water obtained from the wastewater treatment plant exhibited the highest yield, followed by plants irrigated with well water, while those irrigated with river water showed the lowest yield. These results suggest that the quality of irrigation water significantly influences the essential oil yield of *Mentha piperita* plants.

Table 2. Essential Oil Yield of the Three Plantations.

Essential Oil	EO1	EO2	EO3
Essential oil yield (% *w*/*w*)	0.98 ± 0.06 a	0.84 ± 0.05 b	0.66 ± 0.02 c

All values in this table represent mean ± SD (n = 3). Significant differences are indicated by different letters (a, b, and c) within the same row ($p < 0.05$). EO1: essential oil extracted from plants irrigated with treated wastewater; EO2: essential oil extracted from plants irrigated with well water; EO3: essential oil extracted from plants irrigated with river water.

3.3. Qualitative and Semi-Quantitative Analyses of MPEOs

Figure 1 and Table 3 present the qualitative and semi-quantitative analysis results of the three essential oils obtained through GC/MS analysis. EO1, extracted from plants irrigated with water collected from the wastewater treatment plant, consists of 12 compounds. The dominant compounds in EO1 are carvone (51.35%), D-limonene (17.88%), eucalyptol (10.69%), β-caryophyllene (4.39%), and Germacrene D (5.21%). EO2, extracted from plants irrigated with well water, exhibits a more diverse composition with 18 compounds. The major compounds in EO2 are carvone (76.89%), eucalyptol (5.22%), D-limonene (3.27%), β-caryophyllene (3.02%), and Germacrene D (2.97%). EO3, obtained from plants irrigated with river water, is composed of 17 compounds, with carvone (77.17%), *p*-menthane, 1,8-epoxy- (5.06%), D-limonene (3.42%), α-cubebene (2.98%), and β-caryophyllene (2.93%) being the major compounds identified in this essential oil.

The findings of our study reveal variations in the composition of the essential oils compared to previous studies conducted by Scarvoni et al. [37], de Sousa et al. [38], Moghaddam et al. [9], and Mahboubi and Kazempour [39]. For instance, the essential oil extracted from *Mentha piperita* in Brazil by [40] exhibited significant amounts of menthyl acetate (35.01%), menthol (42.32%), menthofuran (4.56%), menthone (4.05%), and 1,8 cineole (5.56%). Similarly, de Sousa et al. [38] reported major components such as menthol (49.97%), menthone (19.08%), methyl acetate (5.29%), isomenthol (4.56%), and isomenthone (4.06%).

Each of the essential oils analyzed in our study exhibited distinct compositions characterized by specific compounds. EO1 contained compounds such as sabinene hydrate, Plinol A, and Germacrene B. EO2 consisted of compounds like *p*-menth-1-en-8-ol, α-Pinene oxide, β-Farnesene, and γ-Cadinene. EO3 contained compounds such as *p*-Menthane, 1,8-epoxy-, α-cubebene, cubenol, and epiglobulol. Additionally, varying concentrations of common components were observed among the three essential oils. These differences in composition could be attributed to the quality of irrigation water used during plant cultivation.

The percentage and synergistic effects of chemical compounds present in essential oils play a crucial role in determining their antioxidant and antimicrobial activities [41,42]. Therefore, the variations in the composition of the essential oils obtained in our study may contribute to differences in their biological properties compared to previous reports.

3.4. Physiochemical and Pharmacokinetic Properties (ADME)

Table 4 presents the results of the physicochemical and drug-likeness analysis conducted on the major compounds identified in the studied essential oils. The analysis aimed to assess the suitability of these compounds for drug development based on various parameters. The compounds listed in the table exhibit a range of characteristics that are important for drug-likeness evaluation. Firstly, the hydrogen-bond donor (HBD) and hydrogen-bond acceptor (HBA) values indicate the potential for forming or accepting hydrogen bonds, which can influence the compounds' interactions with biological targets. In this case, all compounds showed an HBD/HBA value of 0/0 or 0/1, suggesting a low propensity for hydrogen bonding. The topological polar surface area (TPSA) provides an estimate of the molecular surface area that is involved in polar interactions. Compounds with higher TPSA values may have increased solubility and permeability, which are desirable properties for drug-like molecules. Interestingly, most of the compounds in the table exhibited a TPSA value of 0.00 $Å^2$, suggesting minimal polar surface area. The logarithm of the partition coefficient (Log Po/w) provides information about the compound's lipophilicity, which

influences its distribution between aqueous and lipid phases. Compounds with appropriate Log Po/w values are expected to have good permeability through biological membranes. The compounds in the table demonstrated Log Po/w values ranging from 2.17 to 5.20, indicating a diverse range of lipophilicity among the compounds. Solubility (Log S) is another crucial factor affecting a compound's pharmaceutical applicability. Compounds with higher Log S values tend to be more soluble, increasing their potential for effective drug delivery. The Log S values observed in the table ranged from 2.17 to 4.93, indicating varying degrees of solubility among the compounds.

