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Antibiotic Activity of Rosemary Plant (*Rosmarinus officinalis*) Extract against Antibiotic Resistant Bacteria

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Abstract

The aim of the study was to determine the optimum parameters of the Rosmarinic acid (RA) extraction of the Rosemary plant (*Rosmarinus officinalis*) and to investigate the antibiotic activity of the extracted RA against antibiotic-resistant Gram-negative bacterial strains. The bacterial strains were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* as compared with the control strains of ATCC which were identified using the VITEK 2 system as parallel compared with the counter-control strains of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 13883. An extraction protocol using 70% ethanol was optimized for fresh leaves of *Rosmarinus officinalis* grown in Umm Al Quwain, UAE, after which the bacterial strains were exposed to the prepared extract. The absorption spectrum of the extracted RA was scanned between 200 - 450 nm and the peak of the maximum absorption was determined to be at (λmax 330 nm) which was used for RA quantification and standardization. The mean value of the moisture contents of rosemary leaves was determined to be 85.27%. The optimum time of RA-equivalent extraction was determined to be 45 min of extraction in 70% ethanol using both fresh and dry leaves. The local isolates of *Klebsiella pneumoniae* and *Escherichia coli* were resistant to ampicillin (10 µg), while both isolates were susceptible to imipenem (10 µg), ceftriaxone (30 µg), and ciprofloxacin (5 µg). The corresponding ATCC reference strains, *K. pneumoniae* ATCC 13883 and *E. coli* ATCC 25922, were included as quality-control strains and showed the expected susceptibility profiles. The antibiotic activity of the crude RA treatment on the tested local strains *Klebsiella pneumoniae, Pseudomonas aeruginosa, and Escherichia coli* showed no inhibition zone and therefore the strains were considered resistant. The control strain *K. pneumoniae* ATCC 13883 was sensitive to the RA treatment as it showed an inhibition zone of 12.3 mm diameter. The crude rosemary extract also showed

weak antibacterial activity against *P. aeruginosa* ATCC 27853, with inhibition zones increasing from 8.25 ± 0.12 mm at 100 mg/mL to 9.24 ± 0.13 mm at 400 mg/mL. The antibiotic activity of the crude RA treatment on the counter-control strain of *E. coli* ATCC 25922 showed no inhibition zone and therefore the strain was considered resistant. The inhibition zones for the combined treatment of crude extract with antibiotics against the local strains *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* were recorded as 29.34, 27.84, and 27.59 mm, respectively. Furthermore, the inhibition zones for the combined treatment of crude extract with antibiotics against *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 27853, and *E. coli* ATCC 25922 were recorded as 27.95, 22.13, and 30.10 mm, respectively. In the combination assay, neither rosemary crude extract nor equivalent pure RA enhanced the activity of the tested antibiotics, as all combination treatments produced smaller inhibition zones than the antibiotic alone. Overall, UAE-grown rosemary was validated as a quantifiable local source of RA, but its crude extract and equivalent RA exhibited a weak direct antibacterial effect and no antibiotic-enhancing effect against the Gram-negative bacteria under disk diffusion conditions.

Keywords

Rosmarinus officinalis, Rosmarinic Acid, Antibacterial Resistance, Disk Diffusion, Antibiotic Resistant Bacteria

1. Introduction

One of the most urgent challenges to global population health is antimicrobial resistance (AMR). In 2019 alone, drug-resistant bacteria directly caused 1.27 million deaths worldwide, and the United Nations forecasts that the number may rise to 10 million deaths annually by 2050, with the economic losses caused by these bacteria reaching up to 3 trillion a year [1]. More than 2.8 million cases of antibiotic-resistant infections are reported each year in the United States alone, which causes over 35,000 deaths [2]. The main mechanisms of AMR are well characterised. Bacteria use active efflux pumps to expel antibiotics, produce beta-lactamases and carbapenemases that enzymatically degrade drugs, alter antibiotic target sites through genetic mutation, reduce outer membrane permeability, and form biofilms that increase bacterial tolerance to antibiotic treatment [3].

The Gulf Cooperation Council (GCC) region, including the UAE, carries a significant AMR burden. Meropenem resistance in *Pseudomonas aeruginosa* across six GCC countries ranges from around 0% - 46%, and methicillin-resistant Staphylococcus aureus (MRSA) accounts for 25% - 35% of *S. aureus* isolates [4]. The surveillance data indicate substantial cephalosporin resistance among *Escherichia coli* and *Klebsiella pneumoniae*, with *E. coli* showing ceftriaxone/cefotaxime resistance of 33.0%/30.3% in the UAE in 2020 and 37.0%/32.0% in Abu Dhabi in 2023, while *K. pneumoniae* showed corresponding resistance levels of 29.0%/23.0%

and 24.0%/21.4%, respectively. In addition, MRSA prevalence was reported at 35.1% in the UAE in 2020 and 38.7% in Abu Dhabi in 2023 [5]. Nevertheless, AMR is a significant health problem in the United Arab Emirates, and over 200 deaths have been caused due to this resistance since 1990. In 2021, there were an estimated 970 AMR-associated deaths in UAE, with the highest mortality rate among adults aged 50 - 69 [6]. The most lethal combinations of pathogens and drugs were methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter baumannii* [6].

Medicinal plants have gained renewed attention as potential sources of novel antibacterial agents, particularly because the development of new antibiotics has slowed [7]. *Rosmarinus officinalis* L. (rosemary), a perennial aromatic shrub native to the Mediterranean basin, is cultivated successfully across arid Gulf environments. Chemical profiling of Saudi-grown rosemary confirms terpene-rich essential oil profiles comparable to Mediterranean cultivars [8], suggesting that Gulf-cultivated rosemary retains its phytochemical potential. The major water-soluble phenolic of rosemary is Rosmarinic acid (RA), which is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid [9]. RA has several antibacterial effects, including deterioration of bacterial cell membranes, interfering with their defence systems, and reducing their ability to form biofilms [10]. Reports have indicated that RA had an antibacterial effect with *S. aureus* and MRSA, a combination effect with vancomycin, ofloxacin, and amoxicillin with *S. aureus*, and with vancomycin with MRSA, suggesting that RA could be used as an antibiotic adjuvant. [11]. However, no systematic measurements of RA levels of rosemary cultivated under the Gulf climatic conditions and no thorough investigation of antibacterial activity of extracts prepared in the UAE against local isolates native to this region has been conducted. This study therefore, addresses these gaps by optimising ultrasound-assisted extraction and spectrophotometric quantification of RA, characterising the antibiotic resistance profiles of local isolates and evaluating its antibacterial activity.

