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Research article

Antibacterial Activity of *Retama raetem* Plant Extract against Clinically Relevant Bacterial Strains

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Abstract

Background: The rapid emergence of antibiotic-resistant bacteria has intensified the search for alternative antimicrobial agents, particularly those derived from medicinal plants. *Retama raetam*, a perennial shrub traditionally used in North African medicine, has gained attention for its potential antimicrobial properties. **Aim:** This study evaluated the antibacterial activity of methanolic extract of *R. raetam* against clinically important bacteria. **Methods:** Antibacterial activity was assessed using the disc diffusion method, and minimum inhibitory concentrations were determined by broth microdilution following Clinical & Laboratory Standards Institute guidelines. **Results:** The *R. raetam* extract exhibited concentration-dependent antibacterial activity, with the strongest effects observed against *Salmonella typhimurium* and *Staphylococcus aureus*. **Conclusion:** These findings suggest that *R. raetam* represents a promising source of bioactive compounds for antimicrobial drug discovery.

Keywords: *Retama raetam*, antibacterial activity, minimum inhibitory concentration, antibiotic resistance, medicinal plants, Libya

Introduction

Antibiotic resistance is a critical global health problem that threatens the effective prevention and treatment of bacterial infections (Ventola, 2015). The inappropriate use of antibiotics in human medicine and agriculture has accelerated the emergence of resistant strains, leading to increased morbidity, mortality, and healthcare costs (World Health Organization, 2021). Resistance mechanisms such as enzymatic degradation, efflux pumps, target modification, and horizontal gene transfer further complicate infection management (Blair *et al.*, 2015).

In Libya, where antimicrobial resistance surveillance is limited but resistance rates are rising among clinical isolates, the need for alternative therapeutic strategies has become increasingly urgent (Ahmed *et al.*, 2025). Recent reports have documented significant resistance patterns in Libyan healthcare settings, emphasizing the importance of exploring locally available medicinal plants as potential sources of novel antimicrobial agents (Abouzeed *et al.*, 2018). Ahmed *et al.* (2025) highlighted the global initiatives to phase out critical antibiotics in food-producing animals, underscoring the pressing need for safe and effective alternatives derived from natural sources.

Medicinal plants have historically served as a major source of therapeutic agents and remain vital in modern pharmacology (Newman and Cragg, 2020). Plant-derived compounds often exert multi-target antimicrobial effects, which reduce the likelihood of resistance development when compared to conventional antibiotics (Atanasov *et al.*, 2021). Consequently, medicinal plants are increasingly explored as alternative or complementary antimicrobial agents (Silva *et al.*, 2024).

Retama raetam (Fabaceae) is a desert shrub widely distributed in North Africa and the Middle East and has long been used in traditional medicine for treating infections, diabetes, and inflammatory conditions (Alqasoumi *et al.*, 2019). Phytochemical studies have identified flavonoids, alkaloids, phenolic acids, and saponins in *R. raetam*, many of which possess known antimicrobial properties (Hamrouni *et al.*, 2022). Previous screening of Libyan medicinal plants by Abouzeed *et al.* (2018) demonstrated promising antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), providing a strong rationale for investigating regionally abundant species like *R. raetam*. However, scientific evidence regarding its antibacterial activity remains limited and inconsistent (El-Akawi *et al.*, 2010).

Therefore, the present study aimed to evaluate the antibacterial activity of methanolic extracts of *R. raetam* against selected Gram-positive and Gram-negative pathogenic bacteria and to compare its efficacy with standard antibiotics.

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Materials and methods

Plant collection and extraction

Aerial parts (stems, leaves, and flowers) of *R. raetam* were collected from the Aljafara region, Libya, during the flowering season (November 2024). The plant material was identified by a botanist, and a voucher specimen was deposited at the herbarium of the Faculty of Science, University of Tripoli, Libya. Shade drying at room temperature (25±2°C) for two weeks was employed to preserve thermolabile compounds (Sasidharan *et al.*, 2022). The dried material was ground into a fine powder using an electric grinder. Fifty grams of the powdered plant material were extracted by maceration in 500 mL of 70% methanol for 72 hours at room temperature with occasional shaking. Methanol (70%) was selected as the extraction solvent due to its efficiency in extracting a broad range of phytochemicals, including both polar and moderately polar compounds (Abouzeed *et al.*, 2018). The extract was filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C. The resulting crude extract was stored at 4°C in airtight containers until use. The extraction and all subsequent laboratory procedures were conducted at the Libyan Center for Medical Research, Al Zawia.

