



Article Phenolic Composition, Antioxidant and Antibacterial Activities of Extract from Flowers of *Rosa damascena* from Morocco

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Abstract: *Rosa damascena* is referred to as the queen of roses due to its ornamental, flavoring, and perfuming uses, along with its recognized use in therapy. This study aimed to investigate the total phenols and flavonoids contents, the phenolic compounds, and study the antioxidant and antibacterial properties of the hydroethanolic extract from *Rosa damascena* flowers, collected from the Middle Atlas of Morocco (Khenifra). The total phenols and flavonoids were assessed using gallic acid and quercetin as standards, and the phenolic compounds were characterized using HPLC-PDA-ESI/MS. The antioxidant activity was evaluated by two methods, namely ferric reducing assay power and total antioxidant capacity. The broth microdilution method was employed to evaluate the antibacterial activity of extract against four bacteria (*Escherichia coli, Salmonella typhimurium, Staphylococcus aureus,* and *Listeria monocytogenes*). Up to 16 phenolic compounds belonging to tannins and flavonoids were positively identified in the *Rosa damascena* extract. The latter displayed high antioxidant activity and exhibited a bacteriostatic effect against *Escherichia coli* and a bactericidal effect against *Salmonella typhimurium, Staphylococcus aureus,* and *Listeria monocytogenes*. As a result, the flowers of *Rosa damascena* might be employed as natural agents in the pharmaceutical field.

Keywords: Rosa damascena; flowers; HPLC-PDA-ESI/MS; antioxidant activity; antibacterial activity

1. Introduction

Nowadays, the research on medicinal and aromatic plants extracts has received considerable attention, due to their phenolic profile and pharmacological properties (antioxidant, antibacterial, anti-inflammatory). Phenols from medicinal herbs are a promising source of natural antioxidant and antimicrobial agents and they can help overcome two major human health problems: oxidative stress and antibiotic resistance. Studying the phenolic composition of the plant extracts and assessing their antioxidant and antibacterial properties are necessary to estimate their potential as new drug sources.



Citation: Chroho, M.; Bouymajane, A.; Oulad El Majdoub, Y.; Cacciola, F.; Mondello, L.; Aazza, M.; Zair, T.; Bouissane, L. Phenolic Composition, Antioxidant and Antibacterial Activities of Extract from Flowers of *Rosa damascena* from Morocco. *Separations* **2022**, *9*, 247. https:// doi.org/10.3390/separations9090247

Academic Editors: Neli Kinga Olah, Daniela Hanganu and Violeta Turcuş

Received: 22 July 2022 Accepted: 2 September 2022 Published: 5 September 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Damascus rose, *Rosa damascena*, belongs to the genus *Rosa*, which contains 200 species and more than 18,000 varieties. It is a hybrid species between *Rosa gallica* and *Rosa phoenicia*. It is referred to as the "queen of roses" and it is popular as a decorative plant [1,2]. It is called Damascus rose because it originated from Damascus in the Middle East and was introduced into Europe [3,4].

Damascus rose is exploited for the attainment of several products, e.g., dried flower buds and essential oil. The latter is one of the most costly essential oils in the world due to its unique combination of odorant constituents and its low yield, which does not exceed 0.049% [1,5]. Rose water is used mainly in cosmetic industry in various blonde and facial cleansing creams [6].

Morocco, in addition to six other countries (Bulgaria, France, Italy, Turkey, Iran, and India), are the most known countries by the culture of Damascus rose [5,7]. In Morocco, it is called «lward lbaldi» in the Moroccan dialect. The cultivation of Damascus rose was originally located in the Dades valley in the Ouarzazate region in the form of hedges or fences surrounding agricultural fields. It has an important economic impact on the inhabitants of this region, and its economic importance is concretized by a big festival that is organized every year in the region in Kela M'Gouna city. In recent years, seeing the economic impact of the Damascus rose in the region of Dades, its culture was no longer satisfied in this region, and individuals as well as associations and cooperatives working in the field of Medicinal and Aromatic Plants (MAP) began to cultivate it in other regions where the climatic conditions are favorable for its cultivation.