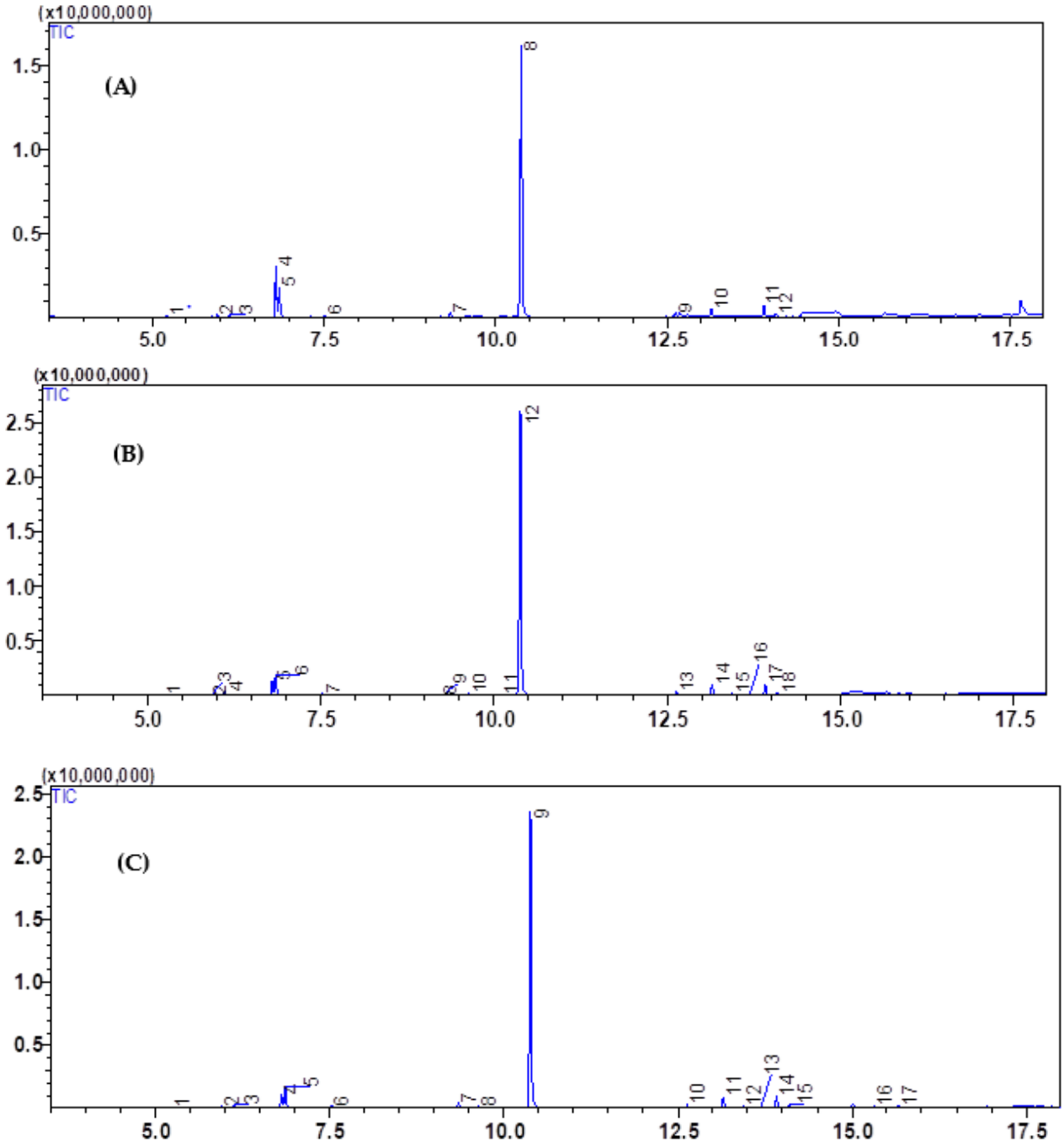

Figure 1. GC/MS chromatogram of the chemical composition of MPEO extracted from plants irrigated with treated wastewater (**A**), well water (**B**), and river water (**C**).

Table 3. Chemical composition of the three essential oils of *M. piperita* obtained under three different irrigation regimes.

N°	Compounds	Formula	RT [1,2,3] (min)	% Area		
				EO1	EO2	EO3
1	α-Pinene	$C_{10}H_{16}$	5.219 [1]; 5.218 [2]	-	0.13	0.130
2	β-Pinene	$C_{10}H_{16}$	5.949 [1]; 5.948 [2];	1.22	0.18	0.350
3	β-Myrcene	$C_{10}H_{16}$	6.122 [1]; 6.120 [2];	0.89	0.71	0.740
4	D-Limonene	$C_{10}H_{16}$	6.804 [1]; 6.803 [2];	17.88	3.27	3.420
5	2-methyl-5-propan-2-ylbicyclo [3.1.0] hexan-2-ol	$C_{10}H_{18}O_4$	7.521 [1]	-	-	0.750
6	*p*-Menth-1-en-4-ol	$C_{10}H_{18}O$	9.348 [1]; 9.346 [2,3]	2.56	1.78	1.720
7	2H-Inden-2-one, octahydro-3a-methyl-, *trans*-	$C_{10}H_{16}O$	9.633 [1,2]	-	0.58	0.660
8	Carvone	$C_{10}H_{14}O$	10.384 [1]; 10.386 [2]; 10.378 [3]	51.35	76.44	77.17
9	α-Bourbonene	$C_{15}H_{24}$	12.632 [1,2,3]	2.47	0.89	0.92
10	β-Caryophyllene	$C_{15}H_{24}$	13.148 [1]; 13.146 [2,3]	4.39	3.02	2.93
11	β-Bisabolene	$C_{15}H_{24}$	13.435 [1]	-	-	0.74
12	8-Isopropyl-5-methyl-2-methylene-1,2,3,4,4a,5,6,7-octahydronaphthalene	$C_{15}H_{24}$	13.700 [1]	-	-	0.51
13	α-Cubebene	$C_{15}H_{24}$	13.915 [1]	-	-	2.98
14	γ-Elemene	$C_{15}H_{24}$	14.091 [1]; 14.089 [2]	-	0.77	0.72
15	Cubenol	$C_{15}H_{26}O$	15.314 [1]	-	-	0.50
16	Epiglobulol	$C_{15}H_{26}O$	15.671 [1]	-	-	0.70
17	Sabinene	$C_{10}H_{16}$	5.874 [2]	-	0.18	-
18	Eucalyptol	$C_{10}H_{18}O$	6.856 1; 6.855 [2]; 6.854 [3]	10.69	5.22	5.06
19	Plinol A	$C_{10}H_{18}O$	7.519 [2]; 7.521 [3]	1.24	0.76	-
20	*p*-menth-1-en-8-ol	$C_{10}H_{18}O$	9.202 [2]	-	0.40	-
21	α-Pinene oxide	$C_{10}H_{16}O$	10.078 [2]	-	0.86	-
22	β-Farnesene	$C_{15}H_{24}$	13.434 [2]	-	0.75	-
23	γ-Cadinene	$C_{15}H_{24}$	13.697 [2]	-	0.48	-
24	Germacrene D	$C_{15}H_{24}$	13.914 [2,3]	5.21	2.97	-
25	Sabinene hydrate	$C_{10}H_{18}O$	5.217 [3]	0.56	-	-
26	Germacrene B	$C_{15}H_{24}$	14.087 [3]	1.54	-	-
	Hydrocarbon monoterpenes			15.72	9.33	9.51
	Oxygenated monoterpenes			0.56	1.33	1.16
	Hydrocarbon sesquiterpenes			0	0	1.26
	Oxygenated sesquiterpenes			81.16	88.73	86.35
	Total identified (%)			**97.44**	**99.39**	**98.28**

[1]: essential oil extracted from plants irrigated with treated wastewater; [2]: essential oil extracted from plants irrigated with well water; [3]: essential oil extracted from plants irrigated with river water.

To further evaluate the drug-likeness of the compounds, Lipinski's Rule of Five and the Veber filter were applied. Lipinski's Rule of Five assesses the drug-likeness of compounds based on their molecular weight, Log Po/w, number of hydrogen bond donors, and number of hydrogen bond acceptors. Violations of this rule, indicated by asterisks in the table, suggest potential challenges in drug development. Most compounds complied with Lipinski's Rule of Five, except for those with MLOGP (Molecular LogP) values exceeding the threshold of 4.15.

The Veber filter evaluates the compound's size and flexibility by considering the number of rotatable bonds. All compounds in the table passed the Veber filter criteria, indicating favorable characteristics in terms of size and flexibility. The analyzed essential oils contain major compounds that exhibit drug-like properties, as evidenced by their compliance with Lipinski's Rule of Five and the Veber filter. The compounds possess desirable physicochemical properties such as low hydrogen-bonding potential, diverse lipophilicity, and varying degrees of solubility. Despite a few violations of Lipinski's Rule of

Five due to higher MLOGP values, these compounds hold promise for further investigation and potential pharmaceutical development.