Bacterial strains

Five clinically relevant bacterial strains were used in this study; *Staphylococcus aureus* (ATCC 25923) as a representative Gram-positive bacterium, and four Gram-negative bacteria included *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (clinical isolate), and *Salmonella typhimurium* (clinical isolate). These pathogens are commonly associated with human infections and are known for increasing antimicrobial resistance patterns (Centers for Disease Control and Prevention, 2019). The clinical isolates were obtained from the culture collection of the Libyan Center for Medical Research and were identified using standard microbiological methods. All bacterial strains were maintained on Mueller-Hinton agar slants at 4°C and subcultured regularly to maintain viability.

Preparation of inoculum

For each bacterial strain, three to five pure colonies were transferred into sterile normal saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units (CFU)/mL. The inoculum was used within 30 minutes of preparation.

Antibacterial activity assay (disc diffusion method)

The antibacterial activity of the *R. raetam* methanolic extract was evaluated using the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022a). The crude extract was dissolved in 10% dimethyl sulfoxide (DMSO) to prepare three concentrations: 25, 50, and 100 mg/mL. Sterile filter paper discs (6 mm diameter, Whatman No. 1) were impregnated with 20 µL of each extract concentration and allowed to dry under sterile conditions.

Mueller-Hinton agar plates were inoculated with the standardized bacterial suspensions using sterile cotton swabs to ensure confluent growth. The impregnated discs were placed on the surface of the inoculated plates using sterile forceps. Chloramphenicol (30 µg/disc) and levofloxacin (5 µg/disc) were used as positive controls, while discs impregnated with 10% DMSO served as the negative control. The plates were incubated at 37°C for 18-24 hours. After incubation, the diameters of the inhibition zones (including the disc diameter) were measured in millimeters using a calibrated ruler. All tests were performed in triplicate, and the results were expressed as mean±standard deviation (SD).

Minimum inhibitory concentration determination

The minimum inhibitory concentration (MIC) of the *R. raetam* extract was determined using the broth microdilution method in 96-well microtiter plates, as recommended by the CLSI (CLSI, 2022b). The extract was serially diluted two-fold in Mueller-Hinton broth to achieve concentrations ranging from 7.8 to 1000 µg/mL. An equal volume (100 µL) of each dilution was dispensed into the wells. Bacterial suspensions (100 µL) adjusted to 5×10^5 CFU/mL were added to each well, resulting in final extract concentrations ranging from 3.9 to 500 µg/mL. Positive controls (wells with bacteria but no extract), negative controls (wells with extract but no bacteria), and sterility controls were included. The plates were incubated at 37°C for 18-24 hours. The MIC was defined as the lowest concentration of the extract that inhibited visible bacterial growth (no turbidity). All tests were performed in duplicate.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as mean±SD. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons using GraphPad Prism version 9 (GraphPad Software, 2023). Statistical significance was set at $p<0.05$.

Ethical approval

No human subjects, live animals, nor protocols or materials need special approval were used in this study. All analytical procedures were performed according to Good Laboratory Practices.

Results

Antibacterial activity of R. raetam extract

The antibacterial activity of *R. raetam* crude extract was evaluated against five clinically important bacteria. The extract demonstrated clear inhibitory zones in a concentration-dependent manner. As shown in table 1, at 100 mg/mL, inhibition zones ranged from 9.0±1.0 mm for *K. pneumoniae* to 20.1±1.4 mm for *S. typhimurium*. Intermediate activity was observed against *S. aureus* (18.7±1.5 mm), *P. aeruginosa* (15.0±1.0 mm), and *E. coli* (14.7±2.1 mm).

The data confirmed a consistent dose–response pattern across most strains. Between 25 and 100 mg/mL, the inhibition zones increased substantially for *S. typhimurium* (+7.8 mm) and *S. aureus* (+6.7 mm), while *K. pneumoniae* showed minimal increase (+1.0 mm), indicating near-complete resistance (Figure 1).

Comparison with standard antibiotics

When compared to standard antibiotics, the plant extract was less potent. Chloramphenicol and levofloxacin produced inhibition zones in the range of 22–28 mm with

MICs as low as 2–8 µg/mL (Table 2). However, against *S. typhimurium*, the extract's inhibition zone at 100 mg/mL (20.1±1.4 mm) approached that of chloramphenicol (22.0±1.5 mm), suggesting promising activity (Figure 2).

