



Bioactive Potential of Moroccan *Ruta chalepensis*: Polyphenol-Rich Extracts with Antioxidant and Anti-*Staphylococcus aureus* Activities

Hajar Sadrati*, Bouchra Ezahidi, Abdelhakim Taha, Tarik Ainane, Ayoub Ainane, Younes Abbas, and Sanaa Sabour Alaoui

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Abstract

The accelerating global crisis of antimicrobial resistance and oxidative stress-related diseases has intensified the search for novel therapeutic agents derived from natural sources. Polyphenolic compounds, abundant in many medicinal plants, have emerged as key candidates due to their potent antioxidant and antimicrobial properties. *Ruta chalepensis* L., a perennial species belonging to the Rutaceae family, is traditionally used in Mediterranean ethnomedicine for its antiseptic, anti-inflammatory, and digestive properties. In Morocco, where phytotherapy forms a cornerstone of rural healthcare practices, this species remains underexplored despite its wide distribution across the Middle Atlas and Rif regions. The present study aims to determine the phytochemical profile and its biological activity of Moroccan *R. chalepensis* by conducting a comprehensive organ-specific analysis. Methanolic extracts from the plant's flowers, leaves, and stems were evaluated for their total phenolic content (TPC) and total flavonoid content (TFC) using the Folin-Ciocalteu and aluminum chloride colorimetric methods. Antioxidant potential was assessed through DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays, while antibacterial activity was tested against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 9721, and *Streptococcus sp.* CCMM/B24 using disc diffusion and minimum inhibitory concentration (MIC) protocols. Among all plant parts, floral extracts exhibited the highest TPC (160 ± 2.38 mg GAE/g DW) and TFC (30.1 ± 1.32 mg QE/g DW), alongside superior antioxidant activity (DPPH $IC_{50} = 23.1 \pm 1.15$ μ g/mL). Notably, Gram-positive strains showed greater susceptibility to the extracts, with *S. aureus* being the most affected. These results underscore the therapeutic potential of *Ruta chalepensis*, particularly from Moroccan chemotypes, as a valuable source of natural antioxidants and antimicrobial agents. They also reinforce the importance of valorizing Morocco's endemic medicinal flora in addressing contemporary biomedical challenges.

Keywords: antimicrobial activity, antioxidant potential, polyphenols, *Ruta chalepensis* L., *Staphylococcus aureus*

1. INTRODUCTION

Morocco's exceptional floristic diversity has supported a centuries-old herbal medicine tradition: over 500 aromatic and medicinal species have been documented, including more than 15 endemics distinguished by their unique bioactive compounds [1]. Polyphenolic compounds, ubiquitous secondary metabolites renowned for free-radical scavenging and modulation of signaling pathways, are central to these traditional remedies, offering protection against oxidative damage implicated in chronic diseases such as atherosclerosis, neurodegeneration and

diabetes [2]-[4]. The escalating burden of oxidative stress-driven pathologies has spurred renewed interest in identifying and standardizing traditional Moroccan phytomedicines, especially those derived from underexplored local chemotypes.

Concurrently, antimicrobial resistance has emerged as a major global public health concern. This phenomenon is largely fueled by the inappropriate use of antibiotics and the rapid spread of resistant microorganisms. In Morocco, surveillance data indicate an increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in both hospital and community environments, with rates reaching nearly 30% in some urban areas [5]. Such trends underscore the pressing need to explore new therapeutic alternatives, particularly those derived from natural bioactive compounds [5][6].

R. chalepensis (Rutaceae), commonly called fringed rue, is a staple of Maghreb folk medicine, prized for its digestive, anti-inflammatory and antiseptic qualities [7][8]. Phytochemical investigations in Tunisian and European populations have characterized a rich array of flavonoids (rutin, quercetin

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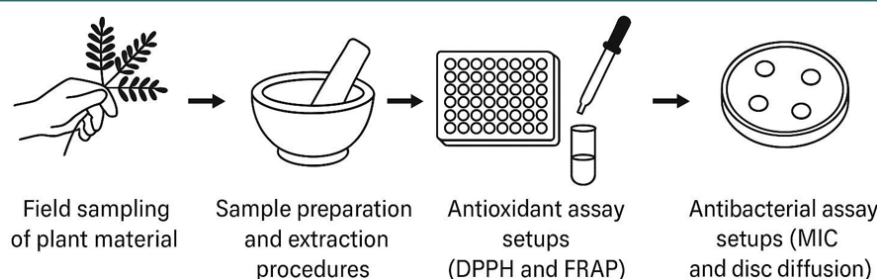


Figure 1. Schematic representation of the experimental workflow for *R. chalepensis* analysis.

derivatives), coumarins (psoralen, bergapten) and alkaloids, which collectively confer strong free-radical scavenging and moderate antibacterial activities [6][9][10][12]. More recently, Althaher et al. [8] reported high total phenolic contents and potent 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity in Moroccan *R. chalepensis* extracts, suggesting that indigenous chemotypes may harbor enhanced bioefficacy. However, despite these findings, no study has yet examined the organ-specific distribution of polyphenolic compounds in Moroccan *R. chalepensis* or evaluated how these variations relate to antioxidant and antibacterial activities. Existing studies from Morocco remain limited to global measurements such as total phenolic or flavonoid contents and general antioxidant assays [13]. We hypothesize that the floral organs of *R. chalepensis* may contain higher concentrations of polyphenolic compounds than vegetative organs, which could be associated with enhanced antioxidant and antibacterial activities.

Accordingly, this study presents the first comprehensive assessment of the phytochemical composition and bioactivity of different organs (leaves, stems, and flowers) of *R. chalepensis* collected from the Middle Atlas region of Morocco. Total phenolic and flavonoid contents were quantified and correlated with antioxidant activity using DPPH and FRAP assays. In addition, antibacterial activity was evaluated against clinically relevant strains, including MRSA, using disc diffusion and minimum inhibitory concentration (MIC) methods.

