Preprints.org

Article

Solvent Polarity Effects on Phenolic Fingerprint and Biological Activities, Using Three Different Extractions Formulation for Examining of *Mentha aquatica* Leaf Extracts: An In Vitro and In-Silico Investigation

Meryem TOURABI [*] , AMIRA Metouekel , Mohammed Jeddi , Mohamed Chebaibi , Nesrine Benkhaira , Kawtar Fikri Benbrahim , Badiaa Lyoussi , Elhoussine Derwich

Article

Solvent Polarity Effects on Phenolic Fingerprint and Biological Activities, Using Three Different Extractions Formulation for Examining of *Mentha aquatica* Leaf Extracts: An *In Vitro* and *In-Silico* Investigation

Meryem Tourabi [1,*], Amira Metouekel [2], Mohamed Jeddi [3], Mohamed Chebaibi [4,5], Nesrine Benkhaira [3], Kawtar Fikri Benbrahim [3], Badiaa Lyoussi [1] and Elhoussine Derwich [1,6]

[1] Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health & Quality of Life, Faculty of Sciences, Sidi Mohamed Ben Abdellah University, Fez, Morocco; lyoussi@gmail.com (B.L.); elhoussinederwich@yahoo.fr (E.D.)

[2] Euromed Research Center, Euromed Faculty of Pharmacy, Euromed University of Fes (UEMF) Route de Meknes, 30000 Fez, Morocco; metouekel.amira@gmail.com

[3] Laboratory of microbial biotechnology and bioactive molecules, science and technology faculty Sidi Mohamed ben Abdellah University, Imouzzer, Road, Fez, Morocco; mohamed.jeddi@usmba.ac.ma (M.J.); nesrine.benkhaira@usmba.ac.ma (N.B.); kawtar.fikribenbrahim@usmba.ac.ma (K.F.B.)

[4] Ministry of Health and Social Protection, Higher Institute of Nursing Professions and Health Techniques, Fez, Morocco; mohamed.chebaibi@usmba.ac.ma

[5] Biomedical and Translational Research Laboratory, Faculty of Medicine and Pharmacy of Fez, Sidi Mohamed Ben Abdellah University, Fez 30000, Morocco

[6] Unity of GC/MS and GC-FID, City of Innovation, Sidi Mohamed Ben Abdellah University, Fez, Morocco

* Correspondence: meryem.tourabi@usmba.ac.ma

Abstract: The main purpose of our research is the evaluation of the efficacity of solvent polarity on phenolic composition, as well as the antibacterial, and antioxidant activities of extract of Moroccan *Mentha aquatica* L., leaves. To attempt these objectives, the HPLC-DAD was used to identify the biomolecules. The total amount of phenolic and flavonoid content was determined using the Folin-Ciocalteu and aluminum trichloride processes as well as the DPPH, RP, and TAC tests were used to evaluate the antioxidant capabilities. In addition, by employing both *in vitro* and *in silico* analysis through the microdilution method, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were assessed against multidrug-resistant bacteria. Furthermore, the High-Performance Liquid Chromatography (HPLC) analysis indicated that all extracts of *M. aquatica* were abundant in noteworthy phytochemical compounds, especially gallic acid, vanillic acid, chlorobenzoic acid, quercetin, and kaempferol. Furthermore, the results indicated that the total phenolic content was higher in hydroethanolic extract (62.19 ± 1.19 mg GAE/g DW) in contrast to the acetonic and aqueous extracts (22.22 ± 0.64, and 22.44 ± 0.57 mg GAE/g DW) respectively. Similarly, the aqueous ethanol extract contained the greatest flavonoid concentration, with values of 29.15 ± 0.09 mg QE/g DW when compared with the other extracts. The seam extract poses excellent antioxidant power with an IC_{50} of 0.06 ± 0.00 mg/mL for the DPPH test, and lower RP with EC_{50} of 80 ± 0.00 µg/ml when compared with acetonic and aqueous extract (370 ± 0.00 and 460 ± 0.01 µg/mL) respectively. Moreover, the hydroethanolic extract has a high level of TAC 75.09 ± 2.00 mg EAA/g DW, followed by aqueous extract carrying a value of 28.53 ± 1.36 mg EAA/g DW. The antibacterial capacity is ranged between 0.78 ± 0.05 mg/mL and 12.60 ± 0.00 mg/L. The results of the *in-silico* study demonstrated that quercetin, vanillic acid, and naringenin components contribute to the antioxidant capability. While gallic acid, kaempferol, and quercetin components are attributed to the antibacterial capacity.

Keywords: *Mentha aquatica*; HPLC-DAD analysis; *in-silico* approach; solvent polarity; antioxidant activity; antibacterial activity.

1. Introduction

Mentha is a distinguished genus of the Lamiaceae family. This genus comprised between 25 and 30 reported species of mint growing almost everywhere in the world [1]. It's well recognized for its medicinal properties, owing to its widespread usage as an antimicrobial, biocide, anti-inflammatory, antiulcer, antiemetic, antidiabetic, antispasmodic, tonic, anti-allergic, and antihypertensive [2]. Likewise, the reported pharmacological and therapeutic benefits have been associated with distinct bioactive phytochemical components, including flavonoids, phenolic acid, carotenoids, tannins, fatty acids, saponins, terpenoids, and volatile compounds[3].

Mentha aquatica L., commonly known as water mint, is an annual herbaceous plant that is a member of the Lamiaceae family. It is distributed extensively across all continents (except in South America and Antarctica). In folk medicine traditions of several nations, is used to treat various diseases, such as respiratory problems cough, and ulcerative colitis [4]. It is taken as a tonic, used as a stimulant, digestive stomachic, carminative, relaxing, antispasmodic, sedative, and analgesic [5]. Solid research has proven some of *M. aquatica*'s pharmacological characteristics, most notably insecticidal [6], antihemolytic [7], anti-inflammatory [3] antimicrobial [8], hepatoprotective [9], anticancer [10], gastroprotective effect [11], and antiemetic [5].

The extraction solvent is one of the key parameters influencing the effectiveness of extracting bioactive molecules from plant materials and their associated beneficial effects on health. Selecting the optimal solvent could maximize the rate of phytochemicals and antioxidants extracted [12]. For phytochemical extraction, acetone, water, ethanol, and a combination of these organic solvents with water are frequently utilized [13]. The solubility and bioactivities of chemical compounds are additionally suggested by the increasing solvent polarity from hexane to distilled water (hexane <chloroform<acetone < ethyl acetate < ethanol < distilled water).

Several prior reports have extracted bioactive chemicals from *Mentha* genus species using methanol, ethanol, chloroform, acetone, and water as the solvents[10,11]. To the best of our knowledge, no previous studies have been exploring how varying solvent polarities affect the bioactive properties and extraction efficiency of antioxidant compounds from the leaves of Moroccan *M. aquatica*. The present study attempts to explore the efficacity of solvent polarities (acetone, ethanol, and distilled water) on the extraction of bioactive molecules, antioxidant potential, and antibacterial activity of *M. aquatica* leaves, in order to determine which solvent is best for extracting more bioactive composites and distinguishing *M. aquatica's* antioxidant and antibacterial capability.

2. Results and Discussion

2.1. Preliminary Solvent Secerning

Selecting an appropriate solvent is the pivotal step in phytochemical research, especially when isolating phenolic compounds and other beneficial constituents from vegetables, fruits, and plants [15]. Overall, the efficiency of phenolic component extraction is affected by various factors, including time of extraction, solvent polarity, and temperature. The results of the preliminary screening solvent extracts for the extraction of total phenolic content and antioxidant ability are displayed in (Figure 1 A, and B), and demonstrated that all three solvents (hexane, chloroform, and ethyl acetate) exhibited little effect on antioxidant activity, reducing power, as well as total antioxidant capacity. The seam three solvent showed a lower level of flavonoids and phenolic amounts. Additionally, the amount of phenolic and flavonoid content ranged between 2.56 ± 0.43 to 62.198±1.65 mg GAE/g DW, and 1.57± 0.20 to 29.157± 0.07 mg QE/g DW respectively. It must be stated that the ETOH 70% extract boosted to extract a great level of TPC and TFC. The results of the preliminary testing indicate that the potent antioxidant power and the high phenolic and flavonoid concentrations of *M. aquatica* were considerably related to the solvent polarity which was confirmed by Tourabi et al [15]. Based on the preliminary screening results, we chose the stronger extracts (aqueous, acetonic, and ETOH 70%) to test for phenolic composition and antibacterial activity. The next part of this work will present and discuss the outcomes of this preliminary screening.
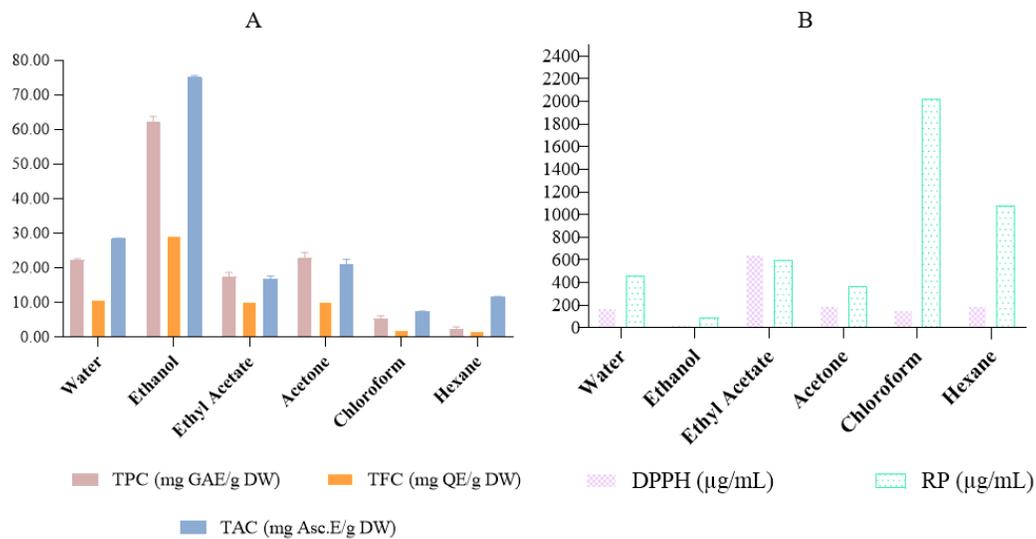
Figure 1. (A): Total phenolic amount (TPC) and flavonoid content (TFC), total antioxidant capacity (TAC), (B): EC_{50} of Reducing power (RP), and IC_{50} of DPPH scavenging activity of different secerned solvents.