2. Materials and Methods

2.1. Plant Material

Plants of *Rosmarinus officinalis* L. were obtained at a local market in Masafi, Ras Al Khaimah, UAE and then grown in Al Atheab, Umm Al Quwain, under greenhouse conditions using UAE sand as a substrate. The harvest was at a uniform vegetative pre-flowering stage of the plants. Fresh healthy leaves were picked in the evening, rinsed with distilled water, dried at 45˚C to constant weight and then ground to fine powder using the short-pulse electric grinding. Sampled material was placed in amber glass jars that were stored under refrigeration. Morphological leaf shape, aroma and growth habit were used to confirm species identity as *R. officinalis*.

2.2. Chemicals and Reagents

The most common extraction solvent was analytical-grade absolute ethanol (99.9%). Calibration was carried out using a RA reference standard (96% purity). All microbiological work was performed using Mueller-Hinton agar, nutrient broth and nutrient agar. Antibiotic discs (MASTDISCS™, Mast Group Ltd., UK) included Penicillin G (10 units), Ampicillin (AMP, 10 μg), Ciprofloxacin (CIP, 5 μg), Ceftriaxone (CRO, 30 μg), Imipenem (IMI, 10 μg), and Erythromycin (E, 15 μg).

2.3. Moisture Content Determination

Fresh rosemary leaves (~5 g per replicate, n = 4) were oven-dried at 100˚C ± 5˚C for 24 hours, cooled in a desiccator, and reweighed. Moisture content (percent) was calculated as

$$\frac{\left(\text{Fresh weight} - \text{Dry weight}\right)}{\text{Fresh weight}} \times 100$$

2.4. Rosmarinic acid Calibration Curve

The primary stock solution of RA was prepared in 70 (v/v) ethanol at 1000 μg/mL, and the purity of 96% was corrected. Ten-fold dilution was used to prepare a 100 μg/mL working solution. UV-Vis scanning (200 - 450 nm) in quadruplicate (Bio-Tek Epoch 2 microplate reader) was used to determine the maximum analytical wavelength (λmax). Calibration standards were prepared between 5 and 30 μg/mL (n = 4 per level) in 70% ethanol and absorbance was recorded at 330 nm with 200 μL per well. Linear regression was used to produce the calibration equation that was used to quantify RA.

2.5. Extraction Optimisation

Three parameters were optimised sequentially using ultrasound-assisted extraction (UAE) in an ultrasonic water bath at 45˚C with 70% ethanol: 1) plant-to-solvent ratio (1:40, 1:30, 1:20, 1:10 w/v, fixed at 10 mL; n = 4 per ratio); 2) extraction time (20, 30, 45, 60 min at 1:20 w/v; n = 4); and 3) number of successive extraction cycles (up to 3 cycles; 0.25 g dry material, 5 mL per cycle; n = 4). All extracts were filtered through 0.2 μm syringe filters, diluted 30-fold with 70% ethanol, and absorbance was measured at 330 nm. RA-equivalent concentrations were calculated from the calibration equation corrected for the 30-fold dilution factor. Precision was assessed by

$$\text{CV\%} = \frac{\text{SD}}{\text{Mean}} \times 100$$

Optimum extraction conditions were selected descriptively based on three criteria: higher RA-equivalent recovery, higher absorbance suitable for RA-equivalent calculation, and lower coefficient of variation among four replicates.

2.6. Large-Scale Extraction, Concentration, and Freeze-Drying

Ten grams of dried rosemary powder was extracted under optimised UAE conditions. The pooled extract was concentrated by rotary evaporation at 35˚C under reduced pressure, and the flask was rinsed with 90% ethanol to recover residual extract. All fractions were transferred to pre-weighed Falcon tubes and freeze-dried (lyophilised) over approximately four days. Crude extract mass was determined gravimetrically using the formula:

$$\text{Extraction yield}(\%) = \frac{\text{Crude extract mass}}{\text{Initial dry plant mass}} \times 100$$

RA-equivalent content in the crude extract was estimated spectrophotometrically and expressed as mg RA equivalents per gram of crude extract. Because rosemary contains other phenolic compounds that may absorb at 330 nm, the values are reported as RA equivalents rather than specific RA-equivalent concentrations.

2.7. Bacterial Strains and Identification

Local isolates of *K. pneumoniae, P. aeruginosa and E. coli* were used alongside ATCC reference strains (LYFO DISK®): *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, and *P. aeruginosa* ATCC 27853. All the 3 tested strains were isolated from urine samples and stored in the microbiology laboratory for routine work with the alphabetical serial number D2, E1, G1. Stocks were maintained at −80˚C in 50% (v/v) glycerol. Local isolates were identified by VITEK® 2 (bioMérieux) using GN identification cards following Gram staining.

2.8. Antibiotic Susceptibility Testing

Susceptibility was assessed by Kirby-Bauer disk diffusion on Mueller-Hinton agar per CLSI M100, 36th edition [12]. Bacterial suspensions were adjusted to 0.5 McFarland (~1.5×10^8 CFU/mL) by DensiCheck. Antibiotic discs were placed on inoculated plates and incubated at 37˚C for 24 h. Zone diameters were measured and interpreted (S/I/R) per CLSI organism-specific breakpoints. Four independent replicates (n = 4) were performed. ATCC reference strains served as quality controls.

2.9. Antibacterial Activity of Crude Extract and Pure RA

The freeze-dried rosemary crude extract was dissolved in 70% ethanol to make four concentrations of 100, 200, 300, and 400 mg/mL. The concentrations of these crude extracts were made depending on the weight of the crude extracts. For 2.50 mL final volume, 0.2500, 0.5000, 0.7500, and 1.0000 g of crude extract were dissolved in 2.50 mL of 70% ethanol to prepare the 100, 200, 300, and 400 mg/mL solutions, respectively.