2. MATERIALS AND METHODS

2.1. Plant Samples: Collection and Identification

Fresh aerial parts (stems, leaves, and flowers) of

R. chalepensis were harvested in June 2024 from wild populations in the Middle Atlas region of Morocco, specifically near the city of Beni Mellal (32°19'N, 6°22'W). Botanical identification was confirmed by Dr. Hamid Khamar, a taxonomist at the Scientific Institute of Rabat (Morocco). The collected plant materials were subjected to shade drying at ambient temperatures (25–30 °C) for 7 days, in accordance with standardized phytochemical preparation protocols [14] to preserve thermolabile compounds, then milled to a fine powder (≤ 0.5 mm) and stored in desiccators at 4 °C until use. A schematic representation of the experimental workflow is provided in Figure 1, summarizing the main steps from plant collection to bioassays.

2.2. Preparation of the Methanolic Extract

Methanolic extracts of *R. chalepensis* leaves, stems, and flowers were prepared following the protocol described by Benbott et al. [15], with slight modifications. Briefly, 50 g of finely powdered plant material from each organ (leaves, stems, and flowers) was subjected to maceration in 250 mL of absolute methanol for 72 h under shade at ambient temperature. The macerates were then filtered through Whatman No. 1 filter paper, and the resulting filtrates were concentrated under reduced pressure at 35 °C using a rotary evaporator (Buchi R-210) to yield the crude methanolic extracts.

2.3. Determination of Total Polyphenol Content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method as described by Singleton et al. [3], with slight modifications. In brief, 100 μ L of methanolic extract (1 mg/mL) was mixed with 500 μ L of Folin–Ciocalteu reagent (diluted 1:10, v/v) and incubated

for 5 minutes at room temperature. Subsequently, 400 μL of 20% (w/v) Na_2CO_3 solution was added, and the reaction mixture was allowed to stand in the dark at 25 $^\circ\text{C}$ for 2 h. Absorbance was measured at 760 nm using a UV-visible spectrophotometer (Shimadzu UV-1500 PC). Quantification was performed using a gallic acid standard calibration curve (0–200 $\mu\text{g/mL}$; $R^2 = 0.998$), and results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g DW). Total polyphenols were computed by Equation 1;

$$T = \frac{C \times V}{M} \quad (1)$$

where C is concentration from calibration (mg/mL), V the extract volume (mL), and M the dry extract mass (g).

2.4. Determination of Total Flavonoid Content

Total flavonoid content (TFC) was quantified spectrophotometrically following the method of Woisky and Salatino [16], with minor modifications. This colorimetric assay is based on the formation of a stable complex between aluminum chloride (AlCl_3) and the hydroxyl groups at positions 4 and 5 of the flavonoid backbone. Briefly, 50 μL of plant extract was mixed with 1.5 mL of 95% methanol and 100 μL of 2% AlCl_3 solution. The reaction mixture was incubated at

room temperature for 1 h in the dark. Absorbance was then measured at 425 nm using a UV-vis spectrophotometer. Quantification was performed using a quercetin calibration curve (0–100 $\mu\text{g/mL}$; $R^2 = 0.997$), and results were expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g DW).

2.5. Antioxidant Activity Assays

2.5.1. DPPH Radical Scavenging Activity

DPPH scavenging was determined following the Oyaizu protocol [2]. A 0.1 mM DPPH solution in absolute methanol was freshly prepared. Extracts were diluted to 10, 25, 50, 100 and 200 $\mu\text{g/mL}$. To 2.8 mL DPPH solution, 200 μL of each extract or methanol blank was added. Ascorbic acid served as positive control. After 30 min in the dark at 25 $^\circ\text{C}$, absorbance at 515 nm was measured. Percent inhibition was calculated as Equation 2.

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (2)$$

IC_{50} values ($\mu\text{g/mL}$) were determined accordingly from dose–response curves ($R^2 > 0.99$).

2.5.2. Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the extracts was evaluated according to the method of Benzie and Strain [17], with slight modifications. A series of extract

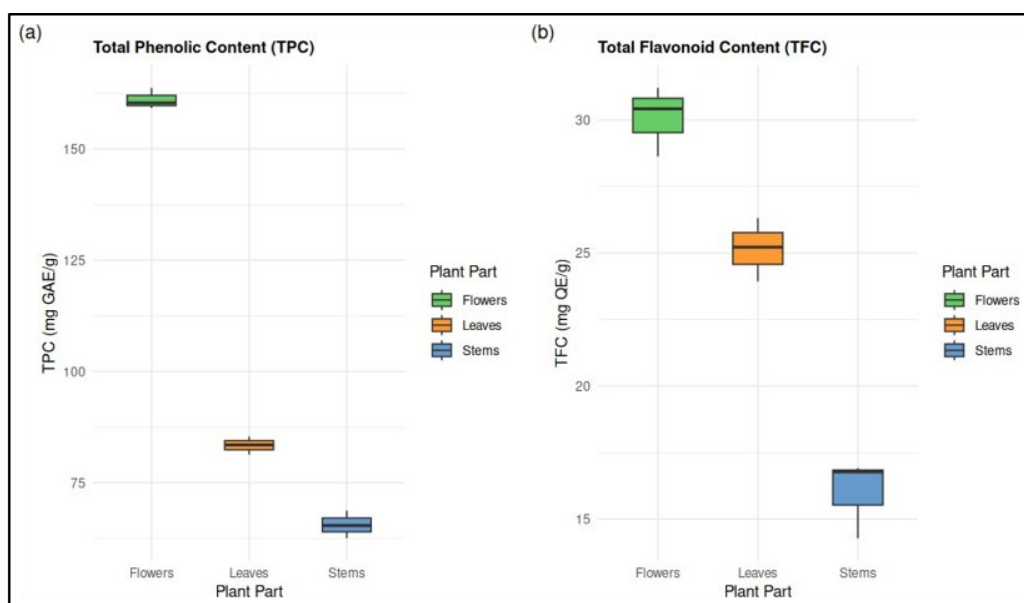


Figure 2. Total phenolic (TPC) and flavonoid (TFC) contents in different aerial parts of the plant (leaves, stems, and flowers). (a): TPC (mg GAE/g), (b): TFC (mg QE/g) concentrations.