Rosa damascena is mainly cultivated for its use in perfumery [8]. It is also used in food and flavoring industries [2,5]. In addition to flavoring and perfuming uses, *Rosa damascena* is used in the treatment of constipation and digestive disorders, liver diseases, and abdominal pain [9]. The pharmacological properties of *Rasa damascena* have been proved and reported, reporting that it has antioxidant, antifungal, antibacterial, antimicrobial, and anti-inflammatory effects [10–12]. Interesting antioxidant and antibacterial properties of extracts from *Rasa damascena* flowers been reported in several studies [13–15].

Several studies have been reported on *Rosa damascena* native from Iran, Bulgaria and Turkey; however, only a few studies have been focused on Moroccan *Rosa damascena* [16–18].

The aim of the present study was the characterization of the phenolic compounds, and the evaluation of the antioxidant and antibacterial activities of the hydroethanolic extract from *Rosa damascena* flowers harvested in the Middle Atlas of Morocco (Khenifra).

2. Materials and Methods

2.1. Plant Material

The flowers of *Rosa damascena* were provided by the cooperative El Hammam for the valuation of Medicinal and Aromatic Plants (Latitude: 33°10′21.9″ N; Longitude: 5°28′22.2″ W; Altitude: 1182 m). The flowers were collected in late May and early June 2019 and kept at room temperature and in darkness. Identification of the species was validated in the Scientific Institute of Rabat.

2.2. Reagents

The employed phenolic standards rutin (Cas No: 153-18-4), quercetin (Cas No: 6151-25-3), kaempferol-3-glucoside (Cas No: 480-10-4), and the HPLC-MS grade solvents; MeOH (Cas No: 67-56-1), water (Cas No: 7732-18-5), acetonitrile (Cas No: 75-05-8), and formic acid (Cas No: 64-18-6) were all purchased from Merck Life Science (Merck KGaA, Darmstadt, Germany).

2.3. Standards Employed

Three phenolic standards (rutin, quercetin, and kaempferol-3-glucoside) were employed for the quantification of the phenolic content in the sample extract. Rutin (0.5, 1, 10, 25, 50) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 10, 25, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25, 25) ppm, y = 10066x + 2176.5; $R^2 = 0.9998$, LoD = 0.014, LoQ = 0.042; Quercetin (0.1, 0.5, 1, 25) ppm, P = 0.014; $R^2 = 0.998$; $R^2 = 0.998$;

2, 10, 50) ppm, y = 20376x + 7053.8, R² = 0.9992, LoD = 0.007, LoQ = 0.022; Kaempferol-3-glucoside (1, 10, 20, 50, 150) ppm, y = 13848x + 2354.1, R² = 0.9995, LoD = 0.090, LoQ = 0.274.

2.4. Phytochemical Screening

The qualitative analysis of extracts based on coloring and/or precipitation reactions derived from a variety of plant extracts produced through decoction, infusion, or maceration are widely used in the photochemical screening. Different families of secondary metabolites were sought in the extract obtained from *Rosa damascena* flowers, according to the method described by Bruneton and N'Guessan et al. [19,20].

2.5. Phenolic Extraction

Phenolic compounds extraction was carried out using a heated solid-liquid extraction. Briefly, 30 g of the dried flowers of *Rosa damascena* were placed on a filter paper into the Soxhlet apparatus, and 350 mL of EtOH/water (70:30, v/v) were added in the flask. After various cycles, the extract was concentrated using a rotary evaporator.

2.5.1. Determination of the Total Phenols Content in Rosa damascena

The Folin-Ciocalteu method was used to determine the total phenols content in the flowers extract of *Rosa damascena* [21]. Briefly, in the flask, 5 μ L of the dried extract were mixed with 1.5 mL of Folin-Ciocalteu reagent (10%) and 1.5 mL of sodium carbonate at 7.5% (*m*/*v*). Afterwards, the flask was completely filled with distilled water. Then, the flask was kept at room temperature for 30 min, and the absorbance was measured at 760 nm. The results were expressed as milligrams of Gallic Acid Equivalent per gram of dry matter (mg GAE/g dm).

2.5.2. Determination of the Flavonoids Contents in Rosa damascena

The flavonoids content was determined using an Aluminum Trichloride (AlCl₃) method [22]. Briefly, 0.1 mL of aluminum trichloride (10%) was mixed with 10 μ L of the extract, and 20 mL of distilled water. Then, a volume of methanol was added to complete 50 mL. Subsequently, the mixture was stored at room temperature and in the darkness for 2 h. The absorbance was measured at 430 nm. The regression equation of quercetin was used to determine the flavonoids content, and the results were expressed as milligrams of Quercetin Equivalent per gram of dry matter (mg QE/g dm).