2.2. Solvent Polarity Effect on the Extraction Yield

The outcomes of the solvent extraction, including acetone, ETOH 70 %, and distilled water were displayed in (Figure 2) and indicated that ETOH 70% had the high extraction yield followed by aqueous extract with a rate of 12 %, however, the acetonic extract showed the lower amount of extraction yield with a value of 9%.

Research findings suggest a positive relationship between the polarity of solvents and their effectiveness in extracting compounds from plant materials [16]. In addition, the solvent's polarity significantly influences the amount of extract obtained it was found that the extract yield was higher in polar solvents (water and aqueous ethanol) than in nonpolar solvents (acetone).
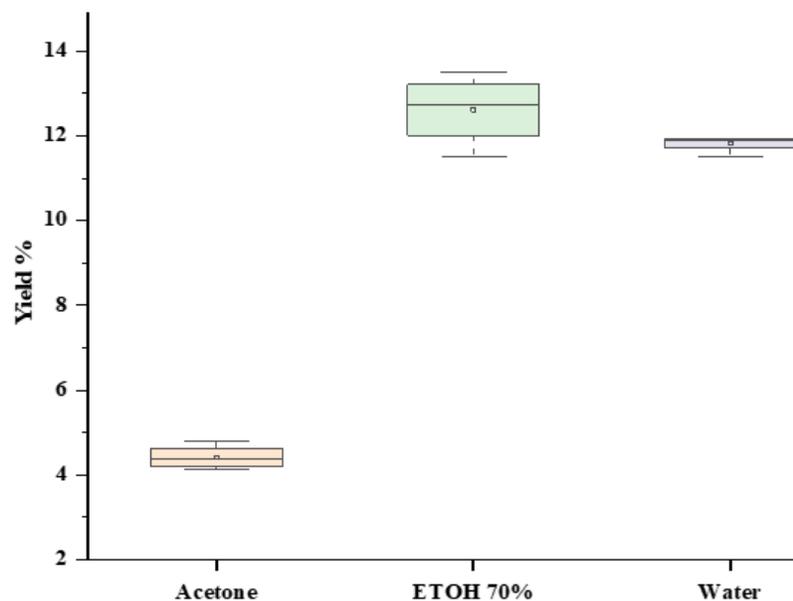

Figure 2. Effect of solvent polarity on the extraction yield of studied solvent extracts of *M. aquatica*. Means values ± SD (n = 3).

2.3. HPLC–DAD expLoration of Individual Phenolic Composition

The findings of the High-performance Liquid Chromatography are displayed in Table 1 for the three solvent extracts (water, ETOH 70%, and acetone). In total, six compounds were discovered in our samples; four phenolic acids (gallic acid, vanillic acid, and chlorobenzoic acid) as well as three flavonoids (quercetin, naringenin, and kaempferol). The acetonic extract is characterized by a low amount of phenolic compounds. Regarding aqueous ethanol extract, four phenolic components; two flavonoids and two phenolic acids were detected. The great concentration is observed for chlorobenzoic acid at 5.61 mg/g DW, followed by naringenin at 2.45 mg/g DW, while kaempferol and gallic acid were detected in low concentrations (Figure 3b). Thus, the aqueous extract comprised four phenolic compounds, which include gallic acid at 1.10 mg/g DW, followed by vanillic acid at a concentration of 1.43 mg/g DW while the flavonoid compounds were also detected in low concentrations (Figure 3 a). The ethanol 70% enhances the extraction of phenolic and flavonoid components of *M. aquatica*. This could result from the synergistic effects of binary solvent extraction (ethanol/water) on the extraction of flavonoids and phenol components. Our findings support those of Teodor et al [17] and Park et al[3], who revealed that the most recognized components of *M. aquatica* are gallic, vanillic acid, kaempferol, naringenin, and quercetin, which are responsible for their biological properties.

Our data highlights that the different solvents can extract diverse chemicals from the same source material due to their particular chemical characteristics such as polarity, solubility, and affinity for specific components.
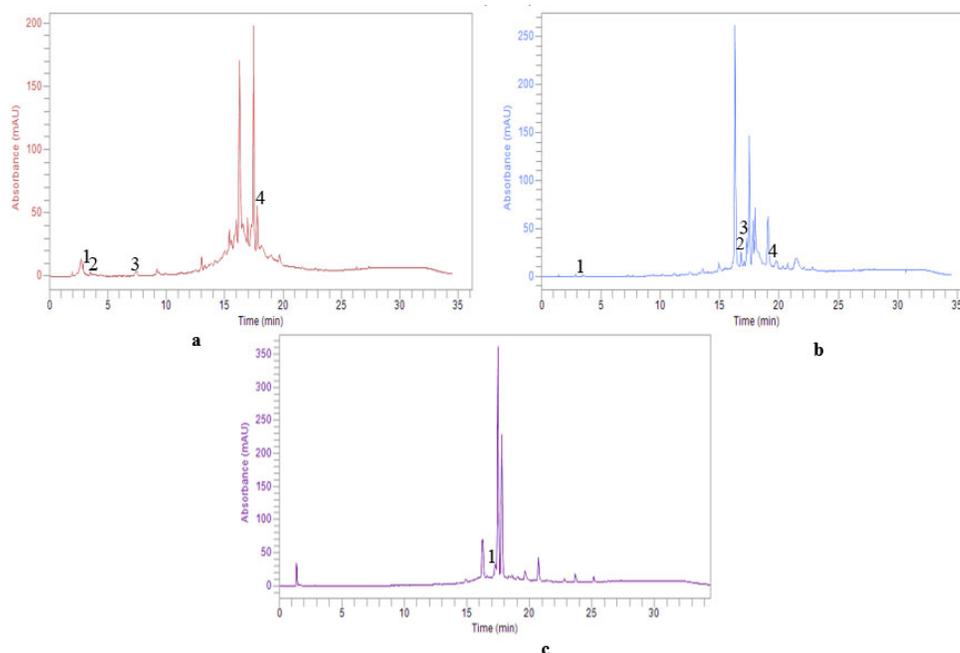
Figure 3. Chromatograms of different solvent extracts of *M. aquatica* using HPLC-DA analysis. (a): aqueous extract; (b) ethanolic extract; (c) acetone extract.

Table 1. HPLC-DAD screening for individual phenolic molecules identified in the solvent extract of *M. aquatica*.

			Area %		
Standards	**MF**	**RT**	**Water**	**EtOH 70%**	**Acetone**
Gallic acid	$C_7H_6O_5$	2.89	1.10	0.22	nd
Vanillic acid	$C_8H_8O_4$	7.428	1.43	nd	nd
Ferulic acid	$C_{10}H_{10}O_4$	12.236	nd	nd	nd

Chlorobenzoic acid	$C_7H_5ClO_2$	16.799	nd	5.61	0.43
Quercetin	$C_{15}H_{10}O_7$	3.307	0.39	nd	nd
Naringenin	$C_{15}H_{12}O_5$	17.130	nd	2.45	nd
Kaempferol	$C_{15}H_{10}O_6$	18.132	0.39	0.9	nd

MF: molecular formula; RT: retention time (min); nd: non-detected.

2.4. Total Phenolic and Flavonoid Content

Phenolic compounds are among the most widespread and frequent groups of plant metabolites including alkaloids, terpenoids, and phenolic compounds, of which (phenolic acids, coumarins, simple and condensed tannins, and flavonoids) are widely used for therapeutic and dietary applications [18]. In addition, the phenolic compound may exhibit biologically pertinent activities including, antioxidant, antimicrobial, anti-inflammatory anti-infectious, and antiproliferative activities [19]. The TPC and TFC of samples are displayed in (Figure 3 A, B). The aqueous ethanol extract had a high level of TPC with a rate of 62.19±1.19 mg GAE/g DW, while the aqueous and acetonic extracts come next with a value of 22.44 ± 0.57 and 22.22 ± 0.64 mg GAE/g DW respectively (Figure 4 A). Considering the outcome of this study, suggests that polar solvents maximize the extraction of phenolic compounds more than non-polar solvents. Our findings are higher than those signaled by Abbas and colleagues [20]. In contrast, Dorman et al found that the aqueous extract had a high level of phenolic content with a rate of 152.5 ± 0.00 mg GAE/g DW [21]. Other research reported by Hoai et al, found that the acetone 50% extract illustrates a high amount of TPC with a rate of 120.92 mg GAE/g DW [22].

Concerning total flavonoid rate, the outcome is presented in (Figure 4B) and indicates that the aqueous ethanol extract (ETOH 70%) extracts a maximum of flavonoid content with a rate of 29.15± 0.09 mg QE/g DW, while the aqueous and acetonic extracts respectively with a rate of (10.93± 0.62 and 10.17± 0.07 mg QE/g DW). These values were in arrangement with those discovered by Tourabi et al [15] and Abbas et al [20].
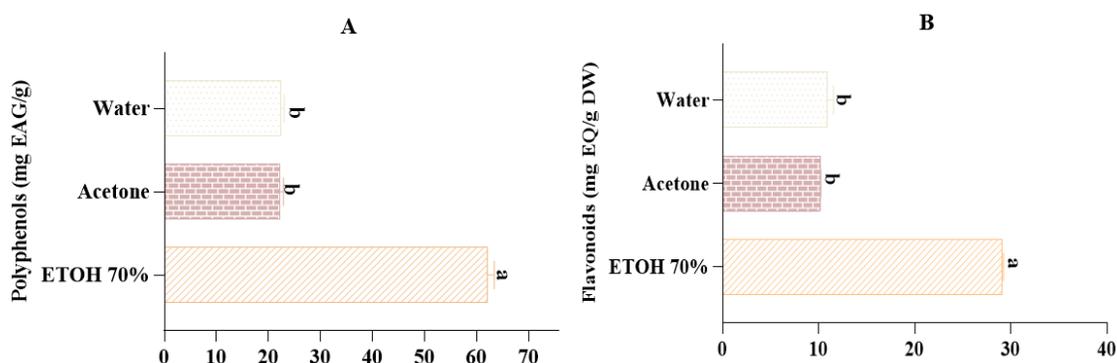

Figure 4. Total phenolic amount (A), and Total flavonoid content of selected solvent extract of *M. aquatica.* There is no statistically significant difference between the same letter findings in the same test (p > 0.05). Means values ± SD (n = 3).