Table 4. Analysis of Physicochemical Properties and Drug-Likeness of Major Compounds in Studied Essential Oils.

N°	Compounds	HBD/HBA	TPSA (Å^2)	Log Po/w (WLOGP)	Log S (SILICO S-IT)	Lipinski's Rule of Five	Veber Filter
1	α-Pinene	0/0	0.00	3.00	2.79	Yes; 1 violation *	Yes
2	β-Pinene	0/0	0.00	3.00	3.08	Yes; 1 violation *	Yes
3	β-Myrcene	0/0	0.00	3.48	3.05	Yes	Yes
4	D-Limonene	0/0	0.00	3.31	2.97	Yes	Yes
5	2-methyl-5-propan-2-ylbicyclo [3.1.0] hexan-2-ol	1/1	20.23	2.19	2.44	Yes	Yes
6	*p*-Menth-1-en-4-ol	1/1	20.23	2.50	2.44	Yes	Yes
7	2H-Inden-2-one, octahydro-3a-methyl-, *trans*-	0/1	17.07	2.55	2.92	Yes	Yes
8	Carvone	0/1	17.07	2.49	2.64	Yes	Yes
9	α-Bourbonene	0/0	0.00	4.27	3.73	Yes; 1 violation *	Yes
10	β-Caryophyllene	0/0	0.00	4.73	4.19	Yes; 1 violation *	Yes
11	β-Bisabolene	0/0	0.00	5.04	4.50	Yes; 1 violation *	Yes
12	8-Isopropyl-5-methyl-2-methylene-1,2,3,4,4a,5,6,7-octahydronaphthalene	0/0	0.00	4.73	4.41	Yes; 1 violation *	Yes
13	α-Cubebene	0/0	0.00	4.27	3.73	Yes; 1 violation *	Yes
14	γ-Elemene	0/0	0.00	4.89	4.61	Yes; 1 violation *	Yes
15	Cubenol	1/1	20.23	3.78	3.22	Yes	Yes
16	Epiglobulol	1/1	20.23	3.47	3.00	Yes	Yes
17	Sabinene	0/0	0.00	3.00	3.23	Yes; 1 violation *	Yes
18	Eucalyptol	0/1	9.23	2.74	2.86	Yes	Yes
19	Plinol A	1/1	20.23	2.36	2.23	Yes	Yes
20	*p*-menth-1-en-8-ol	1/1	20.23	2.50	2.17	Yes	Yes
21	α-Pinene oxide	0/1	12.53	2.21	2.70	Yes	Yes
22	β-Farnesene	0/0	0.00	5.20	4.93	Yes; 1 violation *	Yes
23	γ-Cadinene	0/0	0.00	4.58	4.01	Yes; 1 violation *	Yes
24	Germacrene D	0/0	0.00	4.89	4.01	Yes; 1 violation *	Yes
25	Sabinene hydrate	1/1	20.23	2.19	2.44	Yes	Yes
26	Germacrene B	0/0	0.00	5.18	4.25	Yes; 1 violation *	Yes

* violation: MLOGP > 4.15.

3.5. PASS

The table presents the PASS predictions for the major compounds found in three essential oils, indicating their potential biological activities including antioxidant, antibacterial, and antifungal effects. The compounds are numbered from 1 to 26, and their activity probabilities are represented by Pa (probability 'to be active') and Pi (probability 'to be inactive') (Table 5). The values range from 0 to 0.584 for Pa and from 0.007 to 0.128 for Pi. Compounds with Pa values higher than 0.50 are highlighted in bold, suggesting a higher likelihood

of activity. The table reveals a diverse range of activities among the compounds, with some showing higher probabilities for specific biological activities. For instance, α-Pinene oxide and β-Farnesene demonstrate notable probabilities for antibacterial and antifungal activities. Compound 19, Plinol A, stands out with high Pa values for both antibacterial and antifungal effects, warranting further investigation. The table provides valuable insights into the potential biological activities of the essential oil compounds.

Table 5. PASS prediction of the major compounds found in the chemical compounds of the three essential oils.

N°	Compounds	Biological Activities					
		Antioxidant		Antibacterial		Antifungal	
		P_a	P_i	P_a	P_i	P_a	P_i
1	α-Pinene	-	-	0.326	0.051	0.439	0.042
2	β-Pinene	-	-	0.233	0.093	0.225	0.121
3	β-Myrcene	0.470	0.008	0.398	0.030	**0.584**	0.020
4	D-Limonene	0.157	0.094	0.405	0.029	**0.582**	0.020
5	2-methyl-5-propan-2-ylbicyclo [3.1.0] hexan-2-ol	-	-	0.217	0.103	0.457	0.038
6	*p*-Menth-1-en-4-ol	0.151	0.102	0.328	0.050	0.466	0.036
7	2H-Inden-2-one, octahydro-3a-methyl-, *trans*-	0.145	0.109	0.269	0.073	0.386	0.053
8	Carvone	0.193	0.059	0.396	0.031	**0.562**	0.022
9	α-Bourbonene	-	-	0.319	0.053	0.278	0.091
10	β-Caryophyllene	0.174	0.075	0.437	0.023	**0.582**	0.020
11	β-Bisabolene	0.257	0.034	0.413	0.027	**0.585**	0.020
12	8-Isopropyl-5-methyl-2-methylene-1,2,3,4,4a,5,6,7-octahydronaphthalene	-	-	0.315	0.055	**0.509**	0.029
13	α-Cubebene	-	-	0.278	0.069	0.298	0.082
14	γ-Elemene	0.164	0.086	0.452	0.022	**0.564**	0.022
15	Cubenol	-	-	0.403	0.029	0.356	0.061
16	Epiglobulol	-	-	0.381	0.035	0.484	0.033
17	Sabinene	-	-	0.201	0.117	0.340	0.066
18	Eucalyptol	0.161	0.090	0.298	0.061	0.214	0.128
19	Plinol A	0.170	0.079	**0.519**	0.015	**0.557**	0.023
20	*p*-menth-1-en-8-ol	0.137	0.118	0.369	0.038	0.435	0.042
21	α-Pinene oxide	-	-	0.323	0.052	0.368	0.057
22	β-Farnesene	0.497	0.007	0.415	0.027	**0.607**	0.018
23	γ-Cadinene	-	-	0.447	0.022	0.489	0.032
24	Germacrene D	-	-	0.427	0.025	**0.570**	0.022
25	Sabinene hydrate	-	-	0.217	0.103	0.457	0.038
26	Germacrene B	-	-	0.374	0.037	0.297	0.082

3.6. Results of the Toxicity Predictions

Table 6 displays the findings of a research investigation that aimed to forecast the toxicity and toxic endpoints associated with the primary compounds detected in three *M. piperita* essential oils (likely referring to peppermint). The table provides information on 26 compounds, along with their predicted LD50 values (a measure of acute toxicity), GHS hazard classes, and probabilities of various toxic endpoints. The LD50 values indicate the acute toxicity of the compounds, with lower values suggesting higher toxicity. The compounds with LD50 values above 5000 mg/kg are classified as non-toxic (GHS hazard class VI). Compounds with LD50 values between 2000 and 5000 mg/kg are considered potentially harmful if swallowed (GHS hazard class V), while those with LD50 values between 300 and 2000 mg/kg are classified as harmful if swallowed (GHS hazard class IV). Based on the LD50 values provided in the table, the majority of compounds fall into GHS hazard classes IV and V, indicating that most of them have a moderate level of acute

toxicity. However, none of the compounds are classified as highly toxic (GHS hazard class I-III), as all have LD50 values above 300 mg/kg.