2.6. HPLC-PDA-ESI/MS Analyses

2.6.1. Sample Preparation

The crude ethanolic extract of *R. damascena* was redissolved in the same organic solvent and subjected to dilution (1:40, v/v). An injection volume of 5 µL was employed, and the analysis was performed in triplicate.

2.6.2. HPLC-MS Conditions

Chromatographic analysis was conducted using a Shimadzu HPLC system (Kyoto, Japan) supplied with a CBM-20A controller, a DGU20A5R degasser, two LC-20AD dualplunger parallel-flow pumps, an SPD-M20A photodiode array detector, a SIL-30AC autosampler, an LCMS-2020 single quadrupole mass spectrometer, and a CTO-20AC column oven, and via the use of ESI source operated in negative and positive ionization modes.

Phenolic compounds were separated on an Ascentis Express RP C18 column ($150 \times 4.6 \text{ mm}$; 2.7 µm) (Merck Life Science, Merck KGaA, Darmstadt, Germany). A total of 0.1% of formic acid in water (A), and acetonitrile (B) were used as mobile phase. The flow rate was set at 0.8 mL/min and was split to 0.2 mL/min prior to ESI-MS detection, under a gradient elution of 0–15 min, 0–15% B, 30 min, 20% B, 60 min, 50% B, 70 min, 100% B, and 79 min, 100% B. Five µL was the injected volume. The range of application of diode array detection (DAD) was 200–400 nm and it was monitored at 330 nm wavelength (sampling frequency: 40.0 Hz, time constant: 0.08 s). Mass spectrometry conditions were as

follows: At a mass-to-charge ratio (m/z), the scan range was 100–1000 and the scan speed was set at 2500 amu/s; Event time: 0.3 s, nebulizing gas (N₂) flow rate was 1.5 L/min, and drying gas (N₂) flow rate was 15 L/min. The interface temperature was 350 °C, heat block temperature at 300 °C, and desolvation line temperature at 300 °C, desolvation line voltage: 1 V, interface voltage: -4.5 kV.

2.7. Antioxidant Activity

2.7.1. Ferric Reducing Assay Power (FRAP)

The ferric reducing assay power method was used to measure the antioxidant power reducing ferric iron (Fe³⁺) to ferrous iron (Fe²⁺) [23]. The absorbance was measured at 700 nm and ascorbic acid was used as a control. The Effective Concentration (EC₅₀), which corresponds to an absorbance equal to 0.5, was determined.

2.7.2. Total Antioxidant Capacity (TAC)

The total antioxidant capacity method was used to measure the antioxidant power reducing molybdenum (VI) to molybdenum (V) [24]. The absorbance was measured at 695 nm and the results were expressed as milligrams of ascorbic acid equivalent per gram of extract (mg EAA/g E).

2.8. Antibacterial Activity

2.8.1. Bacterial Strains

The bacterial strains (*Escherichia coli, Salmonella typhimirium, Staphyloccocus aureus,* and *Listeria monocytogenes*) used in this study were provided from the Laboratory of Microbiology and Health, Faculty of Sciences at the Moulay Ismail University of Morocco. Bacterial strains were spread on Mueller Hinton agar (Merck Life Science, Merck KGaA, Darmstadt, Germany), and incubated at 37 °C 24 h. Afterwards, a bacterial suspension equivalent to 0.5 McFarland standard (10⁸ cfu/mL) was prepared.

2.8.2. Broth Micro-Dilution Method

Minimum inhibitory and bactericidal concentrations (MIC, MBC) of extract of *Rosa damascena* flowers were assessed by the broth microdilution method, as described by Bouymajane et al. [25]. The MIC was determined as the lowest concentration of extract that showed no visible bacterial growth. The MBC was determined as the lowest concentration of the extract that shows no bacterial growth. A total of 100 μ L from the well showed no visible bacterial growth, streaked on Petri dishes containing MHA and incubated at 37 °C for 30 min. The MBC/MIC ratio was calculated to deduct the antibacterial effect of the extract. The extract effect is bacteriostatic if MBC/MIC > 4, and bactericidal if MBC/MIC \leq 4. All the experiments were done in triplicate.