Table 1. IC₅₀ values of antiradical activity and reducing power of hydromethanolic extracts of flowers, stems and leaves of *R. chalepensis*.

Aerial parts of <i>R. chalepensis</i>	DPPH IC ₅₀ (µg/mL)	Reducing power (EC ₅₀ mg/mL)
Flowers	23.10 ±1.15 c	1.08 ±0.16 a
Leaves	32.06 ±1.75 b	1.51 ±0.53 a
Stems	70.87 ±1.32 a	1.83 ±0.09 a

Different letters indicate significant differences among plant parts (Tukey HSD, $p < 0.05$)

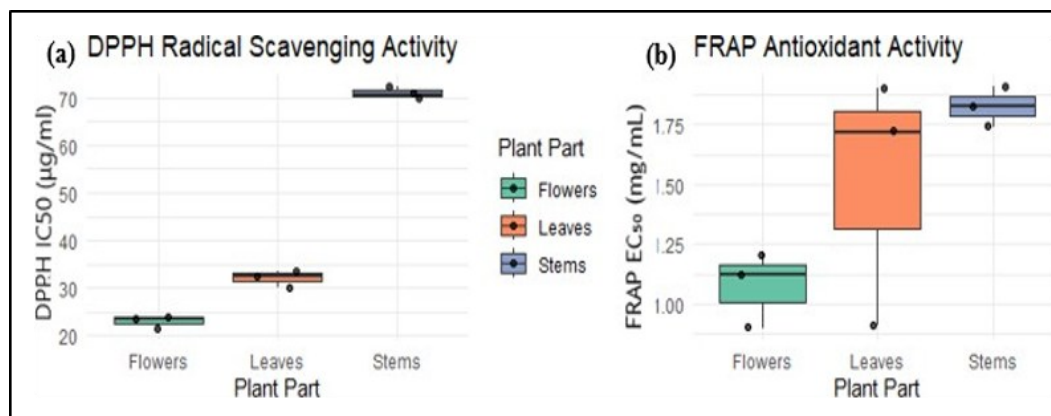


Figure 3. (a): Antioxidant activity assessed using DPPH, (b): FRAP assays of different aerial parts of the plant (leaves, stems, and flowers).

concentrations (10–200 µg/mL) was prepared and mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K₃Fe(CN)₆]. The mixtures were incubated at 50 °C for 20 min, after which 2.5 mL of 10% trichloroacetic acid (TCA) was added to terminate the reaction. The samples were then centrifuged at 3,000 × g for 10 min. From the resulting supernatant, 2.5 mL was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride (FeCl₃). Absorbance was measured at 700 nm using a UV-vis spectrophotometer. The effective concentration at which absorbance reached 0.5 (EC₅₀) was calculated to compare reducing capacity among samples.

2.6. Antibacterial Activity

The bacterial strains used in this study, *Streptococcus* sp. CCMM/B24, *S. aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 9721, are part of the reference microbial collection maintained in our laboratory. These strains are routinely preserved and employed for antimicrobial screening and other microbiological assays due to their well-characterized pathogenic profiles and

relevance in clinical and environmental studies. Their use ensures the reproducibility and reliability of the antibacterial activity evaluations carried out in this work. Prior to antibacterial testing, all methanolic extracts were sterile filtered through 0.22 µm membrane filters, even though the maceration of plant material was carried out at 4 °C to reduce microbial growth. Methanol was used as a solvent control in all tests, and no inhibition zones were seen for the methanol blank.

2.6.1. Disc Diffusion Assay

Mueller-Hinton agar (MHA) plates were uniformly inoculated with standardized bacterial suspensions using sterile cotton swabs to ensure confluent growth. Sterile 6 mm paper discs (Whatman No.1) were aseptically impregnated with 25 µL of plant extract solution (20 mg/mL) and placed onto the surface of the inoculated agar. Discs containing gentamicin (10 µg/disc) and pure methanol were used as positive and negative controls, respectively. The plates were then incubated at 37 °C for 24 h under aerobic conditions. Antibacterial efficacy was assessed by measuring the diameter of the inhibition zones

(mm) surrounding each disc. All assays were conducted in triplicate to ensure reproducibility and statistical reliability.

2.6.2. Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MICs) were determined using the standard broth microdilution method in sterile 96-well microplates. Methanolic extracts were two-fold serially diluted in Mueller–Hinton broth to obtain final concentrations ranging from 1,000 to 7.8 $\mu\text{g/mL}$. Each well was inoculated with 100 μL of a standardized bacterial suspension to achieve a final concentration of approximately 5×10^5 colony-forming units per milliliter (CFU/mL). Plates were incubated at 37 °C for 24 h under aerobic conditions. The MIC was defined as the lowest extract concentration that completely inhibited visible bacterial growth. Streptomycin was included as a positive control antibiotic for comparative purposes.

2.7. Statistical Analysis

All assays were performed in triplicate, (technical replicates) using extracts prepared from pooled plant material, and the results are expressed as mean $\pm$ standard deviation (SD). Statistical analyses were carried out using R version 4.4.2 [18]. Prior to ANOVA, normality of residuals was verified using the Shapiro-Wilk test and homoscedasticity was confirmed using Bartlett's test ($p > 0.05$ for all variables). A one-way

ANOVA followed by Tukey's post-hoc test was applied to evaluate significant differences among groups ($p < 0.05$). The effectiveness of different extracts was ranked based on mean scores. In addition, Pearson's correlation analysis was conducted to assess the relationships between phytochemical contents (TPC, TFC) and bioactivities (antioxidant and antibacterial). Correlation coefficients (r) and associated p -values were computed, and results were visualized as a correlation heatmap. Statistical significance was set at $p < 0.05$ for all analyses.

