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RESEARCH ARTICLE

Exploring the Antibacterial and Antioxidant Activities of *Ruta chalepensis* Before, During, and After Flowering

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SUMMARY

This study investigated the phenolic content, as well as the antibacterial and antioxidant activities of hydromethanolic extracts from Ruta chalepensis (R. chalepensis) collected at three different periods: before flowering (BF), during flowering (DF), and after flowering (AF). Three hydromethanolic extracts were prepared: the before flowering extract (BFE), the during flowering extract (DFE), and the after flowering extract (AFE). A thorough hydromethanolic extract analysis was conducted regarding their total phenolics and flavonoids contents. The AFE displayed the maximum polyphenol and flavonoid levels: 84.54 ± 1.66 µg GAE/mg extract and 25.15 ± 0.88µg QE/mg extract, respectively. Antibacterial activity was evaluated against seven bacterial strains using the disc diffusion (DD) method, and the minimal inhibitory concentration (MIC) was defined. The results of the studied plant showed an important antibacterial activity against both Gram-negative and Gram-positive bacteria. The bacterial strains showed sensitivity ranging from moderate to high. On the other hand, the antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and the total antioxidant capacity (TAC) assays. All extracts exhibited potent scavenging activity with IC50 (Half maximal inhibitory concentration) values ranging between 14.18 ± 1.02 and 30.96 ± 1.80 µg/mL. The strongest antioxidant capacity was exerted by DFE (160.59 ± 5.78 µg AAE/mg extract). In conclusion, the results revealed that R. chalepensis hydromethanolic extracts have strong antibacterial and antioxidant properties, highlighting their potential as natural sources for developing novel antibacterial and antioxidant agents.

Keywords: *Ruta chalepensis, medicinal plants, phenolic compounds, antibacterial activity, antioxidant activity.*

Ruta chalepensis'in Çiçeklenme Öncesi, Sırasında ve Sonrasında Antibakteriyel ve Antioksidan Aktivitelerinin Araştırılması

ÖZ

Bu çalışmada, Ruta chalepensis (R. chalepensis) bitkisinden çiçeklenme öncesi (BF), çiçeklenme dönemi (DF) ve çiçeklenme sonrası (AF) olmak üzere üç farklı büyüme döneminde toplanan hidrometanolik ekstrelerin fenolik içeriği ile antibakteriyel ve antioksidan aktiviteleri araştırılmıştır. Üç hidrometanolik ekstre hazırlanmıştır: çiçeklenme öncesi ekstresi (BFE), çiçeklenme dönemi ekstresi (DFE) ve çiçeklenme sonrası ekstresi (AFE). Hidrometanolik ekstrelerin toplam fenolik ve flavonoid içerikleri kapsamlı bir şekilde analiz edilmiştir. AFE özütü, maksimum polifenol ve flavonoid seviyelerini göstermiştir; sırasıyla 84,54 ± 1,66 µg GAE/mg özüt ve 25,15 ± 0,88 µg QE/mg özüt. Antibakteriyel aktivite, yedi bakteri suşuna karşı disk difüzyon (DD) yöntemi kullanılarak değerlendirilmiş ve minimum inhibitör konsantrasyonu (MİK) tanımlanmıştır. İncelenen bitkinin sonuçları, hem Gram negatif hem de Gram pozitif bakterilere karşı önemli bir antibakteriyel aktivite göstermiştir. Bakteri suşları, orta ile yüksek arasında değişen bir hassasiyet sergilemiştir. Öte yandan, antioksidan aktivite DPPH (2,2-difenil-1-pikrilhidrazil) ve toplam antioksidan kapasite (TAC) analizleri kullanılarak değerlendirildi. Tüm Ekstreler, 14,18 ± 1,02 ile 30,96 ± 1,80 µg/mL arasında değişen IC50 (Maksimum inhibisyonun %50'sini sağlayan konsantrasyon) değerleriyle güçlü antioksidan aktivite gösterdi. En güçlü antioksidan kapasite DFE tarafından uygulandı (160,59 ± 5,78 µg AAE/mg ekstre). Sonuç olarak, elde edilen veriler, Ruta chalepensis hidrometanolik ekstrelerinin güçlü antibakteriyel ve antioksidan özelliklere sahip olduğunu ortaya koymuştur. Bu bulgular, söz konusu ekstrelerin yeni antibakteriyel ve antioksidan ajanların geliştirilmesinde doğal kaynak olarak kullanım potansiyelini vurgulamaktadır.