2.9. Statistical Analysis

The acquired data were provided as the mean \pm SEM (standard error of mean). The Statistical analysis for the antioxidant activity was performed by using one-way analysis of variance (ANOVA), followed by the Tukey–Kramer test, using the SPSS package. All experiments were performed in triplicate and the differences were considered significant at *p* < 0.05.

3. Results and Discussion

3.1. Phytochemical Screening

The results from the phytochemical screening showed that the extract of *R. damascena* flowers are rich with gallic tannins, flavonones, anthocyanes, alcaloids, and triterpenes. Previous studies have shown that the petals of *R. damascena* contain terpenes, anthocyanins, flavonoids, and tannins. In addition, its flowers contain vitamin C, myrcene, carboxilic acid, kaempferol, and quercetin [9,26,27].

3.2. Extraction Yield

The obtained results showed that the extraction yield of the hydroethanolic extract of *Rosa damascena* flowers was 40.5% (Table 1). An extraction yield of 20% was reported for *Rosa canina* by 30% ethanol solvent [28]. Both total yield and yield of particular compounds are affected by the amount of organic solvent present in the water [29]. This good yield of extraction obtained can be linked to the plant's particular phenolic groups as well. For instance, flavonols such as quercetin have a high solubility in alcohols and, as a result, a higher extraction yield, as the ethanol level in water rises from 70% and above [30]. Furthermore, methanol is generally found to be more efficient to extract phenols with a lower molecular weight [30–32].

Table 1. Determination of total phenols and flavonoids contents, and antioxidant activity of the hydroethanolic extract from *Rosa damascena* flowers.

Extraction Yield	Total Phenols Content	Total Flavonoids Content	FRAP	TAC
40.5%	20.07 ± 1.00 mg GAE/g dm	0.987 ± 0.05 mg QE/g dm	$0.20\pm0.1~mg/mL$	213.223 mg EAA/1 g E

3.3. Determination of the Total Phenols and Flavonoids Contents

The total polyphenols and flavonoids contents were calculated using the calibration curves of gallic acid (y = 0.095x + 0.003), and quercetin (y = 0.073x - 0.081) as indicated in Figure 1a,b respectively.

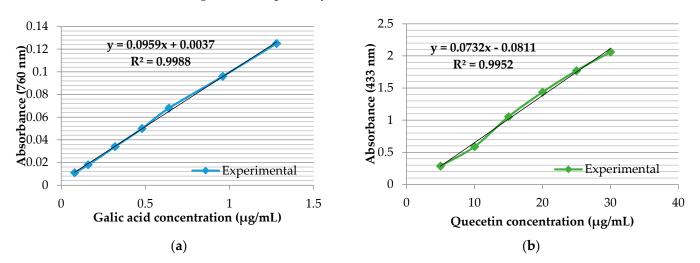


Figure 1. Calibration curves of (a) gallic acid and (b) quercetin (black represent linear curves).

The total phenolic and flavonoids contents for the ethanolic extract of *Rosa damascena* flowers are 20.07 \pm 1.00 mg GAE/g dm and 0.987 \pm 0.05 mg QE/g dm, respectively (Table 1). The total phenolic content in *Rosa damascena* extracts was determined in other studies. In our knowledge, no previous studies have reported the phenols and flavonoids contents from ethanolic extracts of *Rosa damascena* flowers obtained by Soxhlet.

The aqueous extract of *Rosa damascena* flowers from Bulgaria presented 124.86 \pm 1.54 mg GAE/g of extract. The phenolic extraction was done with distilled water at 50 °C under agitation, followed by filtration and water removal by evaporation [33]. The hydro-methanolic extract of *Rosa damascena* from Turkey presented 276 \pm 2.93 mg GAE/g dm for fresh flowers and 248.97 \pm 2.96 mg GAE/g dm for spent flowers. The extract was obtained by mixing (plant powder and solvent), then soaking, followed by filtration and solvent removing by evaporation [14].