Table 6. Prediction of toxicity and the toxic endpoints of the major compounds found in the three essential oils from *M. piperita*. (1) α-Pinene; (2) β-Pinene; (3) β-Myrcene; (4) D-Limonene; (5) 2-methyl-5-propan-2-ylbicyclo [3.1.0] hexan-2-ol; (6) *p*-Menth-1-en-4-ol; (7) 2H-Inden-2-one, octahydro-3a-methyl-, trans-; (8) carvone; (9) α-Bourbonene; (10) β-Caryophyllene; (11) β-Bisabolene; (12) 8-Isopropyl-5-methyl-2-methylene-1,2,3,4,4a,5,6,7-octahydronaphthalene; (13) α-cubebene; (14) γ-Elemene; (15) cubenol; (16) epiglobulol; (17) sabinene; (18) eucalyptol; (19) Plinol A; (20) p-menth-1-en-8-ol; (21) α-Pinene oxide; (22) β-Farnesene; (23) γ-Cadinene; (24) Germacrene D; (25) sabinene hydrate; (26) Germacrene B.

N	Predicted LD_{50} (mg/kg)	Class	Hepatotoxicity		Carcinogenicity		Immunotoxicity		Mutagenicity		Cytotoxicity	
			Predi. *	Prob.**	Predi.	Prob.	Predi.	Prob.	Predi.	Prob.	Predi.	Prob.
1	3700	V	Inactive	0.86	Inactive	0.60	Inactive	0.99	Inactive	0.93	Inactive	0.75
2	4700	V	Inactive	0.80	Inactive	0.66	Inactive	0.97	Inactive	0.95	Inactive	0.71
3	5000	V	Inactive	0.77	Inactive	0.60	Inactive	0.99	Inactive	0.98	Inactive	0.75
4	4400	V	Inactive	0.76	Inactive	0.65	Inactive	0.95	Inactive	0.97	Inactive	0.82
5	2000	IV	Inactive	0.78	Inactive	0.74	Inactive	0.97	Inactive	0.88	Inactive	0.85
6	1016	IV	Inactive	0.80	Inactive	0.72	Inactive	0.99	Inactive	0.83	Inactive	0.88
7	775	IV	Inactive	0.70	Inactive	0.64	Inactive	0.98	Inactive	0.91	Inactive	0.64
8	1640	IV	Inactive	0.65	Inactive	0.83	Inactive	0.99	Inactive	0.97	Inactive	0.80
9	3700	V	Inactive	0.79	Inactive	0.68	Inactive	0.85	Inactive	0.89	Inactive	0.76
10	5300	VI	Inactive	0.80	Inactive	0.70	**Active**	**0.54**	Inactive	0.95	Inactive	0.75
11	4400	V	Inactive	0.82	Inactive	0.74	**Active**	**0.60**	Inactive	0.93	Inactive	0.81
12	5000	V	Inactive	0.80	Inactive	0.73	Inactive	0.87	Inactive	0.70	Inactive	0.70
13	5000	V	Inactive	0.85	Inactive	0.72	Inactive	0.98	Inactive	0.72	Inactive	0.67
14	5300	VI	Inactive	0.82	Inactive	0.79	Inactive	0.99	Inactive	0.70	Inactive	0.82
15	1016	IV	Inactive	0.82	Inactive	0.65	Inactive	0.68	Inactive	0.91	Inactive	0.95
16	2000	IV	Inactive	0.77	Inactive	0.69	Inactive	0.87	Inactive	0.75	Inactive	0.89
17	5000	V	Inactive	0.81	Inactive	0.59	Inactive	0.51	Inactive	0.82	Inactive	0.71
18	2840	V	Inactive	0.86	Inactive	0.68	Inactive	0.99	Inactive	0.96	Inactive	0.75
19	3900	V	Inactive	0.72	Inactive	0.66	Inactive	0.99	Inactive	0.87	Inactive	0.91
20	2830	V	Inactive	0.72	Inactive	0.76	Inactive	0.99	Inactive	0.90	Inactive	0.64
21	5000	V	Inactive	0.85	Inactive	0.53	Inactive	0.98	Inactive	0.89	Inactive	0.74
22	5000	V	Inactive	0.79	Inactive	0.73	Inactive	0.99	Inactive	0.98	Inactive	0.81
23	4400	V	Inactive	0.84	Inactive	0.76	**Active**	**0.55**	Inactive	0.69	Inactive	0.74
24	5300	VI	Inactive	0.80	Inactive	0.73	**Active**	**0.80**	Inactive	0.87	Inactive	0.83
25	2000	IV	Inactive	0.78	Inactive	0.74	Inactive	0.97	Inactive	0.88	Inactive	0.85
26	4390	V	Inactive	0.81	Inactive	0.75	Inactive	0.98	Inactive	0.86	Inactive	0.83

GHS hazard classes: IV—300 mg/kg < LD50 ≤ 2000 mg/kg, harmful if swallowed; V—2000 mg/kg < LD50 ≤ 5000 mg/kg, may be harmful if swallowed; VI—LD50 > 5000 mg/kg, non-toxic compounds. *: Prediction, **: Probability.

The table also provides predicted probabilities for various toxic endpoints associated with exposure to these compounds. The toxic endpoints considered include hepatotoxicity (liver toxicity), carcinogenicity (cancer-causing potential), immunotoxicity (immune system toxicity), mutagenicity (ability to cause genetic mutations), and cytotoxicity (ability to cause cell damage or death). Based on the predicted probabilities, compounds **10**, **11**, and **23** show moderate probabilities of being immunotoxic, while compound **24** showed the highest probability.

It is important to emphasize that the predictions presented in the table regarding the toxicity of the compounds are based on computational models. These models serve as valuable tools for initial assessments, but it is crucial to conduct further empirical research to validate the actual toxicity of these compounds in humans. Empirical studies involving in vitro and in vivo experiments, as well as clinical investigations, are necessary to obtain accurate and reliable data on the potential health implications associated with exposure to these specific compounds found in *Mentha piperita* essential oils. Therefore, while the

results from computational models provide valuable insights, they should be interpreted with caution, and additional research is required to fully understand the potential health effects and establish safe levels of exposure to these compounds.