3. RESULTS AND DISCUSSIONS

3.1. Quantitative Analysis of Total Phenolic and Flavonoid Contents

Methanol extraction yields were measured for each plant organ before the overall phenolic and flavonoid contents were quantified. This revealed organ-dependent variation in extractable chemicals that may affect their future bioactive content. Methanol yields for *R. chalepensis* were 15.26% for stems, 27.30% for flowers, and 32.21% for leaves. Quantitative evaluation of phytochemical constituents revealed a pronounced organ-dependent pattern in the accumulation of phenolic metabolites. The greatest amounts of polyphenols (161.05 ± 2.38 mg GAE g^{-1} DW) and flavonoids (30.09 ± 1.32 mg QE g^{-1} DW) were found in flowers, suggesting that floral tissues exhibit strong biosynthesis, which is probably connected to

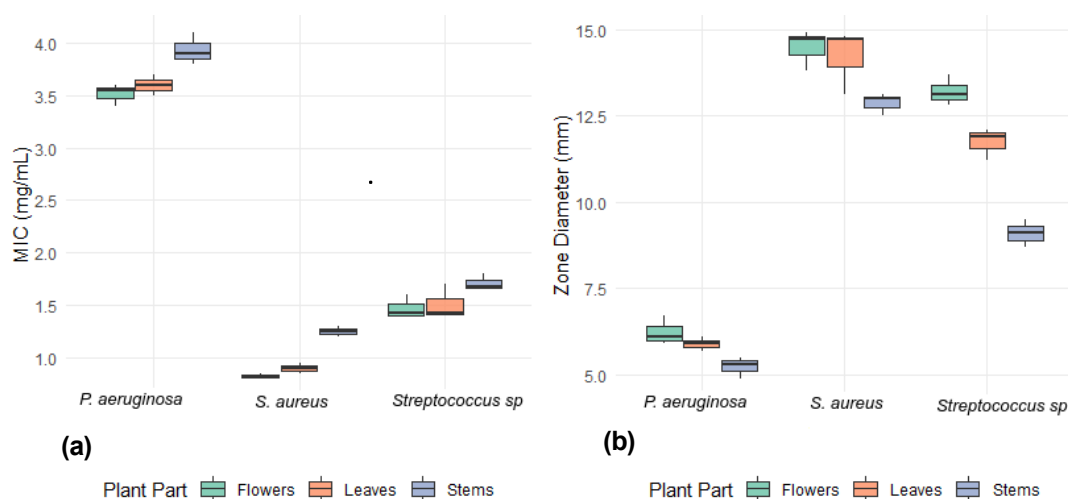


Figure 4. Antibacterial activity of different plant parts against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus sp.*: (a) MIC and (b) Inhibition zone diameter.

Table 2. Evaluation of the antibacterial effect of hydromethanolic extracts from the flowers, stems and leaves of *R. chalepensis*.

Aerial parts of <i>R. chalepensis</i>	<i>S. aureus</i> ATCC 29213		<i>Streptococcus</i> sp. CCMM/B24		<i>P. aeruginosa</i> ATCC9721	
	MIC (mg/mL)	Disc Diff	MIC (mg/mL)	Disc Diff	MIC (mg/mL)	Disc Diff
Flowers	0.82±0.02 ^b	14.47 ±0.59 ^a	1.47 ±0.12 ^a	13.20±0.46 ^a	3.52 ±0.10 ^b	6.23 ±0.42 ^a
Leaves	0.90±0.05 ^b	14.20±0.95 ^a	1.51 ±0.17 ^a	11.73±0.47 ^b	3.60 ±0.10 ^b	5.90±0.20 ^{ab}
Stems	1.25 ±0.05 ^a	12.87±0.32 ^a	1.71 ±0.08 ^a	9.10 ±0.40 ^c	3.93 ±0.15 ^a	5.23 ±0.31 ^b

Different letters indicate significant differences among plant parts (Tukey HSD, $p < 0.05$).

ecological and protective roles. Stems continuously displayed the lowest values, whereas leaves displayed intermediate levels. These results emphasize the significance of *R. chalepensis*'s floral tissues as a major source of bioactive compounds. A visual depiction of these organ-dependent changes in phytochemical content is shown in Figure 2.

From a biochemical perspective, this preferential accumulation may be associated with the activation of the phenylpropanoid pathway in reproductive organs, which leads to the synthesis of phenolic derivatives involved in plant defense and signaling processes [19]. In many angiosperms, floral tissues are metabolically active sites where flavonoids and related phenolic compounds contribute to protection against ultraviolet radiation, oxidative stress, and microbial colonization [20]. Comparative data further highlight the phytochemical richness of *R. chalepensis*, which exhibited significantly higher phenolic content than its congeners *R. graveolens* (41.63 mg GAE/g DW) and *R. montana* (33.68 mg GAE/g DW), as previously reported by Mokhtar and Bouaziz [21] and Ghasemzadeh et al. [22]. Previous phytochemical investigations have shown that species of the genus *Ruta* contain several classes of secondary metabolites, including flavonoids (e.g., quercetin and rutin derivatives), coumarins, and furocoumarins. Flavonoids are particularly relevant because their hydroxylated aromatic structure allows them to donate electrons or hydrogen atoms, thereby stabilizing reactive oxygen species and contributing to antioxidant defense mechanisms [4][23][24].