The RA-equivalent content of the crude extract was determined to be 55.74 mg RA equivalents/g crude extract. Thus, the corresponding pure RA-equivalent concentrations were determined by the following equation:

$$\text{Equivalent pure RA concentration} = \frac{\text{crude extract concentration} \times 55.74}{1000}$$

Based on this, the corresponding equivalent pure RA-equivalent concentrations of 100, 200, 300, and 400 mg/mL crude extract were 5.574, 11.148, 16.722 and 22.296 mg/mL, respectively. As an illustration, the RA-equivalent concentration of 400 mg/mL crude extract was computed as follows:

$$\frac{400 \times 55.74}{1000} = 22.296\,\text{mg/mL}$$

Sterile filter paper discs were loaded with 7.5 µL of each solution, air-dried, and placed on Mueller-Hinton agar plates inoculated with standardised suspensions of six strains: *E. coli* ATCC 25922, *E. coli* local (E1), *P. aeruginosa* ATCC 27853, *P. aeruginosa* local (G1), *K. pneumoniae* ATCC 13883, and K. *pneumoniae* local (D2). The negative control was a 70% ethanol disc and the positive control was a standard antibiotic disc. Incubation of plates was done at 37˚C over 24 h and inhibition zones were measured.

2.10. Combination Disk Diffusion Assay

The highest crude extract concentration (400 mg/mL) and its equivalent pure RA were combined with antibiotics selected by strain susceptibility: CIP 5 µg for *E. coli* and *Pseudomonas*, CRO 30 µg for *Klebsiella*. Three-disc conditions were tested per plate: 1) antibiotic alone; 2) antibiotic + crude extract (7.5 µL of 400 mg/mL applied onto the antibiotic disc); 3) antibiotic + equivalent RA. Assays were conducted on three ATCC strains and three local isolates in triplicate.

2.11. Statistical Analysis

Findings are presented in the form of mean ± standard deviation. Coefficient of variation (CV percent) was calculated as:

$$\text{CV\%} = \frac{\text{SD}}{\text{Mean}} \times 100$$

Linear regression was used to construct a calibration curve. The extraction optimisation and antibacterial assays were evaluated descriptively based on mean values, CV%, and inhibition-zone comparisons. No inferential statistical test was applied. All calculations were performed using Microsoft Excel.

3. Results

3.1. Moisture Content of Fresh Rosemary Leaves

The fresh rosemary leaves were analysed using the gravimetric analysis to identify the moisture content of the leaves in four replicates. Table 1 summarises the results. The values of individual moisture content were between 85.03 and 85.46 percent among replicates. The mean moisture level was 85.27 + 0.18%, which implied that the moisture level was high and reproducible.

Table 1. Moisture content of fresh rosemary leaves (*Rosmarinus officinalis*) (n = 4).

Sample ID	Fresh weight (g)	Dry weight (g)	Moisture content (%)
1	5.0060	0.7281	85.46
2	5.0012	0.7372	85.26
3	5.0074	0.7500	85.03
4	5.0002	0.7336	85.33
Mean ± SD	5.0037 ± 0.0035	0.7372 ± 0.0093	85.27 ± 0.18

3.2. Maximum Analytical Wavelength and Calibration Curve

The UV-Vis absorption spectrums of R measured in the wavelength range of 200-450 nm exhibited similar spectral patterns in four replicates with a maximum absorbance (lmax) of 330 nm (Figure 1). At this wavelength, the measured absorbance values for calibration standards increased with concentration from 5 to 30 μg/mL, yielding mean absorbance values of 0.50 ± 0.01, 0.77 ± 0.01, 1.02 ± 0.01, 1.24 ± 0.01, 1.66 ± 0.02, and 1.82 ± 0.01, respectively (Table 2). The analysis of the calibration data using linear regression yielded the equation y = 0.0542x + 0.2193 with a coefficient of determination (R^2) of 0.9905 (Figure 2).

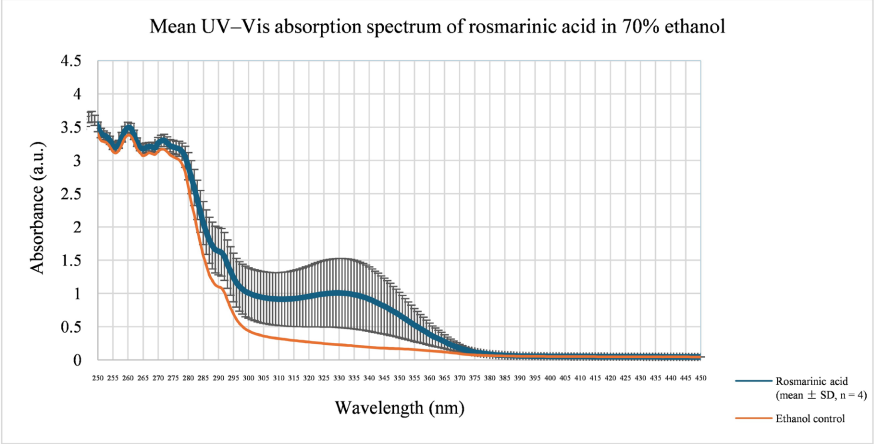

Figure 1. UV-Vis absorption spectrum of Rosmarinic acid in 70% (v/v) ethanol and ethanol as control over the wavelength range of 250 - 450 nm, expressed as mean absorbance ± SD from four independent replicates (n = 4).

Table 2. Rosmarinic acid calibration standards and corresponding mean absorbance values at 330 nm measured using a BioTek Epoch 2 microplate reader (n = 4).