2.5. Antioxidant Activity

Three distinct techniques were used to assess the antioxidant effects, including the DPPH test, reducing power test, and total antioxidant capacity, and results are displayed in Figures 5A, B, and C. Our results showed significant variability between the different extracts, which the aqueous ethanol exhibited a potent antiradical activity and reducing power with the stronger $IC_{50}= 0.06±0.19$ mg/mL for radical DPPH and the heigh $EC_{50} = 0.08±0.01$ mg/mL for RP test, followed by acetonic extract with $IC_{50}= 0.19$ and $EC_{50}= 0.37 ±0.02$ (Figure 5 A and B). Similarly, the aqueous ethanol extract showed a high total antioxidant capacity of 75.09 ± 2.00 mg AAE/g DW, following the aqueous extract with a rate of 28.53 ± 1.00 mg AAE/g DW, however, the acetonic extract exhibited a less total

antioxidant capacity of 21.11± 0.13 mg AAE/g DW (Figure 5 C). This outcome is corroborated with those indicated by Conforti et al who reported that the hydroethanolic extract has a high IC_{50} value of the scavenging activity of 29 μg/mL [23], greater compared to the cited by Thi et al [24] which found that the ethanolic and aqueous extract exhibited the lower antioxidant ability with IC_{50} value of   306.97 ± 13.78 and 142.98 ± 19.93 μg/mL respectively. The obtained result in this work suggests that the high levels of total phenolic and flavonoid content are observed in the aqueous ethanol extract which strongly contributed to their potent antioxidant properties. The result of the ANOVA test indicates that a statistically significant difference ($p < 0.05$) was noted among the three extraction solvents for the *M. aquatica* leaf. Our results agree with those of other studies, which stated that the solvents with a superior polarity namely water, ethanol, methanol, and acetone are mostly used to maximize the extraction of antioxidant components [15,25]. Indeed, the variability in antioxidant potential is achieved by *in vitro* synergism between phytochemical components. Hajimehdithepoor and coworkers reported the synergetic antioxidant capability of the mixture of quercetin, gallic acid, caffeic acid, and the combination of quercetin, gallic acid, and rutin using FRAP assay [26]. Additionally, the tested extract showed powerful antioxidant capacity that may be associated with their antioxidant constituents notably phenolic acids, flavonoids, and terpenoids, especially any potential interactions among them
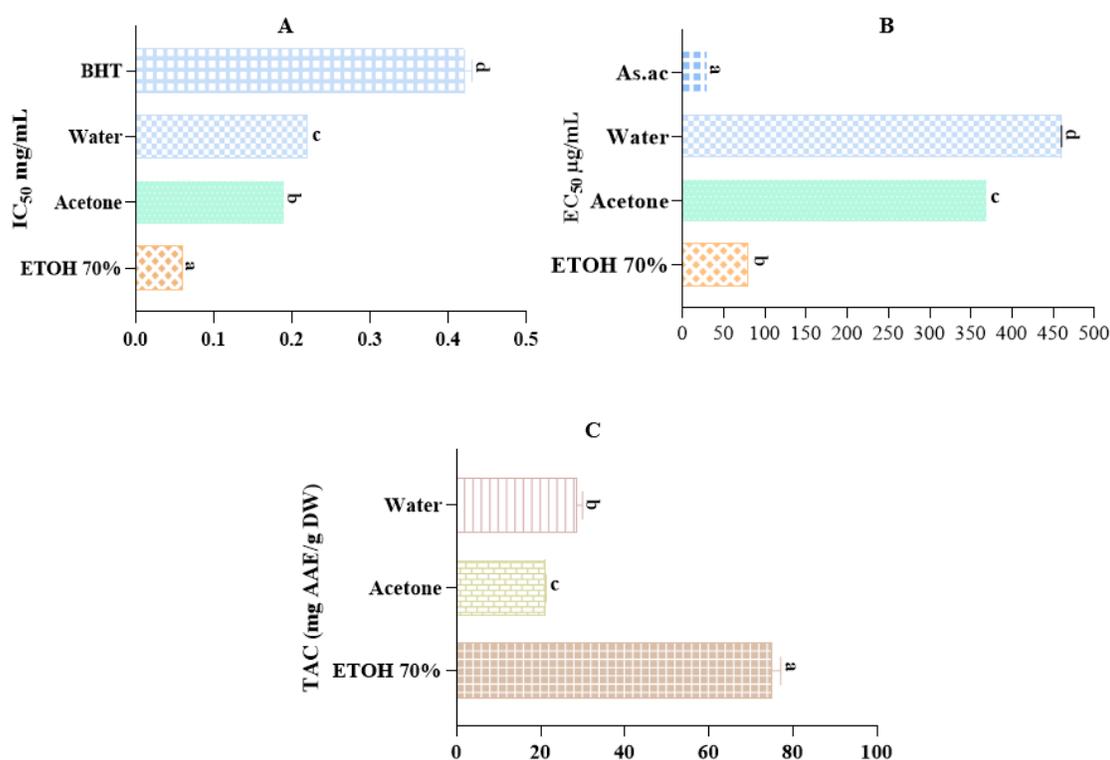

Figure 5. Antioxidant activities of solvent extract of *M. aquatica* (A): IC_{50} of DPPH assay, (B): EC_{50} of reducing power, and (C): Total antioxidant capacity. There is no statistically significant difference between the same letter results in the same test ($p>0.05$). Means values ± SD (n = 3); BHT: Butylated hydroxytoluene; As.ac: Ascorbic acid.

2.6. Assessment of the Antibacterial Capacity

The results presented in Table 2 highlight the potent antibacterial properties of the solvent extract derived from *M. aquatica* across a spectrum of bacterial strains including *E. coli, P. aeruginosa, B. cereus,* and *S. aureus*. Especially, the acetonic extract exhibited high inhibitory concentration against *S. aureus* with MIC= 0.78±0.05 mg/mL and MBC= 1.56±0.33 mg/mL and against *B. cereus* bacteria with MIC=1.56±0.11 mg/mL and MBC=0.78±0.25 mg/mL. Likewise, acetone extract also has great activity

against *E. coli* with MIC=4.68±0.0 mg/mL. Furthermore, aqueous ethanol exhibits potent inhibitory activity against *B. cereus* with a MIC=1.78±0.55 mg/mL. It is important to note that the acetonic and ethanolic extracts had a more potent antibacterial efficiency stronger so as for fighting gram-positive bacteria than gram-negative ones. In the same context, Tourabi et al [15] stated that the acetonic extract of *M. longifolia* expressed the ultimate antibacterial activity, especially against *S. aureus* and *B. cereus*. Ferhat et al [27], examined the antibacterial capacity of chloroform and methanolic extract of *M. aquatica* aerial part against several multi-resistance bacteria, and The findings indicated that the methanolic extract exhibited a potent antibacterial ability with a MIC value of 128 ± 1.30 μg/mL.

The examined extracts except aqueous ethanol showed that the ratio of MBC to MIC (MBC/MIC) was less than 4, indicating an effective bactericidal effect on the strains of *B. cereus* and *S. aureus*. This proposal is based on the observation that compounds with an MBC/MIC ratio of 4 or less are likely to induce bactericidal action, while those with a ratio greater than 4 are more likely to exert bacteriostatic effect [28].

According to the results of our experimental work, Gram-positive bacteria responded to our extracts more readily than Gram-negative strains. The complex architecture of the double membrane found in gram-negative microbes which consists of a lipoprotein and a lipopolysaccharide membrane (LPS) that is a component of the bacterial cell envelope, may be the reason for their reduced sensitivity [29]. Furthermore, the powerful antibacterial capacity of *M. aquatica* leaf extracts is explained by their rich in natural bioactive molecules, namely phenolic acids such as gallic acid, vanillic acid, and chlorobenzoic acid, as well as flavonoids composites namely quercetin, naringenin, and kaempferol (Table 1). Indeed, prior research revealed higher antibacterial efficiency of gallic acid, vanillic acid, quercetin, kaempferol, and naringenin [30–32]. This activity corresponds to their capacity to penetrate the bacterial cytoplasm and transit through the bacterial wall of strains [33].

As previously mentioned, phenolic molecules have been successfully isolated from botanical matter using a range of solvent mixtures. The most frequently utilized solvents are methanol, ethanol, water, and acetone, as well as their aqueous combinations [34]. While acetone dissolves both hydrophilic and lipophilic components, it may be used to extract antimicrobial substances, and this is responsible for the considerable antibacterial influence shown in our experiment [35]. In the seam context, acetonic and aqueous ethanol extracts were the great extractor of molecules with a range spectrum of biological properties notably hydroxycinnamic acid components such as caffeic acid, sinapinic acid, ferulic acid, vanillic acid as well as hydroxybenzoic acids which include gallic acid and chlorobenzoic acid and flavonoids compounds [36]. These molecules have been confirmed to exhibit the strongest antibacterial ability against multiresistant bacteria via several mechanisms of action, notably focusing on bacterial compounds called metabolites walls of cells, membrane destabilization, lipid membranes, receptors in membranes, ion channels, enzyme inhibition, inhibition of ATP production and the development of biofilms. Some combinations of polyphenols and antibiotics have also been shown to have synergistic effects [33]. Campos et al demonstrated that gallic acid alters the hydrophilic nature of pathogens (how well they interact with the surface of both Gram-positive and Gram-negative bacteria), which may lead to an acidification of their cytoplasm through an increase in K+ release and the induction of protein denaturation. These effects can affect the flexibility of the cytoplasmic membrane, trigger the ejection of intracellular materials, and destruction of membrane cells [37]. According to other experiments, the flavonols kaempferol and quercetin have shown strong antibacterial activity against *S. aureus*, with reported minimum inhibitory concentrations that are as low as 7.8 μg/mL and 1.95 μg/mL, respectively [38,39]. Naringenin has displayed antibacterial efficacy against many strains of *S. aureus* with low levels of inhibition that vary from 256 to 512 μg/mL [40].