3.2. Antioxidant Activity: DPPH Radical Scavenging Activity and FRAP Assays

The antioxidant potential of different aerial parts of *R. chalepensis* was evaluated using two complementary *in vitro* assays: DPPH radical scavenging activity and FRAP. As shown in Table 1, the DPPH assay revealed significant inter-organ variability in antioxidant capacity ($p < 0.05$), whereas the FRAP assay showed no statistically significant differences among plant parts ($p > 0.05$). These differences in DPPH activity reflect the organ-specific distribution of bioactive

metabolites, particularly phenolic and flavonoid compounds, which are known to modulate redox behavior. Indeed, flavonoids such as quercetin derivatives are widely recognized for their ability to neutralize free radicals through hydrogen atom transfer and single-electron transfer mechanisms. The presence of catechol groups in the B-ring and conjugated double bonds enhances the capacity of these molecules to stabilize radical intermediates and interrupt oxidative chain reactions [4][25][26].

The DPPH radical scavenging assay (Figure 3 (a)) revealed significant variation in antioxidant capacity among plant organs. Flower extracts exhibited the highest activity, with an IC_{50} of $23.10 \pm 1.15 \mu\text{g/mL}$, followed by leaf extracts ($32.06 \pm 1.75 \mu\text{g/mL}$) and stem extracts ($70.87 \pm 1.32 \mu\text{g/mL}$). This gradient indicates that the antioxidant potential of floral tissues is approximately three times greater than that of stem tissues, suggesting a preferential accumulation of radical-scavenging compounds in reproductive structures. Such a distribution suggests that flavonoid-rich fractions may represent the main contributors to the radical-scavenging activity observed in *R. chalepensis* extracts, which is consistent with numerous studies demonstrating a strong association between flavonoid concentration and

antioxidant performance in medicinal plants [5][23]-[25].

The FRAP assay (Figure 3(b)) revealed a similar trend in reducing capacity. Flower extracts displayed the lowest EC_{50} value ($1.08 \pm 0.16 \text{ mg/mL}$), followed by leaf extracts ($1.51 \pm 0.53 \text{ mg/mL}$) and stem extracts ($1.83 \pm 0.09 \text{ mg/mL}$). However, no statistically significant differences were observed among plant parts ($p > 0.05$, Tukey HSD). Despite the absence of statistical significance, the consistent ranking observed in both assays (stems < leaves < flowers) indicates that floral tissues represent the principal source of antioxidant metabolites in *R. chalepensis*.

The antioxidant profile of *R. chalepensis*, evaluated using both DPPH radical scavenging and FRAP assays, highlights the functional relevance of its phenolic constituents. Floral extracts exhibited the strongest DPPH scavenging activity ($IC_{50} = 23.1 \pm 1.15 \mu\text{g/mL}$), followed by leaf extracts ($32.06 \pm 1.75 \mu\text{g/mL}$), while stem extracts were significantly less active ($70.87 \pm 1.32 \mu\text{g/mL}$). These values notably surpass those reported for *Ruta montana* ($IC_{50} = 82.12 \mu\text{g/mL}$) by Benali et al. [27], confirming the comparatively higher antioxidant potential of *R. chalepensis* within the genus. Importantly, the antioxidant potency recorded in this study is not only statistically significant but also

Table 3. One-way Anova of TPC, TFC, antioxidant, and antibacterial activities in different parts of *Ruta chalepensis*.

Source	df	Sum sq	Mean sq	f-Value	p-Value
<i>Analysis of TPC, TFC, and Antioxidant Activity</i>					
TPC	2	15450.00	7729.00	1191.00	< 0.001 ***
TFC	2	307.18	153.59	86.02	< 0.001 (***)
DPPH	2	3868.00	1934.00	972.50	< 0.001 (***)
FRAP	2	0.85	0.42	4.10	0.0753 ns
<i>Analysis for MIC</i>					
<i>P. aeruginosa</i>	2	0.29	0.14	9.90	0.016*
<i>S. enterococcus</i>	2	0.10	0.05	3.18	0.114 ns
<i>S. aureus</i>	2	0.31	0.15	82.66	< 0.001 (***)
<i>Analysis for disc diffusion</i>					
<i>P. aeruginosa</i>	2	1.55	0.77	7.60	0.0226*
<i>S. enterococcus</i>	2	25.89	12.94	65.47	< 0.001 (***)
<i>S. aureus</i>	2	4.40	2.20	4.87	ns

Significance codes: *** $p < 0.001$, * $p < 0.05$, ns = not significant.

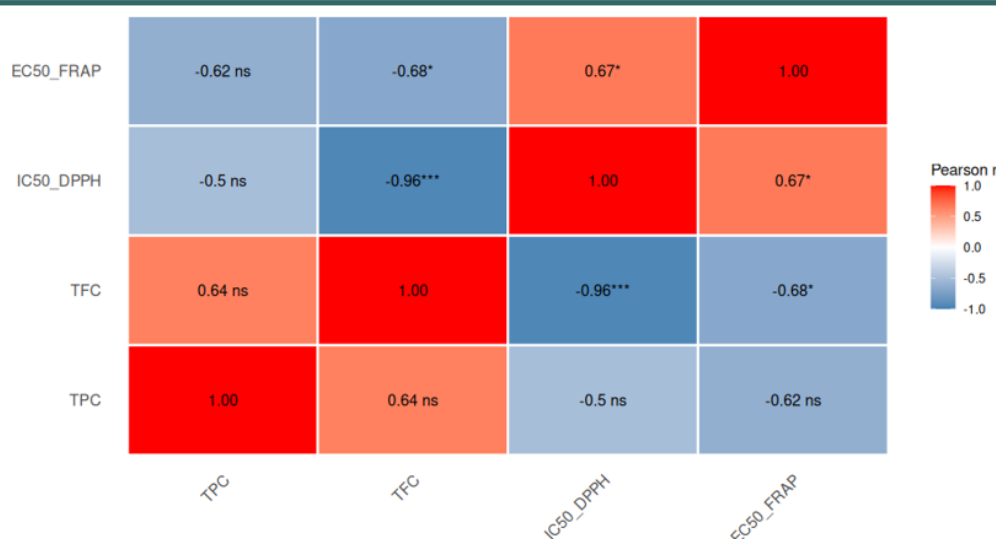


Figure 5. Pearson correlation matrix between total phenolic content (TPC), total flavonoid content (TFC), DPPH IC₅₀ and FRAP EC₅₀ of *R. chalepensis* extracts. Values represent Pearson correlation coefficients (r); *** $p < 0.001$, * $p < 0.05$, ns = not significant.