3.7. Antioxidant Activity of MPEOs

In order to evaluate the antioxidant capacities of the three essential oils (EO1, EO2, and EO3) and determine the IC50 value, which represents the amount of antioxidants required to reduce the level of free radicals by 50%, two techniques were used in this study: DPPH and total antioxidant capacity (TAC). EO1, EO2, and EO3 exhibited significant levels of antioxidant activity. For the DPPH test, the observed IC50 values were 136.89 ± 0.005 µg/mL, 154.88 ± 0.04 µg/mL, and 90.67 ± 0.05 µg/mL, respectively, as shown in Table 7. Regarding the total antioxidant capacity, EO1, EO2, and EO3 showed values of 50.24 ± 0.6 µg AA/mL, 38.81 ± 0.4 µg AA/mL, and 78.26 ± 0.7 µg AA/mL, respectively. These results suggest that all three essential oils possess notable antioxidant activity.

Table 7. Assessment of MPEO antioxidant potential through DPPH and TAC tests (all values in this table represent mean ± SD (n = 3). Significant differences are indicated by different letters (a, b, and c) within the same row ($p < 0.05$).

Antioxidant Test	Inhibitory Concentration 50 $IC_{50)}$			
	EO1	**EO2**	**EO3**	**Ascorbic Acid**
DPPH Assay (µg/mL)	136.89 ± 0.05 a	154.88 ± 0.04 b	90.67 ± 0.05 c	21.06 ± 0.001
TAC (µg AA/mL)	50.24 ± 0.6 a	38.81 ± 0.40 b	78.26 ± 0.70 c	-

The differences in antioxidant activity observed among the three essential oils are likely due to their chemical compositions. It is important to note that these results differ from those mentioned by Amorati et al. [42] but are in line with the findings of Sun et al. [43], who also observed moderate antioxidant activity of MPEOs compared to other essential oils derived from different plants.

3.8. Antibacterial Activity

Essential oils are deemed active when the diameter of microbial growth inhibition is equal to or greater than 15 mm [44]. The essential oils used in this study exhibited moderate to strong antibacterial activities against the tested bacterial strains, as shown in the Results section (Table 8). The diameters of growth inhibition zones varied between 8.3- and 33-mm. Essential oil EO1, extracted from plants irrigated with treated wastewater, exhibited the largest inhibition zone diameter, with values of 33 mm, 27 mm, 22.3 mm, and 11 mm against *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, respectively. A low concentration of 0.0625% (*v/v*) of this EO inhibited the growth of *S. aureus*, indicating a high sensitivity of this bacterium to EO1. Essential oil EO2, extracted from plants irrigated with well water, showed significant antibacterial activity, with inhibition zone diameters of 31 mm, 23.5 mm, 15.3 mm, and 10 mm against *S. aureus*, *M. luteus*, *E. coli*, and *P. aeruginosa*, respectively. The minimum inhibitory concentrations (MICs) for EO2 were slightly higher than those for EO1, indicating slightly lower effectiveness against the tested bacteria. Essential oil EO3, extracted from plants irrigated with river water, exhibited inhibition zone diameters of 29.3 mm, 21.3 mm, 11.2 mm, and 8.3 mm against *S. aureus*, *M. luteus*, *E. coli*, and *P. aeruginosa*, respectively. The MICs for EO3 were the highest among the three essential oils, indicating that EO3 is the least effective against the tested bacteria. The minimum bactericidal concentrations (MBCs) revealed that all the studied essential oils had bactericidal activity at concentrations ranging from 0.5% to 8% (*v/v*). These results indicate that essential oil EO1 appears to be the most effective against the tested bacterial strains, followed by EO2, while EO3 seems to be the least effective. This is consistent with the findings of Satmi [45] and Lim [46], whose studies found significant antibacterial activity of *Mentha piperita* essential oil.

Table 8. Antibacterial Efficacy Evaluation of Three MPEO Variants.

Bacterial Strain		*S. aureus*	*M. luteus*	*E. coli*	*P. aeruginosa*
15 µL [1] of Essential oil, IZ [2]	EO1	33 ± 0.7	27 ± 0.7	22.3 ± 0.9	11 ± 0.7
	EO2	31 ± 0.7	23.5 ± 0.7	15.3 ± 0.9	10 ± 0.7
	EO3	29.3 ± 0.9	21.3 ± 0.9	11.2 ± 0.7	8.3 ± 0.4
15 µL [1] Gentamicine, IZ [2] (1 mg/mL)		19.5	21.5	22.5	20.5
MIC (% *v*/*v*)	EO1	0.0625	0.25	2	4
	EO2	0.125	1	4	4
	EO3	0.5	1	4	4
MBC (% *v*/*v*)	EO1	0.5	1	4	8
	EO2	1	2	8	8
	EO3	1	2	8	8

All values in this table represent mean ± SD (n = 3). [1]: used volume for disc diffusion method. [2]: diameter of inhibition zone (mm). EO1: essential oil extracted from plants irrigated with treated wastewater; EO2: essential oil extracted from plants irrigated with well water; EO3: essential oil extracted from plants irrigated with river water.

These differences may be attributed to the presence of germacrenes in EO1 and EO2, known for their antimicrobial activity [47]. Germacrenes are volatile organic compounds that belong to the terpene family [48]. They are often produced by plants in response to environmental stresses, and these compounds can have protective or adaptive properties [49]. In this context, the occurrence of Germacrene D and Germacrene B in the essential oil of plants irrigated with treated wastewater could be related to the presence of certain elements or compounds in these waters. Despite the antibacterial activity of these compounds, the activity of essential oils cannot be exclusively attributed to a single compound, as the presence of minor compounds could be involved in conferring this characteristic [50]. These results suggest that the water used for irrigating the plants from which the essential oils are extracted influenced the antibacterial efficacy of these oils.

3.9. Antifungal Activity

The results of the antifungal activity of three essential oils (EO1, EO2, and EO3) extracted from plants irrigated with treated wastewater, well water, and river water, respectively, were examined (Table 9). EO1 exhibited superior antifungal activity against all the fungal strains tested, displaying larger inhibition zone diameters and lower minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) compared to the other essential oils, with values of 40 mm, 38.3 mm, 27.7 mm, and 15.7 mm against *Penicillium digitatum*, *Aspergillus niger*, *Candida glabrata*, and *Rhodotorula glutinis*, respectively. A low concentration of 0.0625% (*v*/*v*) of EO1 inhibited the growth of *P. digitatum*, indicating a high sensitivity of this fungus to EO1. EO2 demonstrated significant antifungal activity, with inhibition zone diameters of 29.7 mm, 35 mm, 26.3 mm, and 13.3 mm for *P. digitatum*, *A. niger*, *C. glabrata*, and *R. glutinis*, respectively. The MIC values for EO2 were slightly higher than those for EO1, indicating slightly lower efficacy against the tested fungi. EO3, extracted from plants irrigated with river water, showed inhibition zone diameters of 27.7 mm, 30 mm, 20.3 mm, and 10.3 mm for *P. digitatum*, *A. niger*, *C. glabrata*, and *R. glutinis*, respectively. The MIC values for EO3 were the highest among the three essential oils, indicating that EO3 is the least effective of the three against the tested fungi. This is in line with the findings of (Elkhair et al.), who concluded that MPEO had the strongest antifungal activity compared to three other essential oils (*Thymus vulgaris*, *Cymbopogon citratus*, and *O. majoranum* oils) [51]. Another research study conducted by Freire et al. [52] revealed significant antifungal efficacy against various fungal strains, including *F. oxysporum, A. niger, A. flavus, F. semitectum, C. musae, C. gloeosporoides*, and *A. glaucus*. The observed antifungal activity may be attributed to the presence of terpenes, as noted by

Parveen et al., who reported the potent antifungal properties of these compounds [53]. Terpenes have been found to disrupt fungal membranes, degrade fungal mitochondria, and inhibit electron transport and mitochondrial ATPase, which contribute to their strong antifungal effects [54,55].