Anahtar Kelimeler: *Ruta chalepensis, tıbbi bitkiler, fenolik bileşikler, antibakteriyel aktivite, antioksidan aktivite.*

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INTRODUCTION

Foodborne infections remain a significant global health concern with major impacts on public health systems, economic stability, and food safety. These illnesses primarily originate from two mechanisms: microbial contamination by pathogenic organisms capable of causing severe infections and intoxications despite the protective presence of the human microbiome (Severino et al., 2025), and oxidative spoilage leading to food deterioration (Sökmen et al., 2004). Such hazards affect all nations regardless of development status, with contaminated food consumption enabling pathogen proliferation that results in acute poisoning and significant health risks (Celiktas et al., 2007). Compounding this challenge, the emergence of multidrug-resistant organisms – driven by antibiotic overuse in human medicine, agriculture, and livestock production – has created a parallel public health crisis that further complicates foodborne disease management (Elshobary et al., 2025; Marino et al., 2025). This situation has accelerated the search for new treatment approaches, particularly the investigation of safer bioactive substances, driven by increasing microbial virulence and the potential toxicity of synthetic antimicrobial drugs (Magri et al., 2023; Veiko et al., 2023).

Medicinal plants have been a basis of human and healthcare systems for a long time, with an important position in food, therapeutics, and hygiene in the earliest civilizations of Egypt, China, and Greece (Jindal & Seth, 2022). Recent epidemiological data collected by the World Health Organization (WHO) indicate that 80% of the global population continues to use plant-based medicines in their primary healthcare practices (WHO, 2019). Medicinal and edible plants represent a valuable source of bioactive compounds with multifunctional applications, serving as natural food additives, pharmaceutical agents, and industrial components. Their enduring pharmacological relevance is evidenced by continued incorporation in both traditional medicine systems and modern therapeutic approaches, with phytochemical analyses validating the scientific basis for their historical use (Chihomvu et al., 2024; Pallarés et al., 2025).

Plant extracts are showing renewed promise as effective antimicrobials, especially against antibiotic-resistant pathogens (Manso et al., 2021). Their bioactive components include such as phenolics as flavonoids and phenolic acids that have therapeutic effects, including anticancer, antidiabetic, antioxidant, anti-inflammatory, and antimicrobial activities (Fraga et al., 2019; Gutiérrez-del-Río et al., 2018). These natural compounds are especially valuable as antioxidants, as they diminish free radical damage through mechanisms such as hydrogen donation, singlet oxygen quenching, metal chelation and direct radical scavenging (Kacem et al., 2015).

The *Ruta* genus, native to the Mediterranean region, encompasses around 40 species of fragrant shrubs. Among these are *Ruta graveolens*, *Ruta chalepensis*, and *Ruta montana* (Jianu et al., 2021). *Ruta chalepensis*, commonly known as fringed rue, is widely used in Mediterranean traditional medicine (Yesuf et al., 2023). It was known to treat a variety of ailments, including gastrointestinal disorders (Molares et al., 2023; Usman et al., 2022), migraine and tired eyes (Ahmed et al., 2023), neuralgia, rheumatism, and menstrual disorders (Martínez-Pérez et al., 2017). It has analgesic, antipyretic, anti-inflammatory, spasmolytic, and convulsion treatment properties (Althaher et al., 2024). Several extracts of *Ruta chalepensis* are remarkably rich in diverse bioactive compounds, predominantly phenolic compounds, flavonoids, alkaloids, and essential oils. Phytochemical analyses have confirmed the presence of key compounds like rutin, quercetin, and furanocoumarins such as xanthotoxin and bergapten (Bekkar et al., 2021). These compounds are well-regarded for their potent antioxidant and antimicrobial properties. While traditional applications of *R. chalepensis* are widespread, ongoing research continues to validate and explore its purported therapeutic benefits, including its insecticidal, antioxidant, and antimicrobial activities (Szewczyk et al., 2022; Yücel et al., 2023).