Table 1. Inhibition zones and MIC values of *R. raetam* extract against test bacteria.

Bacterium	25mg/mL *	50mg/mL *	100mg/mL *	MIC
S. aureus	12.0±1.0 (p<0.001)	15.3±1.2 (p<0.001)	18.7±1.5 (p<0.001)	125
S. typhimurium	12.3±0.8 (p<0.001)	16.7±1.0 (p<0.001)	20.1±1.4 (p<0.001)	100
P. aeruginosa	13.0±1.0 (p<0.001)	14.0±1.0 (p<0.001)	15.0±1.0 (p<0.001)	125
E. coli	10.0±1.0 (p<0.001)	12.7±1.5 (p<0.001)	14.7±2.1 (p<0.001)	250
K. pneumoniae	8.0±0.5 (p=0.015)	8.5±0.8 (p=0.008)	9.0±1.0 (p=0.007)	500

*Inhibition zone in mm (mean±SD), MIC in µg/mL.

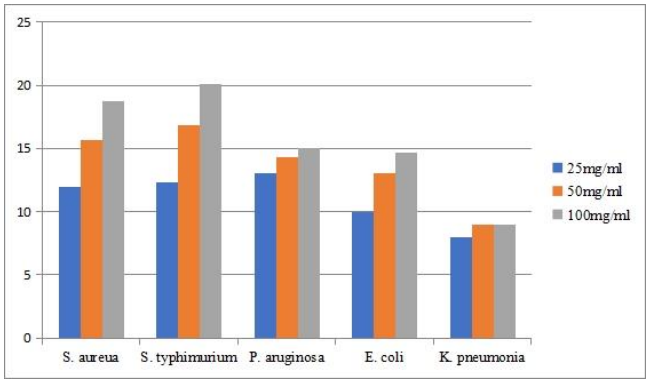


Figure 1. Inhibition zones for all bacteria at 25, 50, and 100 mg/mL concentrations of *R. raetam* extract.

MIC values and potency ranking

The MIC analysis confirmed the disc diffusion findings (Figure 3). The lowest MIC was observed for *S. typhimurium* (100 µg/mL), followed by *S. aureus* and *P. aeruginosa* (125 µg/mL), *E. coli* (250 µg/mL), and *K. pneumoniae* (500 µg/mL). This confirms that *S. typhimurium* and *S. aureus* are the most susceptible species.

Gram-positive versus Gram-negative susceptibility

The extract showed slightly higher efficacy against Gram-positive bacteria overall. The average inhibition zone at 100 mg/mL was 18.7 mm for Gram-positive strains (*S. aureus*) versus 14.6 mm for Gram-negative strains (*S. typhimurium*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*). However, the strong activity against *S. typhimurium* demonstrates that *R. raetam* extract can overcome Gram-negative barriers in certain species.

Table 2. Antibacterial activity of positive and negative controls.

Bacterium	C*	MIC	LEV*	MIC	NC
S. aureus	25.0±1.0	8	28.0±1.2	2	0
S. typhimurium	22.0±1.5	8	25.4±1.8	2	0
P. aeruginosa	20.0±1.2	4	24.0±1.5	2	0
E. coli	23.0±1.3	8	26.0±1.6	2	0
K. pneumoniae	18.0±1.0	4	22.0±1.2	2	0

C: Chloramphenicol, LEV: Levofloxacin, *Inhibition zone in mm (mean±SD), MIC in µg/mL, NC: Negative control.

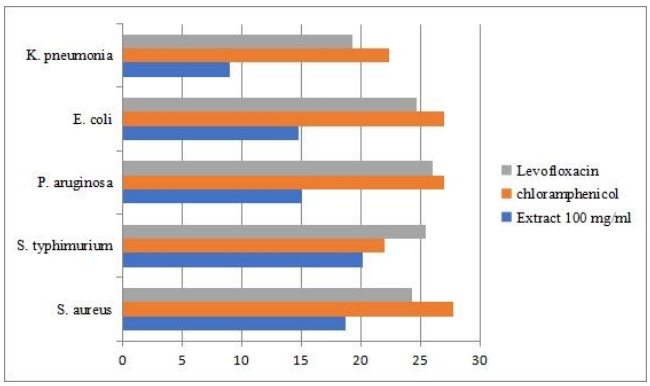


Figure 2. Mean inhibition zones at 100 mg/mL compared to standard antibiotics.