Table 2. Lowest inhibitory (MIC) and bactericidal concentrations (MBC)   of various solvent extracts of different solvent extracts of *M. aquatica* (mg/mL).

Bacterial strains	EtOH 70%				Acetone				Water			
	MIC	MBC	MBC/MIC	Effect	MIC	MBC	MBC/MIC	Effect	MIC	MBC	MBC/MIC	Effect
Gram-negative Bacteria												
P. aeruginosa	6.25±0.00	ND	-	-	6.25±0.00	ND	–	–	6.37±0.16	ND	–	–
E. coli	6.25±0.07	ND	-	-	4.68±0.0	ND	–	–	12.60±0.00	ND	–	–
Gram-positive Bacteria												
S. aureus	6.18±0.08	50±2.03	8.08±0.43	Bacteriostatic	1.56±0.11	1.56±0.33	1	Bactericidal	6.25±0.10	6.25±1	1±0.0	Bactericidal
B. cereus	1.78±0.55	50±1.07	28.07	Bacteriostatic	0.78±0.05	0.78±0.25	1	Bactericidal	3.12±0.06	25±0.0	8.08 ±0.18	Bacteriostatic

ND: non-determined; MBC: Minimal bactericide concentration; MIC: Minimal inhibitory concentration.

2.7. Molecular Docking Assessment

NADPH oxidase plays a pivotal role in producing reactive oxygen species (ROS), with its activity being tightly regulated within cells. Although ROS generated by NADPH oxidase is essential for pathogen defense and cellular signaling, an overproduction can result in oxidative stress, contributing to various diseases. Inhibiting NADPH oxidase represents a promising strategy for enhancing antioxidant activity and protecting against diseases related to oxidative stress. By reducing ROS production, enhancing cellular antioxidant defenses, and decreasing chronic inflammation, NADPH oxidase inhibitors may be crucial in maintaining cellular health and preventing disease progression.

In our *in-silico* study, quercetin, vanillic acid, and chlorobenzoic contained the most active compounds against NADPH oxidase with glide scores of acids -6.587, -6.120, and -6.084 Kcal/mol (Table 2).

In antibacterial activity, gallic acid, ferulic acid, and chlorobenzoic acid were the most active against beta-ketoacyl- [acyl carrier protein] synthase from *E. coli* with a glide score of -7.240, -6.558, and -6.496 Kcal/mol. Furthermore, chlorobenzoic acid, vanillic acid, and ferulic were almost active against *staphylococcus aureus* nucleoside diphosphate kinase with glide scores of -8.448, -8.010, and -7.933 kcal/mol (Table 2).

Table 3. Docking outcomes for multiple receptors ligands.

	Glide score (kcal/mol)		
	Antioxidant activity	Antibacterial activity	
	2CDU	1FJ4	3Q8U
Chlorobenzoic acid	-6.084	-6.496	-8.448
Ferulic acid	-5.401	-6.558	-7.933
Gallic acid	-4.978	-7.24	-5.339
Kaempferol	-5.543	-5.959	-8.984
Naringenin	-6.181	-6.336	-8.209
Quercetin	-6.587	-5.929	-8.991
Vanillic acid	-6.12	-6.217	-8.01

Around the NADPH oxidase active location, quercetin has created two hydrogen bonds with the residues VAL 214 and ASP 179 and a single Pi cation bond with residue LYS 213.

However, gallic acid established three Pi-Pi stacking bonds with residues PHE 392 and HIE 298 in the active site of beta-ketoacyl - [acyl carrier protein] synthase from *Escherichia coli*. During its interaction in the active site of *Staphylococcus aureus* nucleoside diphosphate kinase, chlorobenzoic acid developed a salt bridge with the MG 159 residue (Figures 6 and 7).
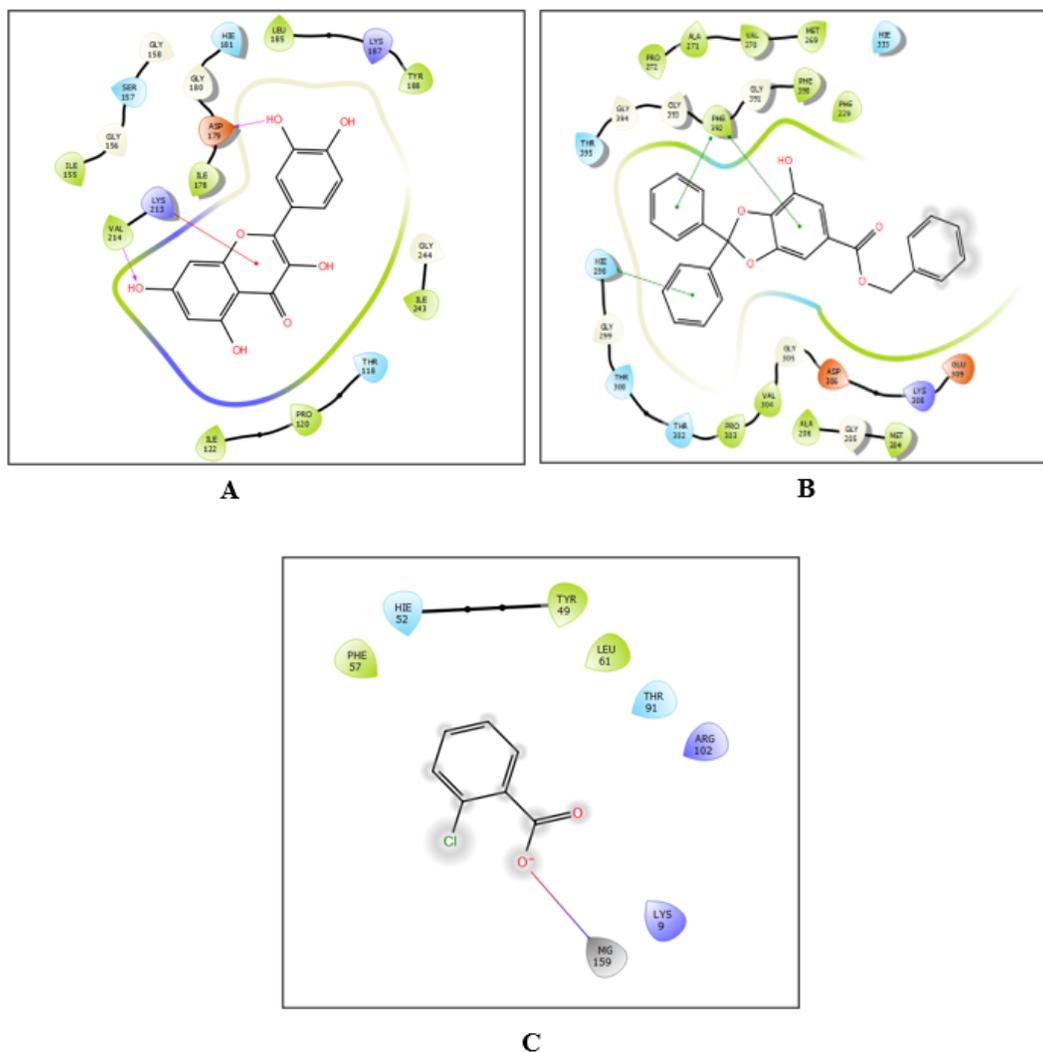