biologically compelling. The DPPH IC₅₀ value of $23.10 \pm 1.15 \mu\text{g/mL}$ and FRAP EC₅₀ of 1.08 mg/mL for flower extracts position *R. chalepensis* as more effective than several benchmark species investigated in the same assays. For comparison, *Enarthrocarpus clavatus* flower methanolic extracts recently profiled in Tunisia had an IC₅₀ near $35 \mu\text{g/mL}$ and a similar FRAP profile [28]. These differences, although subtle, are pharmacologically meaningful, especially in antioxidant screening where low IC₅₀ values are predictive of superior radical scavenging efficiency.

3.3. Antimicrobial Activity Assessment

The antibacterial activity of hydromethanolic extracts obtained from different aerial organs of *Ruta chalepensis* (flowers, leaves, and stems) was evaluated against three clinically relevant bacterial strains: *P. aeruginosa*, *Streptococcus* sp., and *S. aureus*. As illustrated in Figures 4(a) - (b), the extracts displayed clear organ-dependent antibacterial effects. Among the tested plant parts, floral extracts showed the strongest inhibitory activity, as evidenced by the largest inhibition zones and the lowest MIC values. Leaf extracts exhibited moderate antibacterial effects, whereas stem extracts consistently showed the weakest activity. This pattern is particularly evident in the inhibition zone diameters, which were significantly larger for flower and leaf extracts, highlighting their superior

antimicrobial potential compared with stem extracts.

Regarding antibacterial activities, the lowest MICs were observed for *S. aureus* followed by *Streptococcus* sp. (Table 2), indicating a high susceptibility of these strains to the tested extracts. In contrast, the highest MIC was recorded for *P. aeruginosa*, reflecting a greater resistance. Similarly, the inhibition zone diameters reveal that *S. aureus* exhibited the largest zone, followed by *Streptococcus* sp, further confirming their sensitivity. Conversely, *P. aeruginosa* showed the smallest inhibition zone, reinforcing its higher tolerance to the hydromethanolic extracts. Antibacterial assays demonstrated that *R. chalepensis* extracts from all plant parts showed comparable inhibitory effects against *S. aureus* (no significant differences between parts, $p > 0.05$), with inhibition zones measuring $14.47 \pm 0.59 \text{ mm}$ for flowers. For *Streptococcus* sp, flowers and leaves were significantly more effective than stems, with inhibition zones of $13.20 \pm 0.46 \text{ mm}$ and $11.73 \pm 0.47 \text{ mm}$, respectively.

The antimicrobial activities observed in the present study deserve particular attention. Floral extracts of *R. chalepensis* exhibited strong antibacterial effects, with a MIC of 0.82 mg/mL against *S. aureus* and inhibition zones reaching 14.47 mm . These values are comparable to, or

even better than, those reported for several phytochemical-rich medicinal plants previously investigated for anti-staphylococcal activity. For example, essential oils from *Sambucus nigra* showed lower antibacterial efficacy against *S. aureus*, with inhibitory concentrations around 2.5 mg/mL [29]. Such results highlight the notable antibacterial potential of *R. chalepensis*, particularly against Gram-positive bacteria. Comparable antibacterial activities of methanolic extracts from *Ruta chalepensis* against *S. aureus* and *P. aeruginosa* were recently reported by Goshu et al. [30], further supporting the therapeutic potential of *Ruta*-derived bioactive compounds.

In contrast, the activity against *P. aeruginosa* was more moderate (MIC = 3.52 mg/mL). This reduced susceptibility is not unexpected, given the well-documented resistance mechanisms of this pathogen, including the presence of multidrug efflux pumps, reduced outer-membrane permeability, and the ability to form protective biofilms that limit the penetration of antimicrobial agents [30]. Despite this intrinsic resistance, the inhibitory effect observed in the present study still suggests a measurable antibacterial potential of the extracts. From a biochemical standpoint, the antimicrobial activity detected in *R. chalepensis* extracts is likely associated with the presence of phenolic compounds and flavonoids. These secondary metabolites can interact with bacterial cell structures through several mechanisms, including disruption of cytoplasmic membrane permeability, inhibition of essential metabolic enzymes, and interference with nucleic acid synthesis [5][32][33]. Similar mechanisms have been reported for antimicrobial peptides and other antimicrobial molecules that disrupt bacterial membranes and interfere with intracellular metabolic processes [34]. Moreover, species belonging to the genus *Ruta* are known to contain coumarins and

furocoumarins, which may further contribute to antimicrobial activity. These compounds can interact with microbial DNA, alter membrane integrity, and interfere with intracellular metabolic pathways, ultimately leading to bacterial growth inhibition [6][35][36]. Taken together, the combination of phenolic compounds, flavonoids, and coumarin derivatives likely explains the antibacterial effectiveness observed for *R. chalepensis* extracts in the present study. These findings are consistent with recent investigations reporting significant antibacterial activities of *Ruta chalepensis* methanolic extracts against both Gram-positive and Gram-negative bacteria [30].