Koczka et al. compared the total phenolic content from water and ethanolic extracts, obtained by infusion, then filtration and centrifugation, from the hips of four species of *Rosa* (*R. spinosissima*, *R. canina*, *R. rugosa*, and *R. gallica*). For water extracts, the phenolic content values ranged from 150.8 mg to 299.2 mg GAE/100 g DW (dry weight), and for the ethanol extracts, the phenolic content values varied from 255.9 mg to 766.0 mg GAE/100 g DW [34]. The difference in the content of secondary metabolites depends on variety, maturity and harvesting of plant, seasonal variations, geographical conditions, and extraction methods [29].

3.4. HPLC-PDA/ESI-MS Analyses

The phenolic composition analysis was carried out by HPLC-PDA-ESI/MS (Figure 2). As presented in Table 2, a total of 18 phenolic compounds were detected in the extract from *Rosa damascena* flowers, according to standards, times of retention, mass spectrometry, and literature data. The compounds were assigned to tannins (ellagic acid and valoneic acid dilactone) and to flavonoids (quercetin derivatives and kaempferol derivatives).

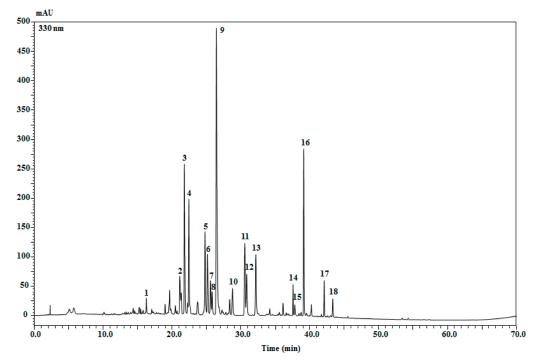


Figure 2. Chromatographic profile of phenolic compounds in the *Rosa damascena* extract (EtOH:H₂O 7:3 v/v) acquired at 330 nm.

The main constituents of rose tannins are primarily monomer or polymer of gallic acid [35]. Valoneic acid dilactone and ellagic acid were identified as peaks 1 and 2. Ellagitannins are hydrolysable tannins formed from gallic acid esterified with hexahydroxydiphenic acid (HHDP). Furthermore, the ellagitannins displayed several pharmacological properties including antiviral, antioxidant, anticancer, and anti-inflammatory activities [36,37]. Quercetin and its glycosides are the most common flavonoids found in may flowers and fruits, and they are essential for preventing the release of proinflammatory mediators [38,39]. Quercetin and kaempferol with their derivatives and glycosides were the major flavonoids detected in the *Rosa damascena* extract, with kaempferol derivatives occurring in a higher amount than quercetin ones.

Peak N°	Compound	t _R (min)	UV (nm)	[M – H] [–]	[M + H] ⁺	Fragments	Quantity (mg/Kg) Extract \pm sd	References
1	Valoneic acid dilactone	16.28	255, 364	469	471	-	Ng	[40]
2	Ellagic Acid	21.13	252, 366	301	303	-	Ng	[28,40,41]
3	Quercetin-3-O-hexoside	21.81	255, 354	463	465	-	7005.05 ± 52.32	[40,41]
4	Quercetin-3-O-hexoside	22.45	255, 353	463	465	-	5837.40 ± 79.04	40,41
5	Kaempferol-O-hexoside	24.81	264, 344	447	449	287(+)	3332.90 ± 37.57	[28, 40, 41]
6	Kaempferol-3-Glucoside-2"- p-coumaroyl	25.17	264, 344	593	595	287(+)	2519.60 ± 3.34	[28]
7	Rutin	25.60	257,348	609	-	303(+)	1778.22 ± 12.79	[28]
8	Quercetin-3-pentoside	25.84	258, 352	433, 151	-	303(+)	1341.14 ± 18.07	[41]
9	Kaempferol-O-hexoside	26.46	264, 344	447	449	287(+)	$13,575.88 \pm 69.39$	[28,40,41]
10	Kaempferol- <i>O</i> -pentoside	28.79	264, 344	417	419	287(+)	1201.45 ± 9.34	[35]
	Kaempferol-3-Glucoside-2"-		,					L - 1
11	p-coumaroyl	30.58	263, 342	593	595	287(+)	2933.56 ± 1.08	[28]
	isomer							L
12	Kaempferol-O-pentoside	30.85	264, 346	417	419	287(+)	1955.01 ± 31.46	[35]
13	Kaempferol-3-O-rhamnoside	32.18	263, 344	431	433	287(+)	2892.49 ± 75.16	[42]
14	Kaempferol acetyldisaccharides	37.57	263, 343	635	637	287(+)	758.59 ± 16.88	[42]
15	Ouercetin	37.84	254, 369	301	303	-	271.96 ± 6.84	[28, 40, 41]
16	Ũnknown	39.13	266, 313	593	595	-	Ng	[42]
17	Unknown	42.09	295	582	584	-	Ng	-
18	Kaempferol	43.33	263, 365	285	287	-	Nq	[41]