Figure 6. The two-dimensional representation of ligand interactions with the active site. (A) Interactions between quercetin and the NADPH oxidase active site (B): Gallic acid interacts with the active site of *Escherichia coli* beta-ketoacyl-[acyl carrier protein] synthase. Interactions between *Staphylococcus aureus* nucleoside diphosphate kinase and chlorobenzoic acid at the active site (C).
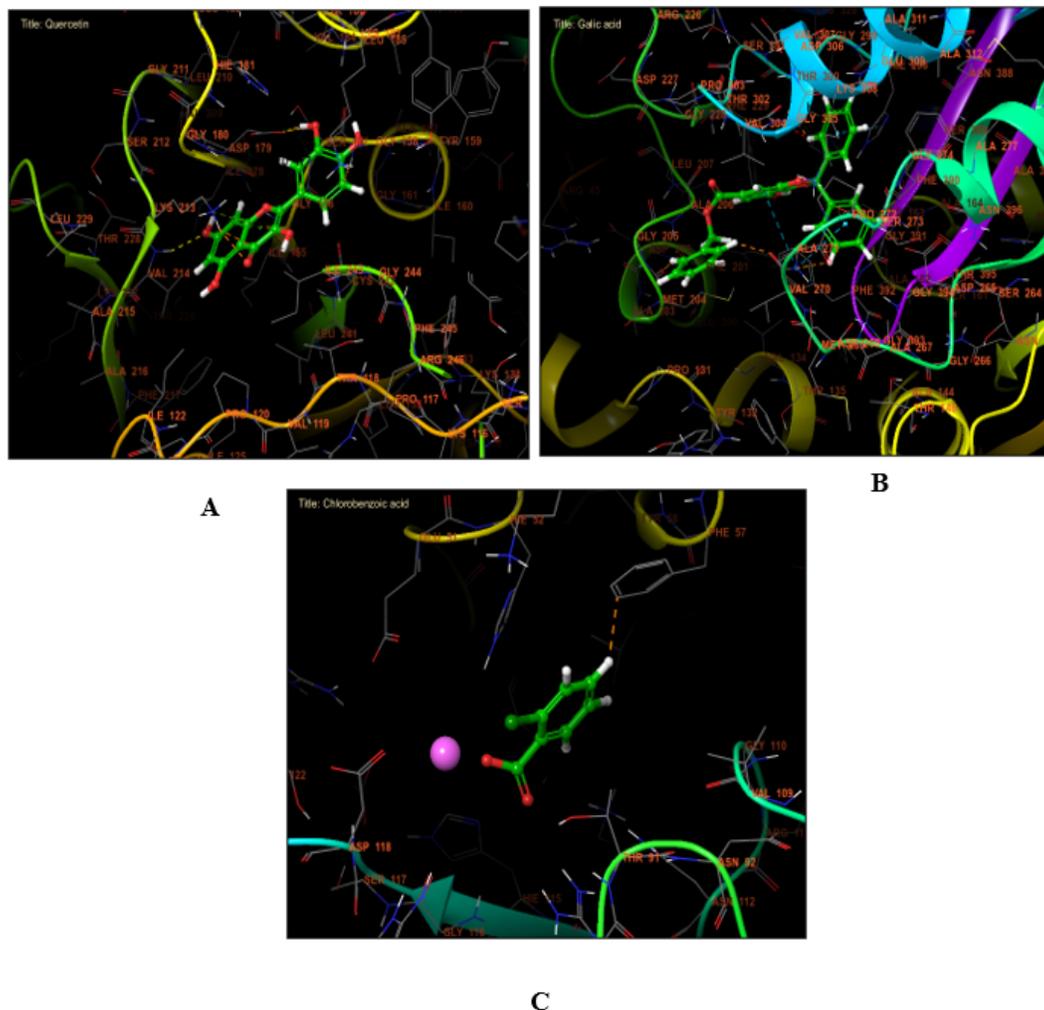
Figure 7. The three-dimensional display of ligand interactions with the active site. (A): Interactions among quercetin and the NADPH oxidase active site, (B): Gallic acid interacts with the active site of *Escherichia coli* beta-ketoacyl-[acyl carrier protein] synthase. (C): The interactions between Staphylococcus aureus nucleoside diphosphate kinase and chlorobenzoic acid at the active site.

3. Statistical analysis

3.1. Correlation Analysis

To determine any relationship between the many factors under study, a correlation test is considered to be an effective tool. In the current examination, we assessed the correlation between antioxidant content and antibacterial activities as well as the correlation between antioxidant properties and amount employing the mean antioxidant value of all solvent extracts tested. The findings of the correlation assay are displayed in Figure 8. There was a substantial positive correlation ($r^2=1$ and $r^2=0.99$) found between the levels of flavonoids, phenolics, and total antioxidant capacity (TAC). However, phenolic compounds flavonoids, and antioxidant capability as measured by DPPH, and RP assays were found to be strongly negatively correlated. Furthermore, there was little correlation found between the antibacterial efficiency of the *M. aquatica* extracts and the polyphenolic and flavonoid content. The obtained data were in line with those mentioned by several reports that indicate the potent correlation between antioxidant activity and phenolic compounds as well as antibacterial ability [15,41].
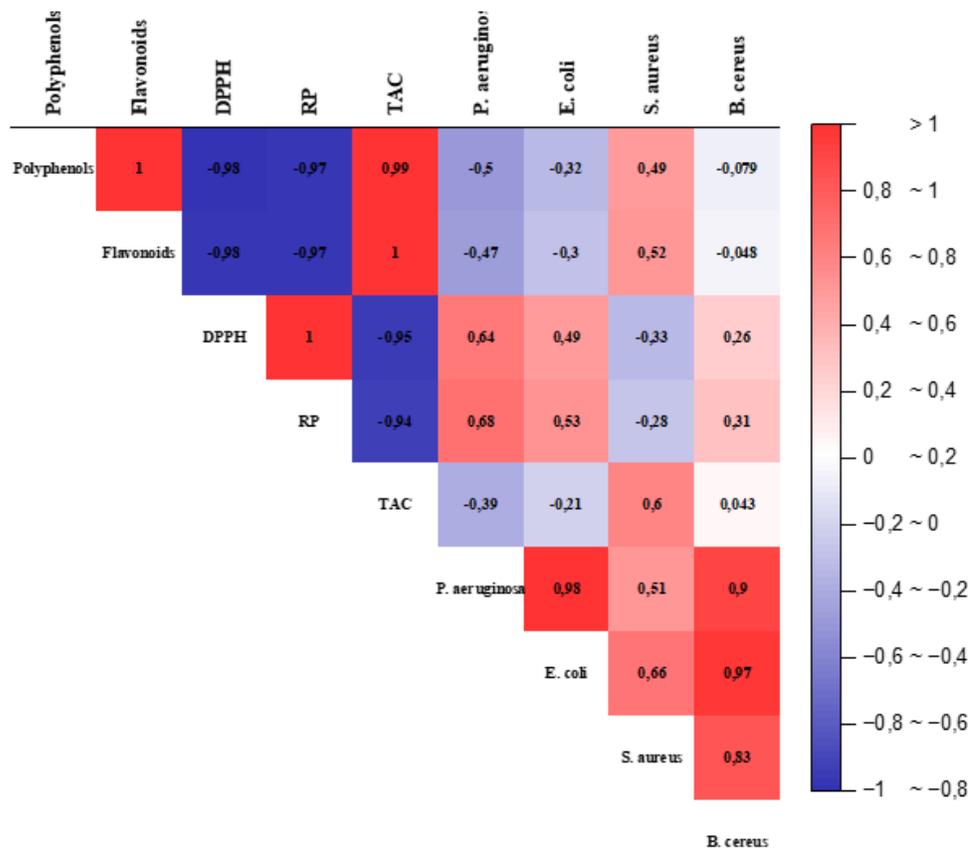
Figure 8. Pearson correlation coefficients of antioxidant content (polyphenols, flavonoids), DPPH, RP, TAC, and antibacterial capacity of *M. aquatica* solvent extract. Pearson correlation values are illustrated by different squares.

3.2. Polar Heatmap Analysis

Figure 9 shows the Polar Heatmap representation based on the data of the correlation test between individual components, antioxidant, and antibacterial activity. Lower amounts were shown in yellow color, while higher amounts were shown in black color. Regrouping various samples according to similarities is made harder by the polar heatmap analysis. It is noticed that the solvent extract has been separated into two groups. One was comprised of ETOH 70 % extract, and the second group was composed of aqueous and acetonic extracts. Our polar heatmap also indicated the correlation between individual phenolic components, total phenolic, flavonoid content, antioxidants, and antibacterial activities.
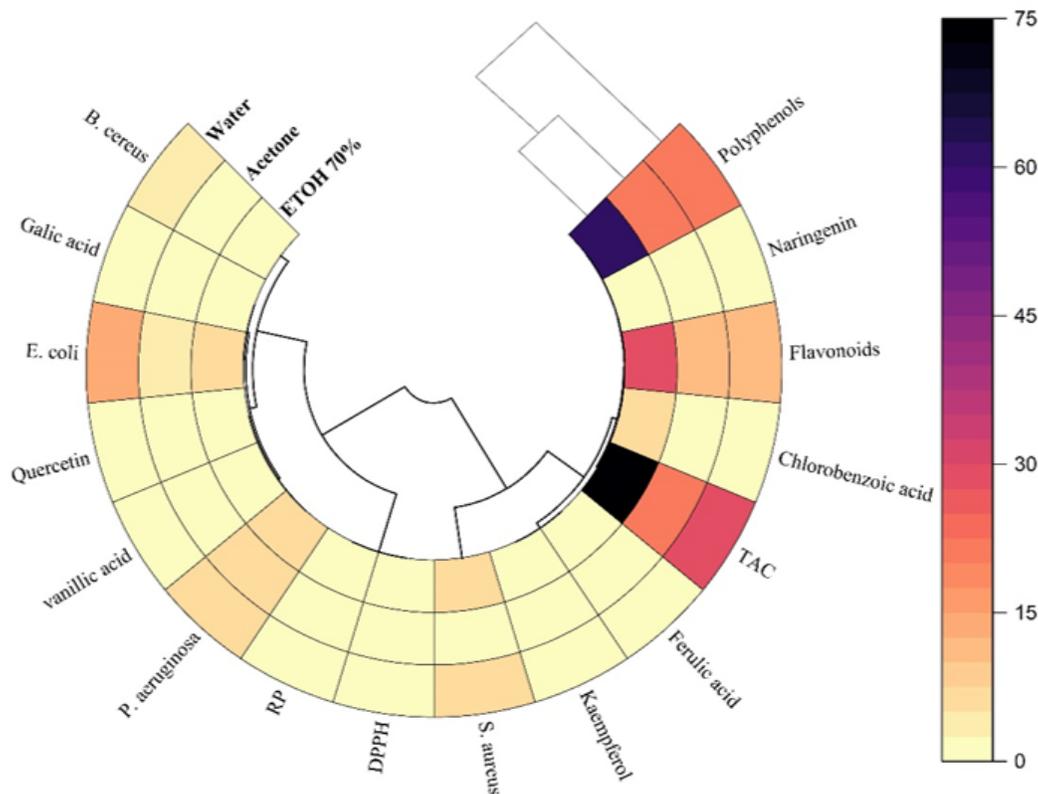
Figure 9. Polar Heatmap with circular dendrogram representation of individual component, polyphenol, flavonoid, antioxidant, and antibacterial correlations of solvent extract.

3.3. Principal Component Analysis (PCA)

The principal component analysis (PCA) was applied to analyzing the prior collected findings, and Figures 7 A and B show the main outcomes. Indeed, Figure 10 (A) displays the main component analysis including TPC, TFC contents, individual phenolic compounds, and antioxidant potency of the solvent extracts. The key finding was that PC1 and PC2 adequately represented all of the chosen variables, accounting for 100% of the variance. The PC1 versus PC2 score plot shows a statistically significant positive correlation between content on TPC, TFC, and TAC, as well as between kaempferol, naringenin, and chlorobenzoic acid. This suggests that the extracts with the greatest antioxidant capacity also contained the greatest amount of TPC, TFC, and specific phenolic components. It was discovered that these variables had a negative relationship with acetone and water and a positive correlation with the hydroethanolic extraction solvents. Furthermore, the total phenolic and flavonoid content is adversely correlated with the DPPH and RP tests.