Standard ID	Concentration (μg/mL)	Mean absorbance ± SD (330 nm)
S1	5	0.50 ± 0.01
S2	10	0.77 ± 0.01
S3	15	1.02 ± 0.01
S4	20	1.24 ± 0.01
S5	25	1.66 ± 0.02
S6	30	1.82 ± 0.01

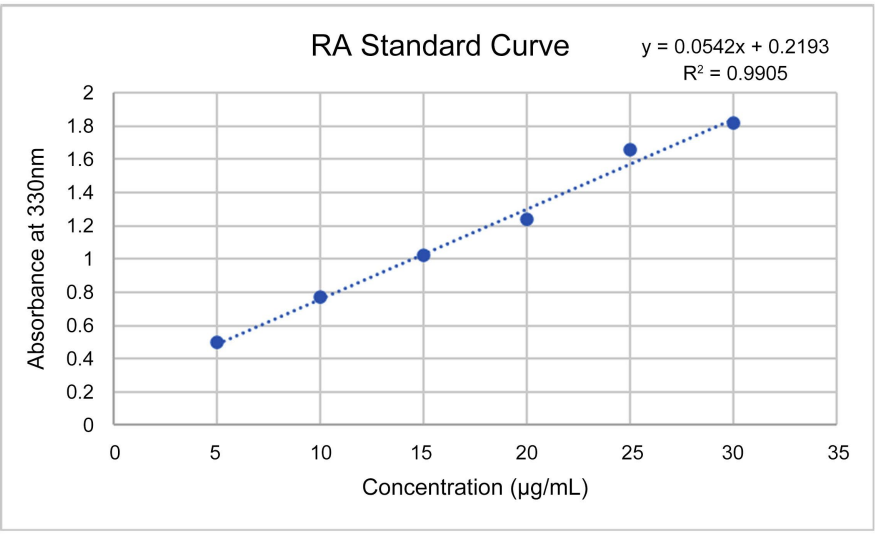

Figure 2. Standard curve of Rosmarinic acid at 330 nm.

3.3. Comparison of Fresh versus Dried Plant Material for RA Extraction

When extraction was performed on equivalent dry matter (0.50 g dry matter per 10 mL 70% ethanol), dried rosemary produced a marginally higher mean absorbance (0.912 ± 0.058; CV = 6.41%) than fresh rosemary (0.854 ± 0.092; CV = 10.79%) (Table 3). Converting these absorbance values using the calibration equation, the RA-equivalent yield from dried material was 7.67 mg/g dry weight compared to 7.03 mg/g dry weight from fresh material.

Table 3. Comparison of absorbance values (330 nm) and calculated RA-equivalent yields from fresh and dried rosemary extracts on a dry-weight basis (n = 4).

Parameter	Fresh rosemary	Dried rosemary
Mean absorbance ± SD	0.854 ± 0.092	0.912 ± 0.058
CV (%)	10.79	6.41
RA-equivalent concentration in diluted sample (µg/mL)	11.71	12.78
RA-equivalent concentration in original extract (mg/mL)	0.351	0.383
Total RA-equivalent content extracted (mg)	3.51	3.83
Yield (mg/g dry matter)	7.03	7.67

3.4. Optimisation of Plant-to-Solvent Ratio

Four plant-to-solvent ratios were evaluated to determine the optimal balance between total RA-equivalent recovery and extraction efficiency (Table 4). Mean absorbance at 330 nm increased progressively from 0.521 ± 0.082 (1:40 ratio, 0.25 g dry mass) to 0.901 ± 0.038 (1:10 ratio, 1.00 g dry mass). However, when efficiency was expressed as RA-equivalent yield per gram of dry plant material, the inverse

relationship was observed: yield decreased from 6.68 mg/g at the 1:40 ratio to 3.77 mg/g at the 1:10 ratio, consistent with solvent saturation effects at higher solid loadings. The 1:20 ratio (0.50 g dry mass, 10 mL 70% ethanol) achieved an acceptable balance between total RA-equivalent recovery (2.53 mg per extraction) and extraction efficiency (5.07 mg/g).

Table 4. Effect of solid-to-solvent ratio on absorbance at 330 nm, total RA-equivalent recovery, and extraction yield from dry rosemary powder (n = 4).

Ratio (w/v)	Dry weight (g)	Mean absorbance ± SD	CV (%)	Total RA-equivalent content (mg)	Yield (mg/g)
1:40	0.25	0.521 ± 0.082	15.7	1.67	6.68
1:30	0.33	0.583 ± 0.115	19.7	2.01	6.10
1:20	0.50	0.677 ± 0.081	12.0	2.53	5.07
1:10	1.00	0.901 ± 0.038	4.2	3.77	3.77

3.5. Optimisation of Extraction Time

Mean absorbance values obtained at 20 min (0.712 ± 0.060, CV = 8.40%), 30 min (0.718 ± 0.049, CV = 6.80%), and 45 min (0.719 ± 0.036, CV = 5.00%) were closely comparable, suggesting that the majority of extractable RA-equivalent content is released within the first 20 minutes of sonication (Table 5). The longest extraction time (60 minutes) yielded the largest mean absorbance (0.804 ± 0.095) but with a significantly higher CV (11.80%).

Table 5. Absorbance values at 330 nm obtained from the first extraction of dry rosemary using ultrasound-assisted extraction at different extraction times (n = 4).

Extraction time (min)	Mean absorbance ± SD	CV (%)	RA concentration (μg/mL)	RA yield (mg/g)
20	0.712 ± 0.060	8.4	272.71	5.44
30	0.718 ± 0.049	6.8	276.03	5.50
45	0.719 ± 0.036	5.0	276.59	5.54
60	0.804 ± 0.095	11.8	323.63	6.45

3.6. Optimisation of Successive Extraction Cycles

Three successive extraction cycles were performed on the same 0.25 g dry rosemary material using 5 mL 70% ethanol per cycle (Table 6). The highest percentage of RA-equivalent content was obtained in the first extraction (mean absorbance 0.8945 ± 0.0438, CV = 4.90%; yield 7.47 mg/g), which recovered 61.04% of the total RA-equivalent content recovered in three cycles. The second extraction yielded an additional 25.38% (mean absorbance 0.500 ± 0.030, CV = 6.0%), and the third cycle contributed only 13.58% of the total (mean absorbance 0.3695 ± 0.0343, CV = 9.3%). The cumulative RA-equivalent recovery after two cycles was 86.42% of the total.

Table 6. Mean absorbance values and calculated RA-equivalent yields from successive ultrasound-assisted extraction cycles of dry rosemary (n = 4, per cycle).