This study investigates the phenolic content, antibacterial activity, and antioxidant potential of *Ruta chalepensis* extracts. To achieve this, we analyzed extracts collected at three distinct phenological stages: before, during, and after flowering. Antibacterial activity was assessed using the disc diffusion method, and the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined. Antioxidant potential was evaluated using two established methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and total antioxidant capacity (TAC). Our findings aim to provide valuable insights into the potential of *R. chalepensis* as a natural food preservative.

MATERIALS AND METHODS

Plant material

Aerial parts of *R. chalepensis* were collected from Djebel Messaad, Algeria (Latitude 34° 59′ 28″ N and Longitude 4° 5′ 33″ East), during three periods: before flowering (BF), during flowering (DF), and after flowering (AF) in 2021. A voucher specimen (N°: RC-1726QS28DjM) was placed in the Department of Nature and Life Sciences' herbarium after the plant was recognized by botanist Dr. Sarri Djamel.

Preparation of *Ruta chalepensis* extracts

The aerial parts of three samples were air-dried at room temperature in dark conditions for one week, and then they were powdered using a blender apparatus. 50 g of each powdered sample was mixed with 500 mL of a methanol/water solution (3:1) and macerated for 24 hours at room temperature in the shade under continuous agitation. The extraction was performed three times. The resulting mixture was then filtered through Whatman No. 4 filter paper and concentrated using a rotary evaporator. The yield of the extraction was then measured, and the dried extracts were stored at 4°C until use (Nouasri et al., 2022).

Determination of the total phenolic content

For the purpose of determining the total phenolic content of each sample, the colorimetric method described by Li et al., (2007) using the Folin-Ciocalteu reagent was adopted. 500 µL of 10% Folin-Ciocalteu reagent was combined with the samples (100 µL). 400 µL of a 7.5% sodium carbonate (Na_2CO_3) solution was added after 4 minutes. After that, the mixture was incubated for 2 hours at room temperature. A wavelength of 765 nm was used to measure the absorbance. Gallic acid (0-140 µg/mL) was used as a standard, and all tests were executed in triplicate. The amount of gallic acid equivalents in micrograms per milligram of extract (µg GAE/mg extract) was used to calculate the quantity of total phenolic compounds in the extracts.

Determination of the total flavonoid content

The total flavonoid content was determined using the method described by Bahorun et al., (1996). Briefly, 1 mL of 2% $AlCl_3$ reagent in methanol was added to an equal volume of extract or quercetin. Following that, the absorbance was measured at 430 nm using quercetin (0–20 µg/mL) as the reference. The extracts' flavonoid concentration was measured in micrograms of quercetin per milligram of extract.

Antibacterial activity

Strains

Bacterial strains subjected to the antibacterial activity of the plant extracts were four Gram-positive: *Staphylococcus aureus* (ATCC 65380), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis*, *Bacillus cereus*, and three Gram-negative: *Escherichia coli* (ATCC 7835), *Klebsiella pneumonia* (ATCC 13076), and *Proteus vulgaris*. These strains were generously supplied by the University Hospital Center (UHC) of Sétif, Algeria.

Disc diffusion method

The disc diffusion method was used to evaluate the antibacterial activity of *R. chalepensis* extracts. Bacterial dilution at 0.5 McFarland turbidity reference suspensions were inoculated into Müller-Hinton agar (MHA) plates. Afterwards, filter paper discs (6 mm) infused with 20 µL of each extract (200 mg/mL) in dimethyl sulfoxide (DMSO) were placed on the inoculated agar. DMSO-soaked discs served as neg-

ative controls, and Ampicillin antibiotic discs (AMP) were used as positive controls. All plates were then incubated at 37°C for 24 h. The inhibition zone (IZ) diameters were measured to determine the antibacterial efficiency of the extracts. Each experiment was performed in triplicate (Szewczyk et al., 2022).

Determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The MIC values are the minimum plant extract concentrations that are vital to totally stop the microorganism development. Those values were determined by the microdilution method using 96-well plates as described by Yèhouenou et al., (2010). Briefly, 100 µL of Müller-Hinton broth (MHB) medium with phenol red (0.2%) was added to each well of a 96-well-plate, 100 µL of extract (500 mg/mL) was added to the first column wells, except the second row. Half-dilutions were successively applied to wells, up to the 12^{th} well in each row, and 100 µL from the last well was discarded. The first well of the second row was filled with 100 µL of MHB that did not include phenol red. After that, successive dilutions of 2 wells per well were carried out up to the 12^{th} well in each row and, the 100 µL of the last well was discarded. All wells were inoculated with 100 µL of bacterial suspension (10^5 CFU/mL) except for the first row which was replaced by MHB without phenol red. Finally, the incubation was carried out at 37°C for 18 to 24h. Bacterial growth is recognized by a yellow color, while the persistence of the original red color indicates the absence of microbial growth. To determine the MBC, 0.1 mL from each well with no visible growth was sown onto MHA and incubated for 24 h at 37°C. The concentrations on plates that showed no signs of bacterial growth were determined to be the MBCs.

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) test

The antioxidant action of *R. chalepensis* extracts was determined regarding the hydrogen atom-donating ability of the plant or the plant radical-scavenging ability, using the stable radical DPPH (Que et al., 2006). Following the addition of 500 µL of the DPPH solution (0.1 mM) to 500 µL of extract solution or the standard butylated hydroxytoluene (BHT) at different concentrations (0-120 µg/mL), the mixture was allowed to stand at room temperature for 30 min in the dark. After that, the absorbance was measured at 517 nm. Based on the following formula, the DPPH scavenging activity was determined:

$$\text{Scavenging activity (\%)} = (A_0 - A_1) \times 100/A_0$$

where A_0 and A_1 represent the absorbances of the control and the test /BHT, respectively.

Total antioxidant capacity (TAC)

The phosphomolybdenum technique was used to assess this test for *R. chalepensis* extracts (Ben Abbes et al., 2021). In Brief, 0.1 mL of each extract sample was added to 0.9 mL of reagent solution, which is composed of the following: 0.6 M sulphuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate. The mixes were left at 95°C for 90 min, then allowed to cool to room temperature. Absorbance of the solution was determined at a wavelength of 695 nm against a blank. The amount of the standard ascorbic acid (AA) equivalent per milligram of extract (µg AAE/mg extract) was used to express the total antioxidant capability.

Statistical analysis

Results are mean ± SD. An ANOVA test and a Dunnett/Tukey test for multiple comparisons were used to assess the significance of the differences between tests/the control and the different tests at $p \leq 0.05$. Prism 9.00 computer software (GraphPad, Boston, USA) was used.

RESULTS AND DISCUSSION

Extraction yield and phenolic contents

Secondary metabolites are organic components found in any life form and are widely exploited in medicinal plants. They are not directly involved in the growth, reproduction, and development of the organism; however, they increase its survival and

fecundity. There are numerous advantages to using medicinal plants, including the ability to treat illnesses, to produce pharmaceutical medications, and to create cosmetics (Aboalhaija et al., 2022). Extraction of these compounds is necessary to evaluate their biological activities. The present study investigated three extracts from the *R. chalepensis* medicinal plant: before (BFE), during (DFE), and after (AFE) flowering. The yield of extraction varied between 16% and 28.97% depending on the collection period, with the following order: AFE > DFE > BFE. These results exhibited greater yields compared to those reported by Bekkar et al., (2021), with the *Ruta* plant grown in Mascara, western Algeria. They were also higher than those shown in similar studies in Tunisia by Khlifi et al., (2013) and Kacem et al., (2015). Moreover, they showed values slightly higher compared to those mentioned in a study conducted in Morocco (Neighboring country) on the same plant (Barbouchi et al., 2024). In a study by El Ouardi et al., (2025), the yield of the hydro-methanolic extract from Moroccan *Ruta montana* was 17.91%, which is comparable to the yields obtained in our DFE and AFE samples. As these results clearly indicate, the extraction yield is subject to variation due to a complex interplay of factors. These include both the plant species itself and external factors, such as environmental conditions, climate, and the extraction solvent.