Table 9. Antifungal Potency Assessment of Three MPEO Variants.

Fungal Strains		*P. digitatum*	*A. niger*	*C. glabrata*	*R. glutinis*
15 µL [1] of Essential oil, IZ [2]	EO1	40.00 ± 0.70	38.30 ± 0.40	27.70 ± 0.40	15.70 ± 0.10
	EO2	29.70 ± 0.90	35.00 ± 0.70	26.30 ± 0.90	13.30 ± 0.40
	EO3	27.70 ± 0.90	30.00 ± 0.70	20.30 ± 0.40	10.30 ± 0.40
15 µL [1] Cycloheximide, IZ [2] (1 mg/mL)		22.90	22.30	21.50	21.00
MIC (% *v/v*)	EO1	0.0625	0.125	0.25	1
	EO2	0.25	0.125	0.5	2
	EO3	0.25	0.125	0.5	2
MFC (% *v/v*)	EO1	0.50	1	2	8
	EO2	1	2	4	8
	EO3	2	2	4	8

All values in this table represent mean ± SD (n = 3). [1]: used volume for disc diffusion method; [2]: diameter of inhibition zone (mm); EO1: essential oil extracted from plants irrigated with treated wastewater; EO2: essential oil extracted from plants irrigated with well water; EO3: essential oil extracted from plants irrigated with river water.

These results suggest that the use of different water sources for irrigation has a significant impact on the chemical composition of MPEO and, therefore, on their antifungal activity.

4. Conclusions

In conclusion, this study emphasizes the significance of irrigation water quality in the context of *Mentha piperita* cultivation and the production of secondary metabolites. The observed variations in water quality have notable implications for the chemical composition, yield, antioxidant properties, and antimicrobial properties of the extracted essential oil. These findings hold potential importance for both the agricultural and pharmaceutical industries. The in vitro experimentation assessing the antioxidant activity of the three MPEOs, using a DPPH radical scavenging assay and the TAC method, demonstrated a strong radical scavenging potential and a high antioxidant capacity for the essential oil extracted from plants irrigated with river water. Additionally, the essential oils showed moderate to strong antimicrobial activities against all four bacterial strains tested and four fungal strains employed in this study.

The results of this study can contribute to the development of improved irrigation strategies aimed at optimizing and targeting the production of secondary metabolites. This is particularly relevant given the growing resistance of numerous pathogens to synthetic drugs. However, further research is necessary to gain a deeper understanding of the precise mechanisms through which irrigation water quality influences secondary metabolite production in *Mentha piperita* and other plant species. Additionally, exploring the impact of different abiotic stresses on metabolite production is an avenue for future investigation. Such knowledge can contribute to the advancement of agricultural practices and the utilization of plant-derived compounds in various applications.

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References

1. Fennane, M.; Tattou, M.I. Statistiques et Commentaires Sur l'inventaire Actuel de La Flore Vasculaire Du Maroc. *Bull. De L'institut Sci. Rabat Sect. Sci. De La Vie* **2012**, *34*, 1–9.
2. Faysse, N.; Raïs, I.; Ait El Mekki, A.; Jourdain, D. Prospects for a Certified Mint Supply Chain in Morocco Based on an Assessment of Consumers' Willingness to Pay. *New Medit.* **2017**, *16*, 47–54.
3. Edris, A.E. Pharmaceutical and Therapeutic Potentials of Essential Oils and Their Individual Volatile Constituents: A Review. *Phytother. Res. Int. J. Devoted Pharmacol. Toxicol. Eval. Nat. Prod. Deriv.* **2007**, *21*, 308–323. [CrossRef] [PubMed]
4. Lawrence, B.M. *Mint: The Genus Mentha*, 1st ed.; CRC Press: Boca Raton, FL, USA, 2006; pp. 185–216. [CrossRef]
5. Singh, R.; Shushni, M.A.M.; Belkheir, A. Antibacterial and Antioxidant Activities of *Mentha piperita* L. *Arab. J. Chem.* **2015**, *8*, 322–328. [CrossRef]
6. Rosato, A.; Carocci, A.; Catalano, A.; Clodoveo, M.L.; Franchini, C.; Corbo, F.; Carbonara, G.G.; Carrieri, A.; Fracchiolla, G. Elucidation of the Synergistic Action of *Mentha piperita* Essential Oil with Common Antimicrobials. *PLoS ONE* **2018**, *13*, E0200902. [CrossRef] [PubMed]
7. Al-Mijalli, S.H.; Mrabti, N.N.; Ouassou, H.; Sheikh, R.A.; Abdallah, E.M.; Assaggaf, H.; Bakrim, S.; Alshahrani, M.M.; Awadh, A.A.A.; Qasem, A.; et al. Phytochemical Variability, In Vitro and In Vivo Biological Investigations, and In Silico Antibacterial Mechanisms of *Mentha piperita* Essential Oils Collected from Two Different Regions in Morocco. *Foods* **2022**, *11*, 3466. [CrossRef] [PubMed]
8. O'Neill, J. *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations*, Government of the United Kingdom: London, UK, May 2016.
9. Moghaddam, M.; Pourbaige, M.; Tabar, H.K.; Farhadi, N.; Hosseini, S.M.A. Composition and Antifungal Activity of Peppermint (*Mentha Piperita*) Essential Oil from Iran. *J. Essent. Oil Bear. Plants* **2013**, *16*, 506–512. [CrossRef]
10. Hayani, M.; Bencheikh, N.; Ailli, A.; Bouhrim, M.; Elbouzidi, A.; Ouassou, H.; Kharchoufa, L.; Baraich, A.; Atbir, A.; Ayyad, F.Z.; et al. Quality Control, Phytochemical Profile, and Antibacterial Effect of Origanum Compactum Benth. Essential Oil from Morocco. *IJPB* **2022**, *13*, 546–560. [CrossRef]
11. Borodin, N.; Bagrin, N.; Bogonina, Z. Nutrients in Waters of the Prut River, Lower Sector. In Proceedings of the Actual Problems of Protection and Sustainable Use of the Animal World Diversity, Chișinău, Moldova, 10–12 October 2013; p. 193, ISBN 978-9975-66-361-8.
12. Gonzalez, C.; Greenwood, R.; Quevauviller, P. (Eds.) *Rapid Chemical and Biological Techniques for Water Monitoring*; John Wiley & Sons: Hoboken, NJ, USA, 2009.
13. Bouyakhsass, R.; Souabi, S.; Rifi, S.K.; Taleb, A.; Pala, A.; Madinzi, A. Optimization of Coagulation-Flocculation for Landfill Leachate Treatment: An Experimental Design Approach Using Response Surface Methodology. *Environ. Nanotechnol. Monit. Manag.* **2023**, *20*, 100841. [CrossRef]
14. Gherairi, F.; Hamdi-Aissa, B.; Touil, Y.; Hadj-Mahammed, M.; Messrouk, H.; Amrane, A. Comparative Study between Two Granular Materials and Their Influence on the Effectiveness of Biological Filtration. *Energy Procedia* **2015**, *74*, 799–806. [CrossRef]
15. Artigas, J.; Majerholc, J.; Foulquier, A.; Margoum, C.; Volat, B.; Neyra, M.; Pesce, S. Effects of the Fungicide Tebuconazole on Microbial Capacities for Litter Breakdown in Streams. *Aquat. Toxicol.* **2012**, *122*, 197–205. [CrossRef] [PubMed]
16. El Kharraf, S.; Farah, A.; Miguel, M.G.; El-Guendouz, S.; El Hadrami, E.M. Two Extraction Methods of Essential Oils: Conventional and Non-Conventional Hydrodistillation. *J. Essent. Oil Bear. Plants* **2020**, *23*, 870–889. [CrossRef]
17. Alam, A.; Jawaid, T.; Alam, P. In Vitro Antioxidant and Anti-Inflammatory Activities of Green Cardamom Essential Oil and in Silico Molecular Docking of Its Major Bioactives. *J. Taibah Univ. Sci.* **2021**, *15*, 757–768. [CrossRef]
18. Linde, G.; Gazim, Z.; Cardoso, B.; Jorge, L.; Tešević, V.; Glamočlija, J.; Soković, M.; Colauto, N. Antifungal and Antibacterial Activities of Petroselinum Crispum Essential Oil. *Genet. Mol. Res.* **2016**, *15*. [CrossRef]