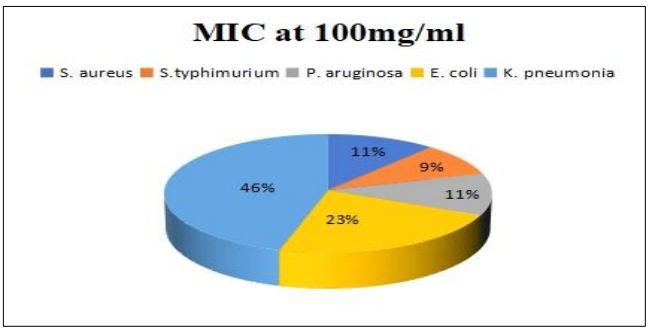


Figure 3. Comparison of MIC values against bacterial strains.

Discussion

The present study demonstrated that the methanolic extract of *R. raetam* possesses measurable antibacterial activity against both Gram-positive and Gram-negative bacteria, though the degree of susceptibility varied considerably among the tested strains. A consistent dose-dependent effect was observed, with higher extract concentrations producing larger zones of inhibition and

lower MIC values. This finding supports the idea that the antimicrobial activity of *R. raetam* is driven by bioactive phytochemicals present in the plant tissues, and that these compounds exert their effects in a concentration-dependent manner (Barka *et al.*, 2019).

Among the tested strains, *S. typhimurium* and *S. aureus* were the most susceptible, with MIC values of 100 and 125 µg/mL, respectively, and inhibition zones exceeding 18 mm at 100 mg/mL. These results are consistent with earlier reports from North Africa and the Middle East. For instance, Barka *et al.* (2019) reported strong inhibition of S. aureus by methanolic extracts of *R. raetam*, with inhibition zones ranging from 15-22 mm depending on the concentration and plant part used. Similarly, Hamza *et al.* (2020) observed significant activity against enteric pathogens, including *Salmonella* species, and attributed this activity to the presence of flavonoids and alkaloids in the extract. The susceptibility of *S. aureus* is particularly noteworthy given its clinical importance as a leading cause of hospital-acquired infections and its increasing resistance to multiple antibiotics, including methicillin (MRSA).

The activity against *P. aeruginosa* (MIC: 125 µg/mL; inhibition zone: 15.0 mm at 100 mg/mL) and *E. coli* (MIC: 250 µg/mL; inhibition zone: 14.7 mm at 100 mg/mL) was moderate but still notable. This is particularly important because *P. aeruginosa* is intrinsically resistant to many antibiotics due to its low outer membrane permeability, constitutive expression of efflux pumps, and ability to form biofilms. Similar results were reported by Sahli *et al.* (2021), who found moderate inhibition of *P. aeruginosa* using *R. raetam* extracts from Tunisia, with MIC values ranging from 128-256 µg/mL. The activity against *E. coli* is also clinically relevant, as this bacterium is a common cause of urinary tract infections, foodborne illnesses, and neonatal meningitis, with increasing rates of resistance to extended-spectrum beta-lactamases (ESBL).

By contrast, *K. pneumoniae* showed very weak susceptibility, with an MIC of 500 µg/mL and an inhibition zone of only 9.0 mm at the highest concentration tested. This limited effect has also been indicated in other regional studies, where *K. pneumoniae* consistently emerged as the most resistant among Gram-negative species tested with *R. raetam* extracts (Hamza *et al.*, 2020). The thick polysaccharide capsule, which provides a physical barrier against antimicrobial agents, and multiple efflux pump systems characteristic of *K. pneumoniae* likely contribute to this observed resistance, as these structures effectively limit the penetration of plant-derived compounds. Additionally, *K. pneumoniae* is known to produce various enzymes that can inactivate antimicrobial compounds, further contributing to its resistance profile.

When compared with commercial antibiotics (chloramphenicol and levofloxacin), our plant extract was clearly less potent, with MIC values 12.5 to 250 times higher than the standard antibiotics. This is expected, as crude extracts contain complex mixtures of compounds, some of which may act synergistically while others might dilute overall potency, and the active compounds are present in relatively low concentrations. Nevertheless, the fact that the *R. raetam* crude extract approached chloramphenicol in inhibition zone diameter against *S. typhimurium* (20.1 mm vs. 22.0 mm) suggests that crude extracts could be useful sources of lead molecules for drug discovery (Newman and Cragg, 2020). Furthermore, the observed activity is comparable to or better than many other medicinal plant extracts reported in the literature, supporting the potential of *R. raetam* as a source of antimicrobial compounds.