Table 1 lists the total amounts of flavonoids and polyphenolics in the *R. chalepensis* extracts under study. These contents depend on the collection period, with levels being high before flowering, decreasing during flowering, and rising again after flowering. The extracts contained polyphenols ranging from 70.36 to 84.54 µg GAE/mg extract. These are lower than those reported in the Tunisian *R. chalepensis* study by Kacem et al., (2015). Indeed, a strong polyphenolic content was mentioned in wild-grown *R. chalepensis* (168.91 mg caffeic acid E/g) cited by Ouerghemmi et al., (2017). The plant content of flavonoids was found to be between 17.15 and 27.15 µg QE/mg. The BFE of *R. chalepensis* showed the main amount, followed by AFE with a slightly lower amount, while DFE exhibited the lowest content. These results values are, in contrast to the polyphenols, higher than those mentioned by Kacem et al., (2015), and lower than those cited by Benchikh et al., (2019) and Althaher et al., (2024).

Table 1. Extraction yields, and polyphenolic and flavonoid contents of *R. chalepensis* extracts.

Extract	Yield (%, w/w)	Polyphenols (µg GAE/mg)	Flavonoids (µg QE/mg)
BFE	28.97 ± 0.15	78.00 ± 0.12	27.15 ± 0.28
DFE	18.54 ± 0.21	70.36 ± 0.04	17.15 ± 0.34
AFE	16.00 ± 0.18	84.54 ± 1.66	25.15 ± 0.88

Values are mean ± SD (n = 3). Significant difference was found between values in each column according to Tukey's multiple comparisons test, $p<0.05$.

Antibacterial activity of *R. chalepensis* extracts

The pathogens' development of antibiotic resistance has prompted the scientific community to undertaken significant research to identify alternative treatment approaches. The rich diversity of plant secondary metabolites has been acknowledged as a valuable resource in this context (Vaou et al., 2021). In this study, the antibacterial activity of the *R. chalepensis* extracts was evaluated against Gram-positive and Gram-negative bacteria. The inhibition zone (IZ) diameters and the MIC values were determined by the disk diffusion and microdilution techniques, respectively. Based on the relevant results (Table 2), and the bacterial inhibition halo criteria mentioned by Hendel et al., (2024), generally, a bacterial strain is classified as sensitive when the inhibition zone (IZ) ranges

from 8 to 14 mm, at 14 to 20 mm, it is very sensitive, and when exceeding 20 mm, it is extremely sensitive. According to these classifications, all tested bacterial strains were sensitive to *R. chalepensis* extracts, except for *E. coli*, which was resistant to the BFE, and *P. vulgaris*, which was resistant to both BFE and AFE. The Gram-positive bacterial strains *E. faecalis* and *B. cereus* were the most sensitive, generally classified as very sensitive, except for *B. cereus* with the BFE, which exhibited a 10 mm inhibition zone. In terms of extract potency, the *R. chalepensis* DFE extract was found to be the most effective, exhibiting the strongest inhibitory action against all tested bacterial strains, and in particular versus gram-negative strains. This finding aligns with the data reported by Althaher et al., (2024); the ethanolic extract of *R. chalepensis* exhibited antibacterial effect against *B. cereus*, *S. aureus*, *E. coli*, and *P. penneri*. A study conducted by Mokhtar et al., (2022) reported antibacterial activity of a *Ruta graveolens* extract against *Staphylococcus aureus*, with an inhibition zone of 14 mm, which is slightly higher than that observed in our results.

The antibacterial activity of the plant extracts is likely mediated by alkaloids, flavonoids, polysaccharides, and other phenolic substances (Álvarez-Ordóñez et al., 2013; Musyimi et al., 2007). One of the actions acts on the plasma membrane H+-ATPase (Trigui et al., 2013). Polyphenols exhibit antibacterial properties by targeting bacterial cell structures. They can trigger membrane condensation and morphological alterations, such as cell deformation and cell wall rupture. This disruption of the cell envelope leads to the release of cytoplasmic material and membrane debris. The fundamental process is the disruption of the bacterial cell wall and membrane, leading to increased permeability (Álvarez-Martínez et al., 2020; Wang et al., 2017).

Table 2. Antibacterial activity of *R. chalepensis* extracts and the ampicillin antibiotic (AMP), as shown with inhibition zone diameter (mm).