Extraction No.	Mean absorbance ± SD	CV (%)	Yield this cycle (mg/g)	% of total RA-equivalent content
1	0.8945 ± 0.0438	4.90	7.47	61.04
2	0.500 ± 0.030	6.00	3.11	25.38
3	0.3695 ± 0.0343	9.30	1.66	13.58

3.7. Crude Extract Yield and RA-Equivalent Content

Large-scale extraction of 10.0296 g dried rosemary yielded 1.69025 g freeze-dried crude extract (Table 7). The crude extract (diluted 1:30) was spectrophotometrically quantified at 330 nm with an absorbance of 0.320. The use of the calibration equation gave an RA-equivalent concentration of 1.858 μg/mL in the diluted sample, which is equivalent to 55.74 mg RA equivalents/g crude extract (0.55738 mg/0.01 g). The detailed calculation is presented in Table 8.

Table 7. Weights of freeze-dried crude rosemary extract collected in individual Falcon tubes and calculated total yield.

Tube No.	Empty tube weight (g)	Final tube weight (g)	Net crude extract mass (g)
1	12.6757	13.1703	0.4946
2	12.87585	13.2695	0.39365
3	12.8375	13.6071	0.7696
4*	12.6365	12.6689	0.0324
Total	—	—	1.69025

* Tube 4 represents the additional fraction recovered from rinsing the rotary evaporator flask with 90% ethanol.

Table 8. Calculation of crude extract yield and RA-equivalent content of freeze-dried rosemary extract.

Parameter	Calculation	Result
Initial dry rosemary powder mass	—	10.0296 g
Total freeze-dried crude extract mass	—	1.69025 g
Extraction yield (%)	(1.69025/10.0296) × 100	16.85%
Crude extract used for RA analysis	—	0.0100 g
Solvent volume used	—	10.00 mL
Absorbance of undiluted sample at 330 nm	—	2.133
Dilution factor	—	1:30
Absorbance of diluted sample at 330 nm	—	0.320
Calibration curve equation	y = 0.0542x + 0.2193	—
RA-equivalent concentration in diluted sample	x = (0.320 − 0.2193)/0.0542	1.858 μg/mL
RA-equivalent concentration in original solution	1.858 × 30	55.738 μg/mL
Total RA-equivalent content in prepared solution	55.738 × 10.00	557.38 μg = 0.55738 mg
RA-equivalent content in crude extract	0.55738/0.0100	55.74 mg/g crude extract

3.8. Antibiotic Susceptibility Profiles

Tables 9-11 provide data on antibiotic susceptibility between local isolates and ATCC reference strains. The three local Gram-negative isolates were all ampicillin-resistant (zone diameter < 13 mm). The local *E. coli* and *K. pneumoniae* isolates were susceptible to imipenem, ceftriaxone, and ciprofloxacin. Intermediate susceptibility of local *P. aeruginosa* to imipenem and strong susceptibility to ciprofloxacin were observed. However, ceftriaxone was also tested against *P. aeruginosa*; however, no S/I/R interpretation was assigned because no CLSI breakpoint was applied for this organism-antibiotic combination. All ATCC QC strains performed within acceptable CLSI ranges (CV% < 7%).

Table 9. Antibiotic susceptibility of local *Klebsiella pneumoniae* versus ATCC 13883.

Antibiotic (disc load)	Local isolate (mm)	CV%	ATCC 13883 (mm)	CV%	CLSI breakpoint (mm)	Local S/I/R
Imipenem (10 µg)	26.48 ± 0.98	3.7	24.90 ± 0.84	3.4	S ≥ 23/R ≤ 19	S
Ceftriaxone (30 µg)	30.01 ± 2.35	7.8	27.55 ± 0.45	1.6	S ≥ 23/R ≤ 19	S
Ciprofloxacin (5 µg)	26.28 ± 0.23	0.9	28.75 ± 0.65	2.3	S ≥ 21/R ≤ 15	S
Ampicillin (10 µg)	5.23 ± 3.56	68.1	6.22 ± 4.25	68.3	S ≥ 17/R ≤ 13	R
Penicillin G (10 µg)	0.00 ± 0.00	0.00%	0.00 ± 0.00	0.00%	-	-
Erythromycin (15 µg)	0.00 ± 0.00	-	8.72 ± 0.32	3.6	-	-

S = susceptible; I = intermediate; R = resistant.

Table 10. Antibiotic susceptibility of local *Escherichia coli* versus ATCC 25922.

Antibiotic (disc load)	Local isolate (mm)	CV%	ATCC 25922 (mm)	CV%	CLSI breakpoint (mm)	Local S/I/R
Imipenem (10 µg)	28.82 ± 0.83	2.9	28.57 ± 1.01	3.5	S ≥ 23/R ≤ 19	S
Ceftriaxone (30 µg)	31.17 ± 0.39	1.3	31.24 ± 0.87	2.8	S ≥ 23/R ≤ 19	S
Ciprofloxacin (5 µg)	29.82 ± 0.80	2.7	33.16 ± 0.98	3.0	S ≥ 21/R ≤ 15	S
Ampicillin (10 µg)	9.90 ± 0.57	5.8	21.40 ± 0.51	2.4	S ≥ 17/R ≤ 13	R
Penicillin G (10 µg)	0.00 ± 0.00	0.00%	0.00 ± 0.00	0.00%	-	-
Erythromycin (15 µg)	8.67 ± 0.46	5.3	9.31 ± 0.46	4.9	-	-

S = susceptible; R = resistant.

Table 11. Antibiotic susceptibility of local *Pseudomonas aeruginosa* versus ATCC 27853.

Antibiotic (disc load)	Local isolate (mm)	CV%	ATCC 27853 (mm)	CV%	CLSI breakpoint (mm)	Local S/I/R
Imipenem (10 µg)	18.98 ± 1.17	6.2	19.10 ± 0.58	3.1	S ≥ 22/R ≤ 18	I
Ceftriaxone (30 µg)	20.05 ± 1.24	6.2	10.24 ± 0.20	1.96	-	-
Ciprofloxacin (5 µg)	34.37 ± 0.16	0.5	29.16 ± 1.81	6.2	S ≥ 21/R ≤ 15	S
Ampicillin (10 µg)	0.00 ± 0.00	-	0.00 ± 0.00	-	-	-
Erythromycin (15 µg)	0.00 ± 0.00	-	0.00 ± 0.00	-	-	-

I = intermediate; R = resistant.