The differential susceptibility observed among the Gram-negative bacteria is noteworthy. While *S. typhimurium* was highly susceptible, *E. coli* showed moderate susceptibility, and *K. pneumoniae* was relatively resistant, despite all three being *Enterobacteriaceae* with similar outer membrane structures. This suggests that factors beyond the simple Gram-negative barrier, such as specific efflux pumps, porin proteins, or enzymatic degradation, may be involved in determining susceptibility. Alternatively, the active compounds in *R. raetam* may have specific targets that are present or accessible in some species but not others. This species-specific activity is clinically relevant, as it indicates that the extract might be particularly useful against certain pathogens (such as *S. typhimurium* and *S. aureus*) while being less effective against others (such as *K. pneumoniae*).

The phytochemical basis of the observed antibacterial activity is likely multifactorial. Previous phytochemical investigations of *R. raetam* have identified various bioactive compounds, including alkaloids (such as retamine and sparteine), flavonoids (such as quercetin and kaempferol derivatives), tannins, phenolic acids, and saponins (Alqasoumi *et al.*, 2019; Hamrouni *et al.*, 2022). Many of these compound classes have documented antimicrobial properties through various mechanisms. For example, flavonoids can disrupt bacterial cell membranes, inhibit nucleic acid synthesis, and interfere with energy metabolism. Alkaloids can intercalate with DNA and inhibit topoisomerases, while tannins can bind to and inactivate bacterial enzymes and cell wall proteins. The presence of multiple classes of bioactive compounds in *R. raetam* suggests that its antibacterial activity likely results from synergistic interactions between different phytochemicals, which could also explain the species-specific activity patterns observed.

Recent advances in formulation technology have shown promise in enhancing the activity of plant extracts. For example, nano-phytosome formulations of *R. raetam* combined with colistin have demonstrated enhanced antibacterial activity compared to crude extracts alone, suggesting potential avenues for improving efficacy through modern drug delivery systems. Such approaches could potentially overcome the lower potency of crude extracts compared to pure antibiotics and make plant-based antimicrobials more clinically viable.

This study has several limitations that should be acknowledged. First, the use of crude extracts means the observed activity results from a complex mixture of compounds, some of which may act synergistically while others might dilute overall potency. Previous phytochemical investigations of *R. raetam* have identified alkaloids, flavonoids, tannins, and phenolic acids, but the specific compounds responsible for the

antibacterial effects remain unidentified. Second, the study was conducted entirely *in vitro*, which cannot fully represent biological complexity in living systems. Third, the bacterial panel, while clinically relevant, was limited in size and did not include a wide range of multidrug-resistant clinical isolates that would better represent the current AMR landscape in Libyan healthcare settings.

Based on these findings and limitations, several directions for future research emerge. Optimization of extraction methods using acidified solvents could enhance the solubility of alkaloids and phenolic compounds, potentially yielding stronger bioactivity. Complete phytochemical profiling using GC-MS and HPLC would enable accurate identification of active constituents. Studies investigating synergistic effects with conventional antibiotics could provide alternative approaches to lowering effective antibiotic doses. Most importantly, *in vivo* studies using animal models are needed to determine therapeutic efficacy, bioavailability, and toxicity under physiological conditions. Given that *R. raetam* is widely available in North Africa, future work should also assess the cost-effectiveness and feasibility of developing this plant extract as a locally accessible antimicrobial option, particularly for underserved communities where access to conventional antibiotics may be limited.

Conclusion

The methanolic extract of *R. raetam* exhibited dose-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria, with the strongest effects against *S. typhimurium* and *S. aureus*. While the extract was less potent than standard antibiotics, its activity suggests potential as a source of antimicrobial lead compounds. Further studies are needed to isolate and identify the active constituents and to evaluate their mechanisms of action.

Author contributions

Bushra E. Aboukhadeer contributed to the study conception and design, coordinated data collection, performed data interpretation, and drafted the manuscript. Maryam K. Altounsi and Khaled M. Aburas contributed to the study design, data interpretation, and critical revision of the manuscript. Abdulgader A. Dhawi contributed to interpretation and provided critical intellectual input. Arwa S. Areebe, Nada A. Aboflejah, and Duaa A. Ammar contributed to data collection and preliminary data analysis. All authors reviewed and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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