Bacterial strains	Inhibition zone diameter (mm)			
	BFE	DFE	AFE	AMP
S. aureus	11.67 ± 0.58^{a}	12.67 ± 1.08^{a}	10.00 ± 1.00^{a}	29.67 ± 1.53
B. subtilis	8.67 ± 0.58^{b}	8.67 ± 0.58^{b}	8.67 ± 0.58^{a}	19.67 ± 1.53^{a}
E. faecalis	15.33 ± 0.58^{a}	17.00 ± 1.00^{c}	14.67 ± 0.58^{a,b}	23.00 ± 1.00^{b}
B. cereus	10.33 ± 0.58^{a,b}	18.00 ± 1.00^{c}	17.67 ± 0.58^{a}	24.33 ± 1.53^{b}
E. coli	6 ± 0.47^{c}	12.67 ± 0.47^{a}	9.67 ± 0.47^{a}	17.67 ± 0.47^{a,c}
K. pneumonia	11.58 ± 0.47^{a}	10.00 ± 1.00^{b}	8.67 ± 0.58^{a}	17.33 ± 1.53^{c}
P. vulgaris	6 ± 0.47^{c}	12.67 ± 1.00^{a}	6 ± 0.47^{a}	18.67 ± 1.53^{a,c}

Values are mean ± SD (n = 3). No significant difference when the same superscript letter appears in the same column, at $p<0.05$ of Tukey's multiple comparisons test.

Minimum inhibitory concentration (MIC)

Our investigation into the antibacterial activity of *Ruta chalepensis* extracts revealed MIC values ranging from 1.95 to 31.25 mg/mL. The DFE and AFE were particularly effective against *Escherichia coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus* (Table 3). These findings align with previous research, such as Althaher et al., (2024), who reported an *R. chalepensis* ethanolic extract with an *E. coli* MIC of 50 µg/mL, and Haddouchi et al., (2013), who observed MICs from 18-163 µg/mL for the essential oil against various bacteria. While MBC values were generally higher than MICs, indicating a bactericidal effect at higher concentrations, notably for *Enterococcus faecalis* and *Bacillus subtilis*, MICs and MBCs were equal, demonstrating a direct bactericidal action at growth-inhib-

itory concentrations. This aligns with Nazzaro et al., (2013), who noted stronger bactericidal effects of plant extracts against Gram-positive bacteria. The potent antibacterial activity against key pathogens, along with favorable MICs, highlights *R. chalepensis* as a promising natural antimicrobial agent, especially relevant in the context of rising antibiotic resistance. The observed variations in activity across phenological stages (DFE and AFE being more efficacious) suggest that harvest timing significantly influences the extract's antimicrobial potential, likely due to differences in bioactive compound profiles.

Table 3. MIC and MBC values of *R. chalepensis* hydromethanolic extracts.

Bacterial strains	MIC (mg/mL)			MBC (mg/mL)		
	BFE	DFE	AFE	BFE	DFE	AFE
S. aureus	15.62	1.95	1.95	31.24	7.8	7.8
B. subtilis	1.95	7.81	15.62	3.9	15.62	15.62
E. faecalis	3.9	7.81	7.81	3.9	15.62	15.62
B. cereus	3.9	7.81	7.81	7.8	15.62	15.62
E. coli	31.25	1.95	1.95	62.5	7.8	15.62
K. pneumonia	7.81	1.95	1.95	15.62	3.9	7.8
P. vulgaris	–	3.9	–	–	7.8	–

– Not defined

Antioxidant activity

DPPH test

The present study investigated the free radical scavenging activity of *R. chalepensis* extracts using the DPPH assay, a widely recognized method for evaluating the ability of extracts to neutralize stable radicals through electron or hydrogen atom donation (Tepe et al., 2005). Our results demonstrate that all three *R. chalepensis* extracts-BFE, DFE, and AFE-exhibited concentration-dependent inhibition of the DPPH radical (Figure 1). This indicates a clear dose-response relationship, where higher concentrations of the extracts lead to increased scavenging activity.

A crucial finding of this investigation is the significant influence of harvesting time on the DPPH free radical scavenging power. The AFE showed the highest antioxidant activity with an IC_{50} value of 14.18 ± 1.02 µg/mL, followed by BFE (IC_{50} = 22.04 ± 1.16 µg/mL) and DFE (IC_{50} = 30.96 ± 1.80 µg/mL). These values were statistically distinct, highlighting that the plant's antioxidant potential varies considerably throughout its phenological stages. Interestingly, the DFE's antioxidant activity (IC_{50} = 30.96 ± 1.80 µg/mL) did not differ significantly ($p < 0.05$) from that of the synthetic antioxidant BHT (IC_{50} = 12.89 ± 0.38 µg/mL). This suggests that the DFE possesses a comparable level of antioxidant efficacy to a well-established synthetic standard.