3.4. Statistical Relationships between Phytochemical Composition and Biological Activities

3.4.1. One-Way ANOVA of TPC, TFC, Antioxidant, and Antibacterial Activities in Different Parts of *R. chalepensis*

Prior to statistical analysis, Shapiro-Wilk and Bartlett's tests confirmed that all data met the assumptions of normality and homoscedasticity ($p > 0.05$), justifying the use of one-way ANOVA. The ANOVA analysis revealed marked differences in the biological activities of the different aerial parts of *R. chalepensis* (Table 3). TPC, TFC, and DPPH radical-scavenging activity all varied highly significantly among plant parts ($p < 0.001$), whereas no significant variation was observed for FRAP values ($p = 0.075$).

The flowers of *R. chalepensis* exhibited the highest TPC, reflecting a significant accumulation of phenolic compounds in this organ. TFC was also markedly elevated in both flowers and leaves. These findings reinforce the status of *R. chalepensis* as a rich source of bioactive compounds, particularly polyphenols and

Table 4. Tukey HSD post-hoc comparisons for differences among *R. chalepensis* plant parts.

Comparison	Mean Difference	95 % CI	Adjusted p-value	Significance
Leaves vs Flowers	-0.97	(-3.10, 1.15)	0.397	ns
Stems vs Flowers	-3.82	(-5.94, -1.69)	0.004	**
Stems vs Leaves	-2.84	(-4.97, -0.72)	0.015	*

Significance codes: ** $p < 0.01$, * $p < 0.05$, ns = not significant.

flavonoids which are known to contribute substantially to antioxidant and antibacterial activities. This observation further supports the hypothesis that specific subclasses of phenolic compounds, particularly flavonoids with catechol structures such as quercetin derivatives, play a dominant role in determining the antioxidant properties of the extracts [4][23]-[25]. Among the plant organs examined, flowers clearly emerged as the most bioactive tissue, containing the highest concentrations of both polyphenols and flavonoids. This heightened bioactivity aligns with the ecological role of flowers, which often serve as sites of intensified secondary metabolite production to protect reproductive structures against environmental stressors and microbial attack.

For antibacterial activity, MIC assays indicated highly significant differences for *S. aureus* ($p < 0.001$), significant differences for *P. aeruginosa* ($p = 0.016$), and no significant variation for *Streptococcus sp.* ($p = 0.114$). Disc diffusion assays showed highly significant differences for *Streptococcus sp.* ($p < 0.001$), significant differences for *P. aeruginosa* ($p = 0.023$), and no significance variation for *S. aureus* ($p = 0.055$). Regarding antioxidant potential, the DPPH assay (IC_{50}) demonstrated a highly significant variation among flowers, leaves, and stems, indicating distinct

radical scavenging potentials among plant organs. In contrast, no statistically significant variation was observed among plant parts for FRAP (EC_{50}) values ($p = 0.075$), suggesting that while radical-quenching activity varies strongly among organs, their overall reducing power remains relatively comparable. The antimicrobial effects observed in the present study are likely associated with the elevated phenolic content of the extracts. Plant-derived phenolics are known to exert antibacterial effects through several complementary mechanisms, including disruption of microbial membranes, inhibition of metabolic enzymes, metal-ion chelation, and interference with DNA replication or biofilm formation [33][35].

Pearson correlation analysis (Figure 5) revealed a strong negative correlation between TFC and DPPH IC_{50} ($r = -0.96$, $p < 0.001$), suggesting that flavonoid content is a major driver of radical-scavenging activity. A moderate negative correlation was also observed between TFC and FRAP EC_{50} ($r = -0.68$, $p = 0.045$), further supporting the role of flavonoids in reducing power. Additionally, a moderate positive correlation was found between IC_{50} and EC_{50} values ($r = 0.67$, $p = 0.049$), indicating concordance between both antioxidant assays. In contrast, TPC showed no statistically significant correlation with any antioxidant parameter ($p > 0.05$), suggesting that

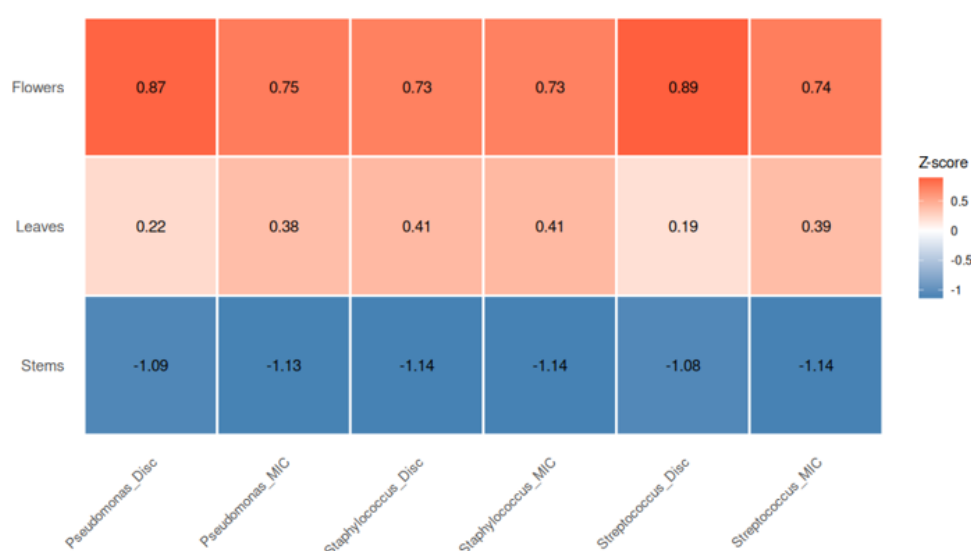


Figure 6. Heat map of normalized antibacterial activity of *R. chalepensis* plant parts. Z-scores are calculated from MIC values (mg/mL, inverted scale) and inhibition zone diameters (mm); red indicates higher efficacy, whereas blue indicates lower antibacterial efficacy

Table 5. Effectiveness ranking of *R. chalepensis* plant parts based on mean antibacterial activity.