The PCA for antibacterial individual phenolic compounds of the examined extracts is displayed in Figure 10 (B), where PC1 and PC2 account for 100% of the variance. The score plot of PC1 versus PC2 demonstrates a statistically significant positive correlation across *E. coli*, *P. aeruginosa*, *B. cereus*, naringenin, vanillic acid, and gallic acid proving that the extracts possessing the greatest level of phenolic composites also had the highest antibacterial capacity. These factors had a positive relationship with water and an adverse correlation with EtOH 70% and acetone extraction solvents.
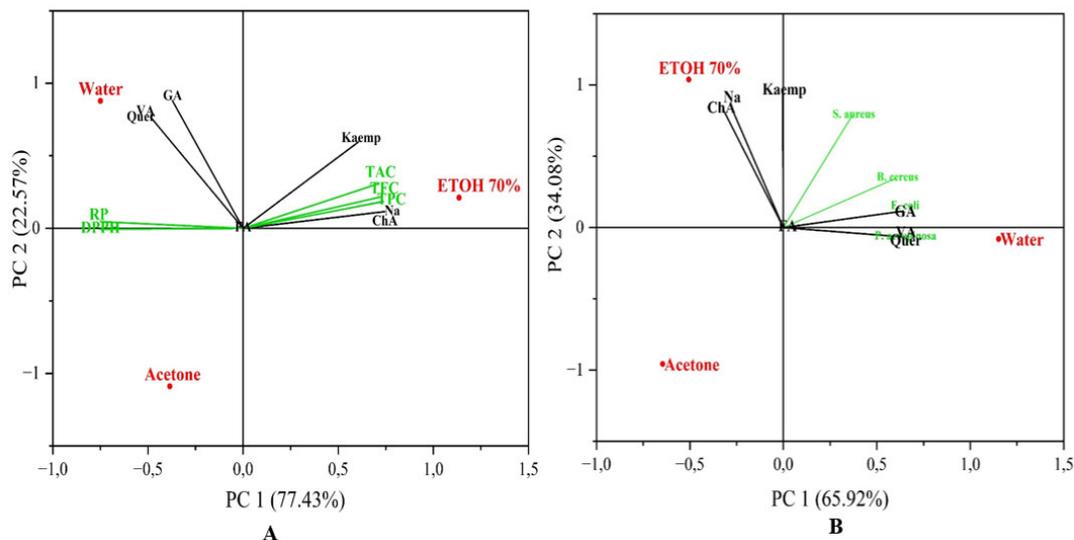
Figure 10. Principal component analysis (PCA). (A) Charts showing the antioxidant properties and phenolic components in the solvent extracts under study. (B) Biplots for individual phenolic compounds, and the antibacterial capacity of examined solvent extracts. Na: naringenin, FA: Ferulic acid, Kamp: Kaempferol, GA: Gallic acid, VA: Vanillic acid, Cha: Chlorobenzoic acid and Quer: Quercetin.

4. Material and Methods

4.1. Botanical Material

The leaves of *M. aquatica* were collected at Merja Zerga (or Moulay Bousselham lagoon) in June 2021 and then prepared into herbarium samples. Professor Amina BARI, a botanist from Sidi Mohamed Ben Abdellah University's Department of Biology, confirmed the following determinations about these specimens. The voucher specimen, with code 002MAMZ2121, has been stored in the faculty herbarium.

4.2. Preparation of Extracts

The present investigation employed diverse solvents with varying polarity to generate the varied extracts, and the extraction procedure was executed in concordance with Tourabi et al.,[15]. In summary, 10 milliliters of each selected solvent (hexane chloroform, ethyl acetate, acetone, hydroethanolic 70% v/v, and distilled water) were combined with one gram of dried plant powder and afterward macerated during seven days with frequent stirring at the ambient temperature. After the extracts had been purified of impurities using a Whatman N°1 filter, they were concentrated at 40 °C in a rotating vacuum evaporator. Before being used, the final crude extracts were obtained and kept in a freezer at -20 °C.

4.3. Estimating the Yield of Extraction

The obtained filtrate was condensed employing a rotating evaporator under a vacuum (BUCHI Rotavapor R200) at 40 °C, to obtain crude extract. Additionally, each extract's yield was estimated, expressed as a percentage, and calculated using the following method.

$$Y_{extract} \% = [((Wext(g))/(Wplant(g)) * 100] \tag{1}$$

Y extract %: extraction yield presented in percentage; W_{ext}: Weight of dry extract; W_{plant}: Weight of dry plant.

14

4.4. Identification of Individual Phenolic Components using High-Performance Liquid Chromatography Coupled with a Diode Array Detector (HPLC-DAD)

Liquid chromatography was used to determine and characterize the solvent extract of *M. aquatica* leaf. A Thermo Scientific HPLC equipment and a MOS-1 HYPERSIL 250 4.6 mm SS Exsil ODS 5 m analytical column were deployed for assessment. In gradient mode, a division was completed by applying two solvents, A (water) and C (acetonitrile). The gradient of elution appeared like this: 80% A, 20% C for one minute, 60% A, 40% C for 2.5 minutes, and 80% A, 20% C for four minutes. A 5 μL volume of injection was employed, and a 1 mL/min flow rate was maintained. By comparing their retention durations and UV spectra against standard profiles, the polyphenolic components namely, ferulic acid, gallic acid, vanillic acid, kaempferol, quercetin, and naringenin were determined. [15].

4.5. Assessment of Total Phenolic Content

The total phenolic amount was determined in accordance with the Tourabi et al. protocol using the Folin Ciocalteu procedure [42]. Gallic acid, at concentrations ranging from 0.016 to 2 mg/mL, provided a point of reference to establish the standard curve ($R^2 = 0.996$). The total phenolic content (TPC) results were then presented as milligrams of gallic acid equivalent per gram of dry plant weight (mg GAE/g DW).

4.6. Assessment of Total Flavonoid Content

The total amount of flavonoid (TFC) was determined by using the colorimetric technique outlined by Tourabi et al., [42]. The standard curve was obtained with quercetin (0.008–1.00 mg/mL; $R^2 = 0.994$), and the TFC data were given in (mg QE/g DW).

4.7. In Vitro Antioxidant Capabilities

Three different prototypes were applied to determine the antioxidant capacity of the solvent extract of *M. aquatica* leaf: The total antioxidant capacity (TAC), the reducing power assay (RP), and the 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•) [1]. All test results were measured using a spectrophotometer made by PerkinElmer Lambda 40.

The inhibitory concentration (IC_{50}) was determined by calculating the percentage of the inhibition curve using BHT as the positive control. The values of IC_{50} were expressed in mg/mL.

The following formula illustrates the calculation of DPPH radical activity in % of inhibition.

$$PI\ (\%) = [(A_{control} - A_{sample}| \ A_{control}) \times 100] \qquad (2)$$

where PI: Percentage of inhibition; $A_{control}$: Absorbance of control; A_{sample}: Absorbance of sample

Ascorbic acid provided the reference for the reducing power test. Using the absorbance graph ($Y = ax + b$; $Y = 0.5$), all results were shown as EC_{50} (Half maximum effective concentration). The values of EC_{50} were expressed in μg/mL.

Ascorbic acid is employed as a standard with an average concentration of 0.00048 to 1 mg/mL ($R2 = 0.9991$) for total antioxidant capability. The data are shown as (mg AAE/g DW).

4.8. Assessment of Antibacterial Capacity

4.8.1. Microbial Testing

The antibacterial capacity of selected solvent extracts was assessed using the microdilution technique. *Staphylococcus aureus* (29,213 (ATCC)), *Escherichia coli* (25,922), *Bacillus cereus* (6633), and *Pseudomonas aeruginosa* (27,853), were employed as a test multiresistant bacteria. Mueller-Hinton agar (MHB) was used for the sensitivity test and nutritional agar (NG) was used to sustain the chosen strains.

4.8.2. Determination of Minimal Inhibitory Concentration (MIC)

The microdilution approach was used to reveal the lowest inhibitory concentration of the selected solvent extracts of *M. aquatica* leaf (water, ethanol 70 %, and acetone) using microplates of 96 wells under sterile conditions [43]. Different extracts were diluted in dimethyl sulfoxide DMSO (2%), to identify the lowest concentration that inhibits. Fifty microliters of each solvent extract at various concentrations from the dilution series were combined with sterilized Mueller–Hinton bouillon and 50 µL of microbial inoculate with a final microbial concentration of 10^8 CFU/mL in microplate wells. Next, the microplates were incubated took place at 37°C for a whole night. To per well, 10 µL of resazurin (0.015%) had been added to reveal the bacterial growth. After two hours of incubation at ambient temperature, microbial growth was indicated by a color change in the microplates from purple to pink.

4.8.3. Assessment of the Lowest Bactericidal Concentration (MBC)

In order to calculate the minimum concentration of bactericide (MBC), transferring 3 µL of the contents from each well showed no visible growth onto MH agar plates, followed by an incubation period of 24 hours at 37°C [44].

4.9. Molecular Docking Study

The molecular docking investigation was done using the Maestro 11.5 tool from the Schrodinger suite. The current research aimed to explore the interaction mechanisms between Phenolic compound of the different solvent extracts of *M. aquatica* and the operating sites of target proteins, including NADPH oxidase, beta-ketoacyl-[acyl carrier protein] synthase from *Escherichia coli*, and nucleoside diphosphate kinase from *Staphylococcus aureus*. The goal was to examine the antioxidant capacity of the phenolic components of the different solvent extracts of *M. aquatica* as well as their antibacterial activities.