19. Filimonov, D.; Lagunin, A.; Gloriozova, T.; Rudik, A.; Druzhilovskii, D.; Pogodin, P.; Poroikov, V. Prediction of the Biological Activity Spectra of Organic Compounds Using the PASS Online Web Resource. *Chem. Heterocycl. Compd.* **2014**, *50*, 444–457. [CrossRef]
20. Daina, A.; Michielin, O.; Zoete, V. SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. *Sci. Rep.* **2017**, *7*, 42717. [CrossRef]
21. Kandsi, F.; Lafdil, F.Z.; Elbouzidi, A.; Bouknana, S.; Miry, A.; Addi, M.; Conte, R.; Hano, C.; Gseyra, N. Evaluation of Acute and Subacute Toxicity and LC-MS/MS Compositional Alkaloid Determination of the Hydroethanolic Extract of *Dysphania ambrosioides* (L.) Mosyakin and Clemants Flowers. *Toxins* **2022**, *14*, 475. [CrossRef]
22. Elbouzidi, A.; Taibi, M.; Ouassou, H.; Ouahhoud, S.; Ou-Yahia, D.; Loukili, E.H.; Aherkou, M.; Mansouri, F.; Bencheikh, N.; Laaraj, S.; et al. Exploring the Multi-Faceted Potential of Carob (*Ceratonia siliqua* Var. Rahma) Leaves from Morocco: A Comprehensive Analysis of Polyphenols Profile, Antimicrobial Activity, Cytotoxicity against Breast Cancer Cell Lines, and Genotoxicity. *Pharmaceuticals* **2023**, *16*, 840. [CrossRef]
23. Banerjee, P.; Eckert, A.O.; Schrey, A.K.; Preissner, R. ProTox-II: A Webserver for the Prediction of Toxicity of Chemicals. *Nucleic Acids Res.* **2018**, *46*, W257–W263. [CrossRef]
24. Kandsi, F.; Elbouzidi, A.; Lafdil, F.Z.; Meskali, N.; Azghar, A.; Addi, M.; Hano, C.; Maleb, A.; Gseyra, N. Antibacterial and Antioxidant Activity of *Dysphania ambrosioides* (L.) Mosyakin and Clemants Essential Oils: Experimental and Computational Approaches. *Antibiotics* **2022**, *11*, 482. [CrossRef]
25. Zrouri, H.; Elbouzidi, A.; Bouhrim, M.; Bencheikh, N.; Kharchoufa, L.; Ouahhoud, S.; Ouassou, H.; El Assri, S.; Choukri, M. Phytochemical Analysis, Antioxidant Activity, and Nephroprotective Effect of the Raphanus Sativus Aqueous Extract. *Mediterr. J. Chem.* **2021**, *11*, 84. [CrossRef]
26. Elbouzidi, A.; Ouassou, H.; Aherkou, M.; Kharchoufa, L.; Meskali, N.; Baraich, A.; Mechchate, H.; Bouhrim, M.; Idir, A.; Hano, C.; et al. LC–MS/MS Phytochemical Profiling, Antioxidant Activity, and Cytotoxicity of the Ethanolic Extract of *Atriplex halimus* L. against Breast Cancer Cell Lines: Computational Studies and Experimental Validation. *Pharmaceuticals* **2022**, *15*, 1156. [CrossRef]
27. Alderman, D.; Smith, P. Development of Draft Protocols of Standard Reference Methods for Antimicrobial Agent Susceptibility Testing of Bacteria Associated with Fish Diseases. *Aquaculture* **2001**, *196*, 211–243. [CrossRef]
28. Remmal, A.; Bouchikhi, T.; Rhayour, K.; Ettayebi, M.; Tantaoui-Elaraki, A. Improved Method for the Determination of Antimicrobial Activity of Essential Oils in Agar Medium. *J. Essent. Oil Res.* **1993**, *5*, 179–184. [CrossRef]
29. Lekbach, Y.; Xu, D.; El Abed, S.; Dong, Y.; Liu, D.; Khan, M.S.; Ibnsouda Koraichi, S.; Yang, K. Mitigation of Microbiologically Influenced Corrosion of 304L Stainless Steel in the Presence of Pseudomonas Aeruginosa by Cistus Ladanifer Leaves Extract. *Int. Biodeterior. Biodegrad.* **2018**, *133*, 159–169. [CrossRef]
30. Lalitha, M. Manual on Antimicrobial Susceptibility Testing. *Perform. Stand. Antimicrob. Test. Twelfth Informational Suppl.* **2004**, *56238*, 454–456.
31. Leghari, S.J.; Wahocho, N.A.; Laghari, G.M.; HafeezLaghari, A.; MustafaBhabhan, G.; HussainTalpur, K.; Bhutto, T.A.; Wahocho, S.A.; Lashari, A.A. Role of Nitrogen for Plant Growth and Development: A Review. *Adv. Environ. Biol.* **2016**, *10*, 209–219.
32. Bolan, N. A Critical Review on the Role of Mycorrhizal Fungi in the Uptake of Phosphorus by Plants. *Plant Soil* **1991**, *134*, 189–207. [CrossRef]
33. Xu, J.; Zhang, J.; Xie, H.; Li, C.; Bao, N.; Zhang, C.; Shi, Q. Physiological Responses of Phragmites Australis to Wastewater with Different Chemical Oxygen Demands. *Ecol. Eng.* **2010**, *36*, 1341–1347. [CrossRef]
34. Blokhina, O. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: A Review. *Ann. Bot.* **2003**, *91*, 179–194. [CrossRef] [PubMed]
35. Urbano, V.R.; Mendonça, T.G.; Bastos, R.G.; Souza, C.F. Effects of Treated Wastewater Irrigation on Soil Properties and Lettuce Yield. *Agric. Water Manag.* **2017**, *181*, 108–115. [CrossRef]
36. Baser, K.H.C.; Buchbauer, G. *Handbook of Essential Oils: Science, Technology, and Applications*; CRC Press: Boca Raton, FL, USA, 2009.
37. Scavroni, J.; Boaro, C.S.F.; Marques, M.O.M.; Ferreira, L.C. Yield and Composition of the Essential Oil of *Mentha piperita* L. (Lamiaceae) Grown with Biosolid. *Braz. J. Plant Physiol.* **2005**, *17*, 345–352. [CrossRef]
38. De Sousa, A.A.S.; Soares, P.M.G.; de Almeida, A.N.S.; Maia, A.R.; de Souza, E.P.; Assreuy, A.M.S. Antispasmodic Effect of *Mentha piperita* Essential Oil on Tracheal Smooth Muscle of Rats. *J. Ethnopharmacol.* **2010**, *130*, 433–436. [CrossRef] [PubMed]
39. Mahboubi, M.; Kazempour, N. Chemical Composition and Antimicrobial Activity of Peppermint (*Mentha piperita* L.) Essential Oil. *Songklanakarin J. Sci. Technol.* **2014**, *36*, 83–87.
40. Delaquis, P. Antimicrobial Activity of Individual and Mixed Fractions of Dill, Cilantro, Coriander and Eucalyptus Essential Oils. *Int. J. Food Microbiol.* **2002**, *74*, 101–109. [CrossRef]
41. Yayi-Ladekan, E.; Kpoviessi, D.; Gbaguidi, F.; Kpadonou-Kpoviessi, B.; Gbenou, J.; Jolivalt, C.; Moudachirou, M.; Accrombessi, G.; Quetin-Leclercq, J. Variation diurne de la composition chimique et influence sur les propriétés antimicrobiennes de l'huile essentielle de Ocimum canum Sims cultivé au Bénin. *Int. J. Bio Chem. Sci.* **2012**, *5*, 1462–1475. [CrossRef]
42. Amorati, R.; Foti, M.C.; Valgimigli, L. Antioxidant Activity of Essential Oils. *J. Agric. Food Chem.* **2013**, *61*, 10835–10847. [CrossRef]
43. Sun, Z.; Wang, H.; Wang, J.; Zhou, L.; Yang, P. Chemical Composition and Anti-Inflammatory, Cytotoxic and Antioxidant Activities of Essential Oil from Leaves of *Mentha piperita* Grown in China. *PLoS ONE* **2014**, *9*, e114767. [CrossRef]