3.9. Antibacterial Activity of Crude Extract and Equivalent RA

The antibiotic control yielded clear inhibition zones in all the bacterial strains tested whereas the 70% ethanol control did not yield any inhibition. No inhibition zones were observed for either rosemary crude extract or equivalent RA-equivalent concentrations (100 - 400 mg/mL) against *E. coli* ATCC, *E. coli* local isolate, *Pseudomonas* local isolate, and *Klebsiella pneumoniae* local isolate, with all measurements recorded as 0.00 mm. However, weak inhibition was observed against *Pseudomonas aeruginosa* ATCC treated with crude extract, with mean inhibition zones of 8.25 ± 0.12 mm, 8.52 ± 0.11 mm, 8.92 ± 0.12 mm, and 9.24 ± 0.13 mm at concentrations of 100, 200, 300, and 400 mg/mL, respectively. Detectable inhibition was also observed only in *Klebsiella pneumoniae* ATCC treated with crude extract, with mean inhibition zones of 11.19 ± 0.31 mm, 11.11 ± 0.60 mm, 11.59 ± 0.57 mm, and 12.30 ± 0.29 mm at concentrations of 100, 200, 300, and 400 mg/mL, respectively. No inhibition was recorded for equivalent RA at any concentration against any of the tested strains (Table 12).

Table 12. Inhibition zone diameters (mm) of crude rosemary extract and equivalent pure rosmarinic acid against selected bacterial strains. Data are expressed as mean ± standard deviation (SD), with coefficient of variation (CV%).

Strain	Sample	Conc. (mg/mL)	Mean ± SD (mm)	CV (%)
E. coli ATCC	Crude extract	100 - 400	0.00 ± 0.00	0.00
E. coli (Local)	Crude extract	100 - 400	0.00 ± 0.00	0.00
P. aeruginosa ATCC	Crude extract	100	8.25 ± 0.12	1.45
		200	8.52 ± 0.11	1.29
		300	8.92 ± 0.12	1.35
		400	9.24 ± 0.13	1.41
P. aeruginosa (Local G1)	Crude extract	100 - 400	0.00 ± 0.00	0.00
K. pneumoniae ATCC	Crude extract	100	11.19 ± 0.31	2.73
		200	11.11 ± 0.60	5.37
		300	11.59 ± 0.57	4.93
		400	12.30 ± 0.29	2.36
K. pneumoniae (Local)	Crude extract	100 - 400	0.00 ± 0.00	0.00
E. coli ATCC	Pure RA	100 - 400	0.00 ± 0.00	0.00
E. coli (Local)	Pure RA	100 - 400	0.00 ± 0.00	0.00
P. aeruginosa ATCC	Pure RA	100 - 400	0.00 ± 0.00	0.00
P. aeruginosa (Local)	Pure RA	100 - 400	0.00 ± 0.00	0.00
K. pneumoniae ATCC	Pure RA	100 - 400	0.00 ± 0.00	0.00
K. pneumoniae (Local)	Pure RA	100 - 400	0.00 ± 0.00	0.00

3.10. Combination Effect of Crude Extract and RA with Antibiotics

The antibiotic control resulted in quantifiable inhibition areas on all strains tested with an average diameter of 24.60 + 0.12 mm to 31.54 + 0.26 mm. When used in combination, both antibiotic + crude extract and antibiotic + RA showed a decrease in the inhibition zones in all strains. For *Klebsiella* ATCC, inhibition zones decreased from 27.98 ± 0.54 mm (antibiotic) to 27.95 ± 0.23 mm (antibiotic + crude) and 25.52 ± 0.12 mm (antibiotic + RA). Similar reductions were observed in *Pseudomonas* ATCC (24.60 ± 0.12 mm to 22.13 ± 0.54 mm and 21.59 ± 0.42 mm), *E. coli* ATCC (31.22 ± 0.66 mm to 30.10 ± 0.06 mm and 27.80 ± 0.27 mm), *Klebsiella* (local) (30.13 ± 0.75 mm to 29.34 ± 0.70 mm and 27.73 ± 0.64 mm), *Pseudomonas* (local) (31.54 ± 0.26 mm to 27.84 ± 0.53 mm and 24.88 ± 0.11 mm), and *E. coli* (local) (30.09 ± 0.92 mm to 27.59 ± 0.60 mm and 25.56 ± 0.53 mm), respectively (Table 13).

Table 13. Inhibition zone diameters (mm) of antibiotic alone and in combination with rosemary crude extract (400 mg/mL) or equivalent pure RA, tested against ATCC reference strains and local isolates.

Strain	Treatment	Mean ± SD (mm)
***Klebsiella* ATCC**	Antibiotic	27.98 ± 0.54
	Antibiotic + Crude	27.95 ± 0.23
	Antibiotic + RA	25.52 ± 0.12
***Pseudomonas* ATCC**	Antibiotic	24.60 ± 0.12
	Antibiotic + Crude	22.13 ± 0.54
	Antibiotic + RA	21.59 ± 0.42
***E. coli* ATCC**	Antibiotic	31.22 ± 0.66
	Antibiotic + Crude	30.10 ± 0.06
	Antibiotic + RA	27.80 ± 0.27
***Klebsiella* (Local)**	Antibiotic	30.13 ± 0.75
	Antibiotic + Crude	29.34 ± 0.70
	Antibiotic + RA	27.73 ± 0.64
***Pseudomonas* (Local)**	Antibiotic	31.54 ± 0.26
	Antibiotic + Crude	27.84 ± 0.53
	Antibiotic + RA	24.88 ± 0.11
***E. coli* (Local)**	Antibiotic	30.09 ± 0.92
	Antibiotic + Crude	27.59 ± 0.60
	Antibiotic + RA	25.56 ± 0.53

4. Discussion

4.1. Extraction and Quantification of RA

The current experiment demonstrated that the *Rosmarinus officinalis* grown in

the UAE is a quantifiable source of RA, with dry leaves containing slightly more RA-equivalent content than fresh leaves (7.67 vs. 7.03 mg/g dry weight) and lower variability. This is in accordance with the ultrasound extraction study by Albu *et al.* [13], who reported the enhancement of the dried rosemary extraction as compared to the fresh material and with Paniwnyk *et al.* [14], who demonstrated that sonication enhances the extraction efficiency of rosemary antioxidants, as well as reducing the time of extraction. Likewise, Zu *et al.* [15] also found that RA is recoverable under the ultrasound-assisted conditions in rosemary and could be observed at 330 nm, which is the same wavelength in the current study.