When compared to other studies on *R. chalepensis*, our observed DPPH scavenging activities appear to be more potent. For instance, an ethanolic extract from Jordanian *R. chalepensis* reported an IC_{50} of 41.2 ± 0.1 µg/mL (Althaher et al., 2024), while a Tunisian *R. chalepensis* extract showed an IC_{50} of 83.42 ± 3.71 µg/mL (Kacem et al., 2015). The significantly lower IC_{50} values obtained in our study, particularly for AFE,

indicate a superior free radical scavenging capacity compared to those previously reported extracts. This enhanced activity could be attributed to variations in geographical origin, environmental factors, extraction methods, or indeed, the specific phenological stage at which the plant material was harvested, as our results strongly suggest. Furthermore, the outcomes reported by Ouerghemmi et al., (2017) for *R. chalepensis* extracts were similar to ours, reinforcing the notion that *R. chalepensis* is a rich source of natural antioxidants.

The robust radical scavenging activity demonstrated by *R. chalepensis* extracts is primarily attributed to their rich content of phenolic compounds. Phenolic compounds, including flavonoids, coumarins, and phenolic acids, are well-known for their antioxidant properties due to their ability to donate electrons or hydrogen atoms to neutralize free radicals (Mokhtar et al., 2022; Nouasri et al., 2022). These compounds possess hydroxyl groups that enable them to scavenge reactive oxygen species (ROS), chelate metal ions, and inhibit lipid peroxidation, thereby protecting cellular components from oxidative damage (Althaher et al., 2023; Barbouchi et al., 2024). The high levels of total phenolic and flavonoid content previously identified in *Ruta* species (Althaher et al., 2024; Barbouchi et al., 2024) directly correlate with their observed antioxidant capacities. The variations in antioxidant activity across different harvesting times (BFE, DFE, AFE) further support the idea that the biosynthesis and accumulation of these phenolic compounds are dynamic processes influenced by the plant's developmental stage. This suggests that optimizing harvest timing could be a critical factor in maximizing the antioxidant potential of *R. chalepensis* for various applications, including nutraceuticals and pharmaceuticals.

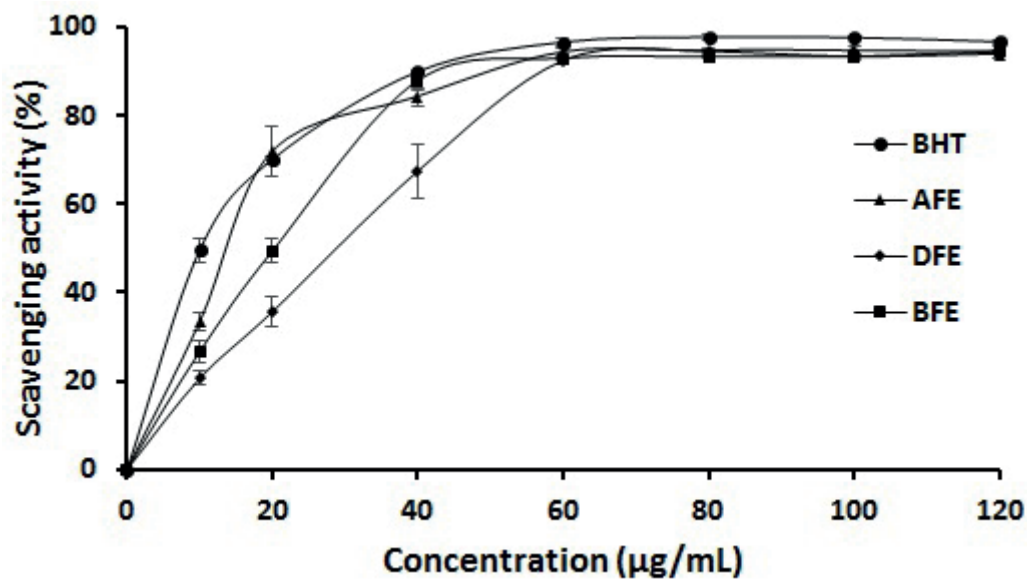

Figure 1. Free radical scavenging activity of BFE, DFE and AFE of *R. chalapensis,* and BHT. Values are mean ± SD (n = 3).