4.9.1. Protein Preparation

The structure of human NADPH oxidase (PDB ID: 2CDU) and the beta-ketoacyl-[acyl carrier protein] synthase from Escherichia coli (PDB ID: 1FJ4), and Staphylococcus aureus nucleoside diphosphate kinase (PDB ID: 3Q8U) were obtained from the RCSB database. Protein preparation was done using Maestro 11.5's Protein Preparation Wizard, comprising preprocessing, refining, and reduction steps. Hydrogen atoms were added, and hydroxyl groups, water molecules, and amino acids were reoriented to address structural flaws like overlapping or missing atoms. The proteins were then gently altered to increase their structures [1]

4.9.2. Ligand Preparation

The Ligprep wizard in Maestro 11.5 from the Schrödinger suite was used to generate ligands. This involves converting 2D structures to 3D, adding hydrogen atoms, resolving bond length and angle problems, and decreasing structures using the OPLS3 force field. Ionization states were adjusted, and chirality was retained throughout the process [45].

4.9.3. Receptor Grid Generation

Receptor grids were developed via Glide molecular docking, discovering active areas based on the coordinates of original ligands. Ligands were positioned within the protein's X-ray crystal structure to evaluate different interaction conformations. The technique also entails defining places, boundaries, rotatable groups, and excluded volumes [46].

4.9.4. Performing Molecular Docking

Docking was carried out in Standard Precision (SP) mode after preparing the ligands and proteins and constructing the grids. This model was chosen to evaluate the binding interactions

between molecules. Binding energies were calculated in kcal/mol, along with internal energy, RMSD, desolvation energy, hydrogen bonding, lipophilic interactions, and π-π stacking interactions. This approach ensures rapid and accurate analysis of ligand-protein interactions, providing detailed insights into binding mechanisms [47].

4.10. Statistical Analysis

The various assays conducted in the examination were carried out three times independently, and the findings are presented as mean ± standard deviation. The program entitled GraphPad Prism 8 was used for the statistical analysis, which included a One-way ANOVA test. The principal component analysis (PCA), the correlation coefficient test, and the polar heatmap analysis were carried out by use of OriginPro 2024 software.

5. Conclusions

The present study highlighted the effect of solvent polarity efficacy on the phenolic profile, antioxidant, and antibacterial activity of *M. aquatica* leaf. The obtained data revealed that the leaf extract of *M. aquatica* comprised a high level of flavonoids and phenols composites. Additionally, the molecular characterization of different solvent polarity extracts showed that the distilled water and aqueous ethanol have a high concentration of phenolic composites. Thus, the greatest antioxidant activity was revealed by aqueous ethanol followed by acetonic extract, while the aqueous extract exhibited the lowest antioxidant ability. The acetonic extract showed great antibacterial potential, especially against Gram-positive bacteria followed by hydroethanolic extract. Overall, this report indicated that This kind of plant offers beneficial levels of bioactive molecules that can be employed in several sectors, including as a conservative agent in food manufacturing, and used in the pharmaceutical sector for the formulation of innovative antibacterial agents.

References

1. Tourabi: M.; Nouioura, G.; Touijer, H.; Baghouz, A.; El Ghouizi, A.; Chebaibi, M.; Bakour, M.; Ousaaid, D.; Almaary, K.S.; Nafidi, H.-A.; et al. Antioxidant, Antimicrobial, and Insecticidal Properties of Chemically Characterized Essential Oils Extracted from *Mentha longifolia*: *in Vitro* and *in Silico* Analysis. *Plants* **2023**, *12*, 3783, doi:10.3390/plants12213783.
2. Shaikh, S. Prospective Role in Treatment of Major Illnesses And Potential Benefits As A Safe Insecticide And Natural Food Preservative of Mint (Mentha Spp.): A Review. *Asian Journal of Biomedical and Pharmaceutical Sciences* **2014**, *4*, 1–12, doi:10.15272/ajbps. v4i35.559.
3. Park, Y.; Baek, S.-A.; Choi, Y.; Kim, J.; Park, S. Metabolic Profiling of Nine *Mentha* Species and Prediction of Their Antioxidant Properties Using Chemometrics. *Molecules* **2019**, *24*, 258, doi:10.3390/molecules24020258.
4. Esmaeili, A.; Rustaiyan, A.; Masoudi, S.; Nadji, K. Composition of the Essential Oils of *Mentha aquatica* L. and *Nepeta Meyeri* Benth. from Iran. *Journal of Essential Oil Research* **2006**, *18*, 263–265, doi:10.1080/10412905.2006.9699082.
5. Gruenwald, J.; Brendler, T.; Jaenicke, C. PDR for Herbal Medicines (Physicians' Desk Reference). *Montvale, New Jersey: Medical Economics Company* **2000**.
6. Singh, P.; Pandey, A.K. Prospective of Essential Oils of the Genus *Mentha* as Biopesticides: A Review. *Front. Plant Sci.* **2018**, *9*, 1295, doi:10.3389/fpls.2018.01295.
7. Ebrahimzadeh, M.; Nabavi, S.M.; Nabavi, S.F.; Eslami, B. Biological Activity of *Mentha aquatica* l. *Pharmacologyonline* **2010**, *2*, 611–619.
8. Mkaddem, M.; Bouajila, J.; Ennajar, M.; Lebrihi, A.; Mathieu, F.; Romdhane, M. Chemical Composition and Antimicrobial and Antioxidant Activities of *Mentha* (longifolia L. and *Viridis*) Essential Oils. *Journal of Food Science* **2009**, *74*, M358–M363, doi:10.1111/j.1750-3841.2009.01272.x.
9. Pereira, O.R.; Macias, R.I.R.; Domingues, M.R.M.; Marin, J.J.G.; Cardoso, S.M. Hepatoprotection of *Mentha aquatica* L., *Lavandula dentata* L. and *Leonurus cardiaca* L. *Antioxidants* **2019**, *8*, 267, doi:10.3390/antiox8080267.
10. Sharma, V.; Hussain, S.; Gupta, M.; Saxena, A.K. In Vitro Anticancer Activity of Extracts of *Mentha* spp. against Human Cancer Cells.
11. Gul, H.; Abbas, K.; Qadir, M.I. Gastro-Protective Effect of Ethanolic Extract of *Mentha longifolia* in Alcohol- and Aspirin-Induced Gastric Ulcer Models. *Bangladesh J Pharmacol* **2015**, *10*, 241–245, doi:10.3329/bjp.v10i1.21689.

12. Fatiha, B.; Khodir, M.; Farid, D.; Tiziri, R.; Karima, B.; Sonia, O.; Mohamed, C. Optimisation Of Solvent Extraction Of Antioxidants (Phenolic Compounds) From Algerian Mint (*Mentha spicata* L.).

13. Iloki-Assanga, S.B.; Lewis-Luján, L.M.; Lara-Espinoza, C.L.; Gil-Salido, A.A.; Fernandez-Angulo, D.; Rubio-Pino, J.L.; Haines, D.D. Solvent Effects on Phytochemical Constituent Profiles and Antioxidant Activities, Using Four Different Extraction Formulations for Analysis of *Bucida buceras* L. and *Phoradendron californicum*. *BMC Research Notes* **2015**, *8*, 396, doi:10.1186/s13104-015-1388-1.

14. Jurić, T.; Mićić, N.; Potkonjak, A.; Milanov, D.; Dodić, J.; Trivunović, Z.; Popović, B.M. The Evaluation of Phenolic Content, *in Vitro* Antioxidant and Antibacterial Activity of *Mentha piperita* Extracts Obtained by Natural Deep Eutectic Solvents. *Food Chemistry* **2021**, *362*, 130226, doi:10.1016/j.foodchem.2021.130226.

15. Tourabi, M.; Metouekel, A.; Ghouizi, A.E.L.; Jeddi, M.; Nouioura, G.; Laaroussi, H.; Hosen, Md.E.; Benbrahim, K.F.; Bourhia, M.; Salamatullah, A.M.; et al. Efficacy of Various Extracting Solvents on Phytochemical Composition, and Biological Properties of *Mentha longifolia* L. Leaf Extracts. *Sci Rep* **2023**, *13*, 18028, doi:10.1038/s41598-023-45030-5.

16. Lapornik, B.; Prošek, M.; Golc Wondra, A. Comparison of Extracts Prepared from Plant By-Products Using Different Solvents and Extraction Time. *Journal of Food Engineering* **2005**, *71*, 214–222, doi:10.1016/j.jfoodeng.2004.10.036.

17. Teodor, E.D.; Gatea, F.; Camelia, A.; Radulescu, C.; Chira, A.; Radu, G. Polyphenols, Radical Scavenger Activity, Short-Chain Organic Acids and Heavy Metals of Several Plants Extracts from `Bucharest Delta`. *Chemical Papers* **2015**, *69*, 1582–1590, doi:10.1515/chempap-2015-0177.

18. Naczk, M.; Shahidi, F. Phenolics in Cereals, Fruits and Vegetables: Occurrence, Extraction and Analysis. *Journal of Pharmaceutical and Biomedical Analysis* **2006**, *41*, 1523–1542, doi:10.1016/j.jpba.2006.04.002.

19. Masibo, M.; He, Q. Major Mango Polyphenols and Their Potential Significance to Human Health. *Comprehensive Reviews in Food Science and Food Safety* **2008**, *7*, 309–319, doi:10.1111/j.1541-4337.2008.00047.x.

20. Abbas, A.; Sultana, B.; Hussain, A.; Anwar, F.; Ahmad, N. Antioxidant Potential, Phenolics Content and Antimicrobial Attributes of Selected Medicinal Plants. *Pak. J. Anal. Environ. Chem.* **2021**, *22*, 307–319, doi:10.21743/pjaec/2021.12.10.

21. Dorman, H.J.D.; Koşar, M.; Kahlos, K.; Holm, Y.; Hiltunen, R. Antioxidant Properties and Composition of Aqueous Extracts from *Mentha* Species, Hybrids, Varieties, and Cultivars. *J. Agric. Food Chem.* **2003**, *51*, 4563–4569, doi:10.1021/jf034108k.

22. Hoai, L.V.N.; Quoc, L.P.T.; Hoang, H.T.; Raes, K.; Mai, D.S.; Thien, L.T. Extraction of Polyphenols from *Mentha aquatica* Linn. Var. Crispa. *Agric. Conspec. Sci.* **2023**, *88*, 37–42.