The optimisation findings were also in agreement with earlier extraction studies. The findings of the current investigation indicated that as the amount of plant loading was increased, the total material recovered rose; however, the yield per gram decreased. 20 - 45 min of extraction times yielded comparable absorbance readings, and two cycles yielded 86.42% of the total extractable RA-equivalent content. Such results are consistent with Paniwnyk *et al.* [14], who determined that ultrasound primarily increases the initial extraction phase, with lesser improvements in extraction, and with Bellumori *et al.* [16], who observed that the recovery of rosemary phenolic under ultrasound is highly condition-dependent and selective instead of merely being dependent on extension of extraction. Overall, these findings justify the chosen protocol as a promising approach to extracting RA-equivalent-rich extracts of UAE-grown rosemary.

The crude extract yield of 16.85 percent and an estimated 55.74 mg /g RA equivalents crude extract also suggest that rosemary grown under the UAE conditions has retained its useful phenolic content. Previously reported rosemary studies have also indicated that ethanol-based ultrasound extraction could yield phenolic-rich fractions expressed as RA equivalents, but with varying absolute values depending on cultivar, pretreatment, solvent composition, and analytical platform due to variation in studies [13]. In this respect, the current information positions rosemary cultivated in the UAE as a viable local source of RA-containing extract in the expanded literature on extraction.

4.2. Antibacterial Activity against Gram-Negative Pathogens

In the current experiment, there was mild direct antibacterial activity of the rosemary preparations against Gram-negative strains tested. Crude extract demonstrated weak inhibition of *Klebsiella pneumoniae* ATCC with zones of approximately 11 - 12 mm, and weak inhibition of *Pseudomonas aeruginosa* ATCC with zones of approximately 8 - 9 mm, whereas pure RA had no inhibition of that strain, and neither preparation inhibited the tested *Escherichia coli* strains or the local *Pseudomonas aeruginosa* isolate in the standalone test. The profile is more similar to the results of Khalil and Al-Lebathi [17], who found only significant direct activity of ethanolic rosemary extract against multi-antibiotic-resistant Gram-negative bacteria and only determined inhibition zones of 7.50 ± 0.50 mm of *P. aeruginosa* and 6.00 ± 0.20 mm of *K. pneumoniae* at 100 mg/ml.

At the same time, the present results are weaker than those of Husein *et al.* [18], who reported measurable antibacterial activity of rosemary extract against urinary isolates, including inhibition zones up to 16 mm in *E. coli*, MIC values of 4 mg/mL for *E. coli* and 32 mg/mL for *Enterococcus faecalis*, and marked biofilm inhibition. This comparison puts the existing results in the literature with great variability in rosemary extract activity depending on the set of strain and assay format. The present study, therefore, aligns more closely with reports of modest Gram-negative activity than with studies showing stronger inhibition in broth-based or biofilm-focused systems.

A further result of the present study is that weak activity was observed with the crude extract against *K. pneumoniae* ATCC and *P. aeruginosa* ATCC, whereas equivalent pure RA showed no activity. This difference is in line with the larger RA literature synthesized by Kernou *et al.* [19], who found that antibacterial effects of RA and its derivatives are strongly reliant on the bacterial model and formulation, and with Ivanov *et al.* [10], who reported RA activity to be variable among organisms and experimental systems. In that scenario, the current evidence indicates that any low activity detected in this case can rather be ascribed to the crude phytochemical mixture than RA.

4.3. Antibiotic Interaction of Crude Extract and RA

The combination disk diffusion assay revealed that the combination of the chosen antibiotics with either rosemary crude extract or the same amount of pure RA did not enhance the antibacterial activity against any of the tested strains. The combined treatments showed no interaction under the current experimental conditions as the inhibition zones of the combined treatments were always equal or smaller than those of the antibiotic alone in both ATCC reference strains and local isolates *of Klebsiella pneumoniae, Pseudomonas aeruginosa, and Escherichia coli.* Furthermore, in several strains, the antibiotic-plus-RA condition had smaller inhibition zones than the antibiotic-plus-crude-extract condition, which suggests that pure RA did not show a greater antibiotic adjuvant effect than the crude extract in this assay. These results are in contrast to the earlier reports of combination effects between rosmarinic acid and antibiotics against *Staphylococcus aureus* and MRSA, suggesting that these effects are highly dependent on bacterial species, antibiotic selection, and assay format [11]. Likewise, differences from more recent rosemary-based combination studies may reflect variation in organism type, extract preparation, and test design [17].

Overall, these results present valuable evidence that the antibiotic-modifying effect of rosemary crude extract and RA is assay- and strain-specific, which provides a clear foundation to continue the research with more sensitive techniques and wider bacterial models.