Table 2. Characterization of the phenolic compounds in the (EtOH:H₂O 7:3 v/v) extract of *Rosa damascena* by HPLC-PDA/MS.

3.5. Antioxidant Activity

3.5.1. Antioxidant Activity of the Rosa damascena Hydroethanolic Extract by FRAP

The hydroethanolic extract of *Rosa damascena* flowers showed a significant antioxidant potential (Figure 3). The EC50 parameter was equal to $0.20 \pm 0.1 \text{ mg/mL}$. For the ascorbic acid tested under the same conditions, the EC50 was equal to 0.031 mg/mL.

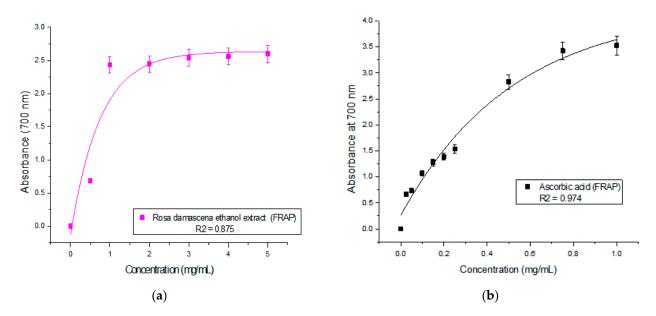


Figure 3. FRAP of the hydroethanolic extract obtained from *Rosa damascena* flowers (**a**) and ascorbic acid (**b**).

The antioxidant capacity of the ethanolic extracts of two parts of the plant was studied using the same method; FRAP activity was found to be higher for the ethanolic extracts from *Rosa damascena* petals (164.23 \pm 1.34 μ M Fe(II)) than receptacles (12.85 \pm 6.19 μ M Fe(II)) [43]. Dudonné et al. reported a ferric reducing power of the aqueous extract of *Rosa damascena* in the order of 5.08 \pm 0.07 mmol Fe²⁺/g [33]. The part of the plant that is studied and the solvent used affect the phenols content of the extract, and consequently also

affect the biological activity. Indeed, it has been established that the phenolic composition of the plant extract is responsible for its biological activities [41,44]. It appears that the hydroethanolic extract from the flowers of *Rosa damascena* displayed the best antioxidant effect, which could be explained by the presence of quercetin and kaempferol derivatives.

3.5.2. Total Antioxidant Capacity (TAC)

The phosphomolybdate technique was used to determine the total antioxidant capability of the hydroethanolic extract obtained from *Rosa damascena* flowers, by converting molybdenum molybdate ions MoO_4^{2-} to molybdenum molybdate ions MoO_4^{2+} , at pH acid. The total antioxidant capacity of the extract was examined as an amount of ascorbic acid equivalent to one gram of dried extract (mg EAA/1 g E). The regression equation of ascorbic acid was as follows: y = 0.0411x + 0.0159 (*R*2 = 0.9966) (Figure 4).

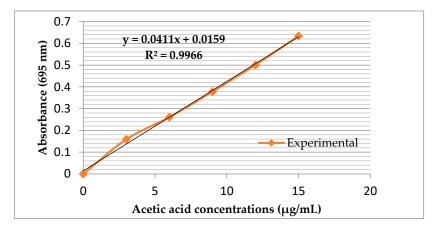


Figure 4. Calibration curve of the acetic acid (black represent linear curve).