Plant Part	Mean Overall Score	Effectiveness Level	Statistical Group
Flowers	1.600	Highest	Group A
Leaves	0.625	Intermediate	Group A
Stems	-2.220	Lowest	Group B

non-flavonoid polyphenols such as tannins and other phenolic compounds may contribute less directly to the measured antioxidant activities, and that flavonoids represent the primary active fraction within the total phenolic pool.

3.4.2. Tukey HSD Post Hoc Comparisons and Effectiveness Ranking of *R. chalepensis* Plant Parts based on Antibacterial Activity

The Tukey HSD post hoc test revealed significant differences in antibacterial efficacy among the aerial organs of *R. chalepensis*. Specifically, stem extracts differed significantly from both flower and leaf extracts, indicating markedly lower antibacterial activity in stems. In contrast, no significant difference was observed between flower and leaf extracts, suggesting comparable antibacterial efficacy between these two plant organs (Table 4).

The heat map in the Figure 6 shows the normalized antibacterial efficacy of flower, leaf and stems extracts, against *P. aeruginosa*, *S. enterococcus* and *S. aureus*. The visualization integrates MIC values and inhibition zone diameters to provide a comparative overview of antibacterial efficacy. Higher color intensity corresponds to greater antibacterial efficacy. In this representation, flower extracts displayed the highest activity, leaf extracts exhibited intermediate activity, and stem extracts consistently showed the lowest antibacterial response.

Based on the mean antibacterial efficacy (Table 5), flowers emerged as the most active plant organ, with the highest overall score (1.600), placing them in statistical group A. Leaves also demonstrated notable antibacterial activity, with a positive score (0.625), and were similarly classified within group A, although their efficacy remained slightly lower than that of flowers. In contrast, stems, obtained a negative score (-2.220) and were assigned to group B, exhibited markedly weaker antibacterial activity.

The statistical analyses collectively emphasize the notable antibacterial activity of *R. chalepensis*, particularly in its floral tissues, which consistently demonstrated superior efficacy across the different evaluation methods. This organ-dependent pattern likely reflects differences in the accumulation and distribution of secondary metabolites within the plant, notably phenolic compounds, flavonoids, and coumarin derivatives. These classes of phytochemicals have frequently been reported as key contributors to antimicrobial activity in numerous medicinal plant species [5][33][35][36]. From a pharmacological perspective, the results obtained in this study suggest that *R. chalepensis* represents a promising natural source of antibacterial agents. Further investigations should therefore focus on isolating and identifying the specific compounds responsible for the observed activity. Advanced analytical techniques, including liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) and nuclear magnetic resonance (NMR), are particularly suitable for this purpose, as they enable precise characterization of bioactive metabolites and facilitate the elucidation of structure-activity relationships [6][37]-[39]. Given the ecological availability of *R. chalepensis* in Morocco and its long-standing use in traditional medicine, these findings reinforce the potential of this species as a valuable resource for the development of plant-derived antimicrobial products.

4. CONCLUSIONS

This study demonstrates that *R. chalepensis*, particularly its floral organs, constitutes a rich source of phenolic and flavonoid compounds associated with significant antioxidant and antibacterial activities. Clear organ-dependent variations were observed, with flowers consistently showing the highest bioactive potential and the

strongest inhibitory effects against *Streptococcus* sp. and *S. aureus*. These findings improve our understanding of the distribution of phytochemicals within the plant and highlight the importance of selecting specific organs when evaluating medicinal plant resources. The results also support the potential of *R. chalepensis* as a promising natural source of bioactive compounds. Future research should focus on the isolation and structural identification of the active molecules, investigation of their mechanisms of action, and *in vivo* evaluation of their efficacy and safety. In addition, exploring the chemotypic diversity of this species across different bioclimatic regions may further enhance its potential for phytopharmaceutical applications.

AUTHOR INFORMATION

Corresponding Author

Hajar Sadrati — Applied Biology for Health, Environment and Sustainable Development Team, University of Sultan Moulay Slimane, Beni Mellal-23000 (Morocco);

 orcid.org/0009-0003-2406-4842

Email: hajarsadrati09@gmail.com

Authors

Bouchra Ezahidi — Applied Biology for Health, Environment and Sustainable Development Team, University of Sultan Moulay Slimane, Beni Mellal-23000 (Morocco);

 orcid.org/0009-0007-7060-9793

Abdelhakim Taha — Applied Biology for Health, Environment and Sustainable Development Team, University of Sultan Moulay Slimane, Beni Mellal-23000 (Morocco);

 orcid.org/0009-0003-5147-218X

Tarik Ainane — High School of Technology – Khenifra, University of Sultan Moulay Slimane, Beni Mellal-23000 (Morocco);

 orcid.org/0000-0001-6743-2666

Ayoub Ainane — High School of Technology – Khenifra, University of Sultan Moulay Slimane, Beni Mellal-23000 (Morocco);

 orcid.org/0000-0002-5004-6416

Younes Abbas — Applied Biology for Health, Environment and Sustainable Development Team, University of Sultan Moulay Slimane,

Beni Mellal-23000 (Morocco);

 orcid.org/0000-0003-2324-7172

Sanaa Sabour Alaoui — Applied Biology for Health, Environment and Sustainable Development Team, University of Sultan Moulay Slimane, Beni Mellal-23000 (Morocco);

 orcid.org/0000-0002-6795-4285

Author Contributions

Conceptualization: H. S., S. S. A.; Methodology: H. S., B. E.; Formal Analysis: H. S., A. T., B. E.; Investigation: H. S., T. A., A. A., S. S. A.; Resources & Funding Acquisition: H. S., Y. A.; Writing – Original Draft Preparation: H. S.; Writing – Review & Editing: H. S., B. E., T. A., A. A., Y. A., S. S. A.; Supervision & Project Administration: Y. A., S. S. A.

Conflicts of Interest

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE AI

Not applicable.

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