5. Conclusion

Conclusively, this research determines that *Rosmarinus officinalis* grown in the

UAE is a local source of RA, and ultrasound-aided extraction with 70% ethanol is a viable and consistent method of extracting and quantifying it. The optimized protocol produced quantifiable amounts of RA-equivalent content in dry plant material and crude extract, which contributes to the phytochemical quality of rosemary grown in the UAE conditions. The microbiological results also revealed that the local Gram-negative isolates possessed unique and clinically pertinent susceptibility profiles, which offered a suitable platform on which rosemary-derived preparations can be assessed. Though the direct activity of crude rosemary extract was limited to weak inhibition of *K. pneumoniae* ATCC and *P. aeruginosa* ATCC, and the effect of pure RA was low in the conditions of disk diffusion employed, the study provides valuable background data on extract dynamics, isolate response, and plant-antibiotic interaction within a UAE environment. Future research should build upon this study by employing broth microdilution checkerboard assays, time-kill dynamics and biofilm-based models to further elucidate antibiotic-modifying possibilities of RA and whole rosemary extract against their clinically significant resistant pathogens.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

[1] Tang, K.W.K., Millar, B.C. and Moore, J.E. (2023) Antimicrobial Resistance (AMR). *British Journal of Biomedical Science*, **80**, Article 11387. https://doi.org/10.3389/bjbs.2023.11387

[2] Sutton, K.F. and Ashley, L.W. (2024) Antimicrobial Resistance in the United States: Origins and Future Directions. *Epidemiology and Infection*, **152**, e33. https://doi.org/10.1017/s0950268824000244

[3] C Reygaert, W. (2018) An Overview of the Antimicrobial Resistance Mechanisms of Bacteria. *AIMS Microbiology*, **4**, 482-501. https://doi.org/10.3934/microbiol.2018.3.482

[4] Alatoom, A., Alattas, M., Alraddadi, B., Moubareck, C.A., Hassanien, A., Jamal, W., *et al.* (2024) Antimicrobial Resistance Profiles of *Pseudomonas aeruginosa* in the Arabian Gulf Region over a 12-Year Period (2010-2021). *Journal of Epidemiology and Global Health*, **14**, 529-548. https://doi.org/10.1007/s44197-024-00191-y

[5] Al Marzooqi, A. and Al Kaabi, S. (2023) Abu Dhabi Antimicrobial Resistance (AMR) Surveillance Report 2023. Abu Dhabi Public Health Center.

[6] Institute for Health Metrics and Evaluation (2021) The Burden of Antimicrobial Resistance (AMR) in the United Arab Emirates. University of Washington.

[7] Allemailem, K.S. (2021) Antimicrobial Potential of Naturally Occurring Bioactive Secondary Metabolites. *Journal of Pharmacy and Bioallied Sciences*, **13**, 155-162.

https://doi.org/10.4103/jpbs.jpbs_753_20

[8] Guetat, A., Al-Ghamdi, F.A. and Osman, A.K. (2014) 1,8-Cineole, α-Pinene and Verbenone Chemotype of Essential Oil of Species *Rosmarinus officinalis* L. from Saudi Arabia. *International Journal of Herbal Medicine*, **2**, 137-141. https://www.florajournal.com/archives/2014/vol2issue2/PartC/30a.1.pdf

[9] Guan, H., Luo, W., Bao, B., Cao, Y., Cheng, F., Yu, S., *et al.* (2022) A Comprehensive Review of Rosmarinic Acid: From Phytochemistry to Pharmacology and Its New Insight. *Molecules*, **27**, Article 3292. https://doi.org/10.3390/molecules27103292

[10] Ivanov, M., Kostić, M., Stojković, D. and Soković, M. (2022) Rosmarinic Acid-Modes of Antimicrobial and Antibiofilm Activities of a Common Plant Polyphenol. *South African Journal of Botany*, **146**, 521-527. https://doi.org/10.1016/j.sajb.2021.11.050

[11] Ekambaram, S., Perumal, S., Balakrishnan, A., Marappan, N., Gajendran, S. and Viswanathan, V. (2016) Antibacterial Synergy between Rosmarinic Acid and Antibiotics against Methicillin Resistant Staphylococcus Aureus. *Journal of Intercultural Ethnopharmacology*, **5**, Article 358. https://doi.org/10.5455/jice.20160906035020

[12] CLSI (2026) Performance Standards for Antimicrobial Susceptibility Testing. 36th Edition, CLSI Supplement M100, Clinical and Laboratory Standards Institute.

[13] Albu, S., Joyce, E., Paniwnyk, L., Lorimer, J.P. and Mason, T.J. (2004) Potential for the Use of Ultrasound in the Extraction of Antioxidants from *Rosmarinus officinalis* for the Food and Pharmaceutical Industry. *Ultrasonics Sonochemistry*, **11**, 261-265. https://doi.org/10.1016/j.ultsonch.2004.01.015

[14] Paniwnyk, L., Cai, H., Albu, S., Mason, T.J. and Cole, R. (2009) The Enhancement and Scale up of the Extraction of Anti-Oxidants from *Rosmarinus officinalis* Using Ultrasound. *Ultrasonics Sonochemistry*, **16**, 287-292. https://doi.org/10.1016/j.ultsonch.2008.06.007

[15] Zu, G., Zhang, R., Yang, L., Ma, C., Zu, Y., Wang, W., *et al.* (2012) Ultrasound-Assisted Extraction of Carnosic Acid and Rosmarinic Acid Using Ionic Liquid Solution from *Rosmarinus officinalis*. *International Journal of Molecular Sciences*, **13**, 11027-11043. https://doi.org/10.3390/ijms130911027

[16] Bellumori, M., Innocenti, M., Binello, A., Boffa, L., Mulinacci, N. and Cravotto, G. (2016) Selective Recovery of Rosmarinic and Carnosic Acids from Rosemary Leaves under Ultrasound- and Microwave-Assisted Extraction Procedures. *Comptes Rendus. Chimie*, **19**, 699-706. https://doi.org/10.1016/j.crci.2015.12.013

[17] Khalil, N.H.A. and Al-Lebathi, H.H. (2025) Evaluating the Synergistic Activity of *Rosmarinus officinalis* Extracts with Antibiotics Against MDR Bacteria. *Universal Journal of Pharmaceutical Research*, **10**, 44-49. https://doi.org/10.22270/ujpr.v10i4.1393

[18] Husein, N., Laban, N.A. and Owais, D.T. (2025) Exploring the Antimicrobial Potential of *Rosmarinus officinalis* against Urinary Tract Infection Isolates in Amman, Jordan. *Iranian Journal of Microbiology*, **17**, 460-469. https://doi.org/10.18502/ijm.v17i3.18829

[19] Kernou, O., Azzouz, Z., Madani, K. and Rijo, P. (2023) Application of Rosmarinic Acid with Its Derivatives in the Treatment of Microbial Pathogens. *Molecules*, **28**, Article 4243. https://doi.org/10.3390/molecules28104243