The total antioxidant capacity of the hydroethanolic extract from *Rosa damascena* flowers was 213.22 mg EAA/1 g E. However, Ozkan et al. found that the ethanolic extract obtained from fresh flowers and spent flowers of *Rosa damascena* exhibited TAC with values varying between 372.26 ± 0.96 and 351.36 ± 0.84 mg/g extract (equivalent to ascorbic acid) [14]. Indeed, our study showed that the extract from *Rosa damascena* flowers exhibited powerful total antioxidant capacity, which may be due to the presence in its extract of quercetin and kaempferol with their derivatives and glycosides. Quercetin has been reported to act as free radical scavengers [2], and kaempferol and its glycosides contained in plants present antioxidant activity, with the ability to reduce the generation of free radicals and such reactive oxygen species [45].

3.6. Antibacterial Activity

In the present study, the antibacterial activity of the hydroethanolic extract obtained from the flowers of *Rosa damascena* was tested against *Escherichia coli* and *Salmonella typhimirium*, *Staphyloccocus aureus*, and *Listeria monocytogenes* through broth microdilution assay. As listed in Table 3, the MIC values of the *Rosa damascena* extract against all tested bacteria ranged from 20.83 ± 0.12 to 41.66 ± 0.15 mg/mL. The extract exhibited a bacteriostatic effect against *Escherichia coli* with an MBC/MIC value of 8, and a bactericidal effect against *Salmonella typhimirium* and *Staphyloccocus aureus* with an MBC/MIC value of 4, and against *Listeria monocytogenes* with an MBC/MIC value of 2.

A previous study reported the resistance of rose petal ethanol extract against *E. coli* [46]. Gram-negative bacteria are more resistant to plant extracts than gram-positive bacteria, because the membrane of gram-negative bacteria consists of phospholipids and lipopolysaccharides, which play an important role against antibacterial drugs [47,48].

Bacteria	MIC	MBC	MBC/MIC
Escherichia coli	20.83 ± 0.12	166.66 ± 0.12	8
Salmonella typhimirium	41.66 ± 0.15	166.66 ± 0.17	4
Staphyloccocus aureus	20.83 ± 0.20	83.33 ± 0.12	4
Listeria monocytogenes	20.83 ± 0.17	41.66 ± 0.19	2

Table 3. Minimum inhibitory and minimum bactericidal concentrations exhibited by the hydroethanolic extract from *Rosa damascena* flowers (mg/mL).

The results were consistent to those found by Talib and Mahasneh [49] who reported that *Rosa damascena* ethanolic extract showed antimicrobial activity against methicillin resistant *Staphyloccocus aureus*, *Listeria monocytogenes*, *Babillus cereus*, and *Candida albicans*.

Besides the type of bacteria, it is well demonstrated that the antimicrobial powers of the plant extract are closely related to its chemical composition and the presence of secondary metabolites (i.e., phenolics, flavonoids, and essential oil, etc.) [41,44].

In this study, the resulting antibacterial activity of the hydroethanolic extract of the flowers of *Rosa damascena* was dependent on the composition of phenolics in the extracts, which are mainly flavonoids (quercetin and kaempferol derivatives). A previous study reported that flavonoids (e.g., myricetin, quercetin, kaempferol, and phenolic acids) are responsible for the antimicrobial effects [44].

4. Conclusions

In this study, the phenolic composition and the antibacterial and antioxidant activities of the hydroethanolic extract obtained from the flowers of *Rosa damascena*, collected in the Middle Atlas of Morocco (Khenifra), were investigated. A total of 16 phenolic compounds belonging to tannins and flavonoids were positively identified in *Rosa damascena* flowers extract. This extract turned out to have an antibacterial and antioxidant power, which may be due to the presence in its composition of quercetin and kaempferol with their derivatives. Therefore, the flowers of *Rosa damascena* might be a promising natural antioxidant and antibacterial agent.

Author Contributions: Conceptualization, M.C., T.Z., and L.B.; methodology, M.C., T.Z., and L.B.; investigation, M.C., T.Z., L.B., Y.O.E.M., M.A., and A.B.; resources, F.C. and L.M.; data curation, Y.O.E.M. and A.B.; writing—original draft preparation, M.C., T.Z., L.B., and A.B.; writing—review and editing, F.C.; supervision, F.C. and A.B.; project administration, L.M. and F.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are thankful to Shimadzu and Merck Life Science Corporations for the continuous support.

Conflicts of Interest: The authors declare no conflict of interest.

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