23. Conforti, F.; Sosa, S.; Marrelli, M.; Menichini, F.; Statti, G.A.; Uzunov, D.; Tubaro, A.; Menichini, F.; Loggia, R.D. In Vivo Anti-Inflammatory and in Vitro Antioxidant Activities of Mediterranean Dietary Plants. *Journal of Ethnopharmacology* **2008**, *116*, 144–151, doi:10.1016/j.jep.2007.11.015.

24. Thi, N.Q.N.; Duc, L.T.; Minh, L.V.; Tien, L.X. Phytochemicals and Antioxidant Activity of Aqueous and Ethanolic Extracts of *Mentha aquatica* L. *IOP Conf. Ser.: Mater. Sci. Eng.* **2020**, *991*, 012027, doi:10.1088/1757-899X/991/1/012027.

25. Alam, Md.N.; Bristi, N.J.; Rafiquzzaman, Md. Review on in Vivo and in Vitro Methods Evaluation of Antioxidant Activity. *Saudi Pharmaceutical Journal* **2013**, *21*, 143–152, doi:10.1016/j.jsps.2012.05.002.

26. Hajimehdipoor, H.; Shahrestani, R.; Shekarchi, M. Investigating the Synergistic Antioxidant Effects of Some Flavonoid and Phenolic Compounds.

27. Ferhat, M.; Erol, E.; Beladjila, K.A.; Çetintaş, Y.; Duru, M.E.; Öztürk, M.; Kabouche, A.; Kabouche, Z. Antioxidant, Anticholinesterase and Antibacterial Activities of *Stachys Guyoniana* and *Mentha aquatica*. *Pharmaceutical Biology* **2017**, *55*, 324–329, doi:10.1080/13880209.2016.1238488.

28. Konaté, K.; Mavoungou, J.F.; Lepengué, A.N.; Aworet-Samseny, R.R.; Hilou, A.; Souza, A.; Dicko, M.H.; M'Batchi, B. Antibacterial Activity against β- Lactamase Producing Methicillin and Ampicillin-Resistants *Staphylococcus aureus:* Fractional Inhibitory Concentration Index (FICI) Determination. *Ann Clin Microbiol Antimicrob* **2012**, *11*, 18, doi:10.1186/1476-0711-11-18.

29. Janakat, S.; Al-Nabulsi, A.A.R.; Allehdan, S.; Olaimat, A.N.; Holley, R.A. Antimicrobial Activity of Amurca (Olive Oil Lees) Extract against Selected Foodborne Pathogens. *Food Sci. Technol (Campinas)* **2015**, *35*, 259–265, doi:10.1590/1678-457X.6508.

30. Adamczak, A.; Ożarowski, M.; Karpiński, T.M. Antibacterial Activity of Some Flavonoids and Organic Acids Widely Distributed in Plants. *Journal of Clinical Medicine* **2020**, *9*, 109, doi:10.3390/jcm9010109.

31. Tian, Q.; Wei, S.; Su, H.; Zheng, S.; Xu, S.; Liu, M.; Bo, R.; Li, J. Bactericidal Activity of Gallic Acid against Multi-Drug Resistance *Escherichia coli*. *Microbial Pathogenesis* **2022**, *173*, 105824, doi:10.1016/j.micpath.2022.105824.

32. Chatterjee, N.S.; Panda, S.K.; Navitha, M.; Asha, K.. . K.; Anandan, R.; Mathew, S. Vanillic Acid and Coumaric Acid Grafted Chitosan Derivatives: Improved Grafting Ratio and Potential Application in Functional Food. *J Food Sci Technol* **2015**, *52*, 7153–7162, doi:10.1007/s13197-015-1874-4.

33. Álvarez-Martínez, F.J.; Barrajón-Catalán, E.; Encinar, J.A.; Rodríguez-Díaz, J.C.; Micol, V. Antimicrobial Capacity of Plant Polyphenols against Gram-Positive Bacteria: A Comprehensive Review. *Current Medicinal Chemistry* **2020**, *27*, 2576–2606, doi:10.2174/0929867325666181008115650.

34. Michiels, J.A.; Kevers, C.; Pincemail, J.; Defraigne, J.O.; Dommes, J. Extraction Conditions Can Greatly Influence Antioxidant Capacity Assays in Plant Food Matrices. *Food Chemistry* **2012**, *130*, 986–993, doi:10.1016/j.foodchem.2011.07.117.

35. Eloff, J.N. Which Extractant Should Be Used for the Screening and Isolation of Antimicrobial Components from Plants? *Journal of Ethnopharmacology* **1998**, *60*, 1–8, doi:10.1016/S0378-8741(97)00123-2.

36. Herrera-Rocha, K.M.; Rocha-Guzmán, N.E.; Gallegos-Infante, J.A.; González-Laredo, R.F.; Larrosa-Pérez, M.; Moreno-Jiménez, M.R. Phenolic Acids and Flavonoids in Acetonic Extract from Quince (*Cydonia oblonga mill.*): Nutraceuticals with Antioxidant and Anti-Inflammatory Potential. *Molecules* **2022**, *27*, 2462, doi:10.3390/molecules27082462.

37. Campos, F.M.; Couto, J.A.; Figueiredo, A.R.; Tóth, I.V.; Rangel, A.O.S.S.; Hogg, T.A. Cell Membrane Damage Induced by Phenolic Acids on Wine Lactic Acid Bacteria. *International Journal of Food Microbiology* **2009**, *135*, 144–151, doi:10.1016/j.ijfoodmicro.2009.07.031.

38. Su, Y.; Ma, L.; Wen, Y.; Wang, H.; Zhang, S. Studies of the in Vitro Antibacterial Activities of Several Polyphenols against Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus. Molecules* **2014**, *19*, 12630–12639, doi:10.3390/molecules190812630.

39. Mokhtar, M.; Ginestra, G.; Youcefi, F.; Filocamo, A.; Bisignano, C.; Riazi, A. Antimicrobial Activity of Selected Polyphenols and Capsaicinoids Identified in Pepper (*Capsicum annuum* L.) and Their Possible Mode of Interaction. *Curr Microbiol* **2017**, *74*, 1253–1260, doi:10.1007/s00284-017-1310-2.

40. Zhang, Y.; Wang, J.; Dong, J.; Wei, J.; Wang, Y.; Dai, X.; Wang, X.; Luo, M.; Tan, W.; Deng, X.; et al. Inhibition of α-Toxin Production by Subinhibitory Concentrations of Naringenin Controls *Staphylococcus aureus Pneumonia. Fitoterapia* **2013**, *86*, 92–99, doi:10.1016/j.fitote.2013.02.001.

41. Matejczyk, M.; Świsłocka, R.; Golonko, A.; Lewandowski, W.; Hawrylik, E. Cytotoxic, Genotoxic and Antimicrobial Activity of Caffeic and Rosmarinic Acids and Their Lithium, Sodium and Potassium Salts as Potential Anticancer Compounds. *Advances in Medical Sciences* **2018**, *63*, 14–21, doi:10.1016/j.advms.2017.07.003.

42. Tourabi, M.; EL Ghouizi, A.; Nouioura, G.; Faiz, K.; Fatemi, H.EL.; Elyagoubi, K.; Lyoussi, B.; Derwich, E.H. Phenolic Profile, Acute and Subacute Toxicity of an Aqueous Extract from Moroccan *Mentha longifolia* L. Aerial Part in Swiss Albino Mice Model. *Journal of Ethnopharmacology* **2023**, 117293, doi:10.1016/j.jep.2023.117293.

43. Fadipe, V.; Mongalo, N.; Opoku, A. In Vitro Evaluation of the Comprehensive Antimicrobial and Antioxidant Properties of *Curtisia dentata* (Burm.f) C.A. Sm: Toxicological Effect on the Human Embryonic Kidney (HEK293) and Human Hepatocellular Carcinoma (HepG2) Cell Lines. *EXCLI J* **2015**, *14*, 971–983, doi:10.17179/excli2015-351.

44. Bachiri, L.; Echchegadda, G.; Ibijbijen, J.; Nassiri, L. Etude Phytochimique Et Activité Antibactérienne De Deux Espèces De Lavande Autochtones Au Maroc : «*Lavandula stoechas* L. et *Lavandula dentata* L.». *European Scientific Journal, ESJ* **2016**, *12*, 313–313, doi:10.19044/esj.2016.v12n30p313.

45. Abdali, Y.E.; Mahraz, A.M.; Beniaich, G.; Mssillou, I.; Chebaibi, M.; Jardan, Y.A.B.; Lahkimi, A.; Nafidi, H.-A.; Aboul-Soud, M.A.M.; Bourhia, M.; et al. Essential Oils of *Origanum compactum* Benth: Chemical Characterization, in Vitro, in Silico, Antioxidant, and Antibacterial Activities. *Open Chemistry* **2023**, *21*, doi:10.1515/chem-2022-0282.

46. Ouahabi, S.; Loukili, E.H.; Daoudi, N.E.; Chebaibi, M.; Ramdani, M.; Rahhou, I.; Bnouham, M.; Fauconnier, M.-L.; Hammouti, B.; Rhazi, L.; et al. Study of the Phytochemical Composition, Antioxidant Properties, and In Vitro Anti-Diabetic Efficacy of *Gracilaria bursa-pastoris* Extracts. *Marine Drugs* **2023**, *21*, 372, doi:10.3390/md21070372.

47. Amrati, F.E.-Z.; Elmadbouh, O.H.M.; Chebaibi, M.; Soufi, B.; Conte, R.; Slighoua, M.; Saleh, A.; Al Kamaly, O.; Drioiche, A.; Zair, T.; et al. Evaluation of the Toxicity of *Caralluma europaea* (C.E) Extracts and Their Effects on Apoptosis and Chemoresistance in Pancreatic Cancer Cells. *Journal of Biomolecular Structure and Dynamics* **2023**, *41*, 8517–8534, doi:10.1080/07391102.2022.2135595.