Total antioxidant capacity

The total antioxidant capacity (TAC) of *Ruta chalepensis* extracts varied significantly depending on the phenological stage of plant collection, as presented in Table 4. The highest TAC was observed in the DFE with a value of 160.59 ± 5.78 µg AAE/mg extract. This was followed by the AFE at 136.03 ± 1.46 µg AAE/mg extract, and the BFE exhibited the lowest capacity at 125.12 ± 2.47 µg AAE/mg extract.

Table 4: Total antioxidant capacity of *R chalepensis* extracts.

Extract	TAC (µg AAE/mg extract)
BFE	125.12 ± 2.47
DFE	160.59 ± 5.78
AFE	136.03 ± 1.46

Values are mean ± SD (n = 3).

The observed variation in TAC across different phenological stages highlights the dynamic nature of bioactive compound accumulation in *Ruta chalepensis*. The maximal antioxidant capacity recorded during the flowering stage suggests that this period is optimal for the synthesis and accumulation of compounds responsible for antioxidant activity in the plant. This aligns with the general understanding that plants, as a rich source of secondary metabolites such as polyphenols, flavonoids, and tannins, produce these compounds as part of their defense mechanisms or physiological processes, which can be influenced by developmental stages (Barbouchi et al., 2024).

Previous studies on *Ruta* species and other medicinal plants have similarly demonstrated that antioxidant capacities can fluctuate based on plant parts, environmental conditions, and growth stages. For instance, Barbouchi et al., (2024) reported a broad range of TAC values for *Ruta* crude extracts (37.67 to 396.80 mg AAE/g CE), indicating the variability in antioxidant potential within the genus and across different preparation methods. Our results, falling within this broad spectrum, reinforce the notion of *R. chalepensis* as a significant botanical source of antioxidants. The enhanced antioxidant activity during flowering might be attributed to an increased synthesis of specific phenolic compounds and flavonoids, which are well-known for their radical scavenging, metal chelating, reducing, and hydrogen-donating properties (Kacem et al., 2015; Ouerghemmi et al., 2017). For example, studies on *R. chalepensis* have confirmed the presence of various polyphenols, with total phenolic content varying significantly between extracts (Ouerghemmi et al., 2017). The correlation between high polyphenol levels and strong antioxidant activity has been previously established (Gali and Bedjou, 2019). Ouerghemmi et al., (2017) also found that *R. chalepensis* leaves and flowers exhibited high total polyphenol content and strong total antioxidant activity, which supports our findings of higher activity during or after the flowering period (DFE and AFE compared to BFE). The pronounced antioxidant potential of *R. chalepensis*, particularly during its flowering stage, reinforces its traditional medicinal uses and suggests its promising application in the food and pharmaceutical industries as a natural preservative and health-promoting agent.

CONCLUSION

This study thoroughly investigated the phenolic content, antibacterial activity, and antioxidant properties of *Ruta chalepensis* extracts across three growth stages (pre-flowering, flowering, and post-flowering). It confirmed that *Ruta chalepensis* is a rich source of polyphenols (70.36 to 84.54 µg GAE/mg extract), with concentrations varying significantly depending on the growth stage. The extracts showed significant antibacterial and antioxidant properties. They exhibited potent broad-spectrum antibacterial activity, with MIC values from 1.95 to 31.25 µg/mL, though *E. coli* showed resistance to the BFE, while *P. vulgaris* was resistant to both BFE and AFE. Antioxidant activity was growth-stage-dependent, with AFE showing the highest DPPH scavenging capacity (IC_{50} of 14.18 ± 1.02 µg/mL) and DFE exhibiting the highest total antioxidant capacity. These findings firmly establish *R. chalepensis* as a promising natural source of bioactive agents for pharmaceutical, nutraceutical, or food preservation applications. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for these beneficial effects to optimize their use in health and food quality formulations.

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AUTHOR CONTRIBUTION STATEMENT

Developing hypothesis (MS, RB), experimenting and statistics (MS), study text, literature research, interpretation of the data, and reviewing (MS, RB).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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