44. Taibi, M.; Elbouzidi, A.; Ou-Yahia, D.; Dalli, M.; Bellaouchi, R.; Tikent, A.; Roubi, M.; Gseyra, N.; Asehraou, A.; Hano, C.; et al. Assessment of the Antioxidant and Antimicrobial Potential of Ptychotis Verticillata Duby Essential Oil from Eastern Morocco: An In Vitro and In Silico Analysis. *Antibiotics* **2023**, *12*, 655. [CrossRef]
45. Satmi, F.R.S. In Vitro Antimicrobial Potential of Crude Extracts and Chemical Compositions of Essential Oils of Leaves of *Mentha piperita* L. Native to the Sultanate of Oman. *Pac. Sci. Rev. A Nat. Sci. Eng.* **2016**, *18*, 103–106. [CrossRef]
46. Lim, H.-W. Antimicrobial Effect of *Mentha piperita* (Peppermint) Oil against *Bacillus cereus*, *Staphylococcus aureus*, *Cronobacter sakazakii*, and *Salmonella enteritidis* in Various Dairy Foods: Preliminary Study. *J. Milk Sci. Biotechnol.* **2018**, *36*, 146–154. [CrossRef]
47. Vagionas, K.; Graikou, K.; Chinou, I.B.; Runyoro, D.; Ngassapa, O. Chemical Analysis and Antimicrobial Activity of Essential Oils from the Aromatic Plants *Artemisia afra* Jacq. and *Leonotis ocymifolia* (Burm. F.) *Lwarsson* Var. *Raineriana (Vision1) Lwarsson Growing In Tanzania. J. Essent. Oil Res.* **2007**, *19*, 396–400. [CrossRef]
48. Gershenzon, J.; Dudareva, N. The Function of Terpene Natural Products in the Natural World. *Nat. Chem. Biol.* **2007**, *3*, 408–414. [CrossRef]
49. Hassanpouraghdam, M.; Gohari, G.; Tabatabaei, S.; Dadpour, M.; Shirdel, M. NaCl Salinity and Zn Foliar Application Influence Essential Oil Composition of Basil (*Ocimum basilicum* L.). *Acta Agric. Slov.* **2011**, *97*, 93–98. [CrossRef]
50. Ambrosio, C. Unraveling the Selective Antibacterial Activity and Chemical Composition of Citrus Essential Oils. *Sci. Rep.* **2019**, *9*, 17719. [CrossRef] [PubMed]
51. Elkhair, E.A. Antidermatophytic Activity of Essential Oils against Locally Isolated Microsporum Canis—Gaza Strip. *Nat. Sci.* **2014**, *6*, 676–684. [CrossRef]
52. Freire, M.M. Composition, Antifungal Activity and Main Fungitoxic Components of the Essential Oil of *Mentha piperita* L. *J. Food Saf.* **2011**, *32*, 29–36. [CrossRef]
53. Parveen, M. Response of Saccharomyces Cerevisiae to a Monoterpene: Evaluation of Antifungal Potential by DNA Microarray Analysis. *J. Antimicrob. Chemother.* **2004**, *54*, 46–55. [CrossRef]
54. Slimani, I.; Nassiri, L.; Boukil, A.; Bouiamrine, E.H.; Bachiri, L.; Bammou, M.; Ibijbijen, J. Inventaire Des Plantes Aromatiques et Médicinales Du Site d'intérêt Biologique et Écologique de Jbel Zerhoun, Région Meknès Tafilalet. *Afr. Sci.* **2016**, *12*, 393–409.
55. Giordani, R.; Kaloustian, J. Action Anticandidosique Des Huiles Essentielles: Leur Utilisation Concomitante Avec Des M ├® Dicaments Antifongiques. *Phytoth Rapie* **2006**, *4*, 121–124. [CrossRef]