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RESEARCH ARTICLE

Evaluation of Antibacterial Effects of Aqueous Extract of *Retama raetam* Plant against MRSA in Different Seasons

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Abstract

Background: The increasing incidence of multidrug-resistant infections, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), is a significant global health challenge that warrants the exploration of alternative antimicrobial agents. This research studied the effectiveness of the aqueous extract of the *R. raetam* plant, from the family *Fabaceae*, against MRSA, with a focus on the effectiveness of plant compounds according to seasonal variation. The research aims to qualitatively analyze the active compounds of the plant and evaluate the effectiveness of these compounds on methicillin-resistant bacterial isolates in different seasons.

Methodology: The leaves and flowers of the plant were collected from the Al'assabiea area of Libya in April–July 2024. These were washed, dried, and crunched to prepare an aqueous extract. The plant parts were screened phytochemically to determine active ingredients. Disc diffusion method was used to determine the antibacterial activity of plant parts against MRSA. Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) were determined.

Results: The results showed that the plant extracts studied in April were highly effective, reaching 25 mm inhibition zone, while the extracts studied in June and July did not produce significant results. The MIC and MBC values were determined to be 100 mg/mL and 200 mg/mL, respectively, indicating the extract's potential to inhibit and destroy MRSA at these concentrations. Thus, *R. raetam* has potential antibacterial activity against MRSA, especially when collected in the spring, because it contains sufficient bioactive flavonoids that affect bacteria.

Conclusion: The findings show *R. raetam* as a potential natural source for managing antibiotic-resistant bacteria. Further studies are recommended to isolate and identify active constituents accountable for antibacterial activity, and evaluate their efficacy *in vivo*. Limitations of the study are the need for a more detailed phytochemical analysis to understand seasonal variations in bioactive properties of the plant.

1. Introduction

Multidrug-resistant pathogens related to healthcare-associated infections (HCA) and community-acquired (CA) diseases have high proportions of resistance, as reported by the World Health Organization (WHO) [1]. In 2014, antibiotic resistance was responsible for an estimated 700,000 deaths globally. Current estimates project that the global population will range from 11 to 444 million in 2050, highlighting the danger of the increasing burden of antimicrobial resistance [1]. From 1970 to 2011, data on antimicrobial resistance (AMR) in Libya was limited, primarily because of insufficient surveillance and scarcity of published studies. Nevertheless, available information indicates persistently high proportion of resistance for *Salmonella* species, a trend observed since the late 1970s and continuing to the present day. Additionally, high prevalence (54–68%) of methicillin-resistant *Staphylococcus*

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aureus (MRSA) has been reported in the last decade among *S. aureus* isolates from patients with burns and surgical wound infections [2].

Addressing this issue requires a two-faceted approach, comprising new antibiotic discovery and enhanced infection control. Historical advances in antibiotics reflect the ongoing difficulty in treating resistant bacteria, which requires ongoing investigations, particularly of natural sources, including medicinal plants [3]. *Retama raetam*, or R'tm as a colloquial designation, is a tough desert plant belonging to the family *Fabaceae*, growing within arid regions of North Africa and the Middle East [4]. It is adapted to survive in extreme conditions [5]. Traditionally, the plant is used to cure many ailments, including diabetes and inflammation [6]. Scientific evidence of *R. raetam* as antimicrobial is limited, which raises the question, can *R. raetam* provide efficient solutions to antibiotic-resistant bacteria [7]. This research aims to uncover new antimicrobial agents from *R. raetam*, address antibiotic resistance, and provide potential alternatives to current treatments. The objectives of this research are to identify biologically active compounds in *R. raetam* and assess seasonal variations affecting these compounds' efficacy as well as evaluate their antimicrobial effectiveness against resistant strains. Notably, *Staphylococcus aureus* is a spherical, Gram-positive bacterium that often takes the shape of a grape cluster, is catalase-positive, and grows selectively on mannitol salt agar [8], often associated with hospital-acquired infections (HAIs) producing MRSA and vancomycin-resistant *Staphylococcus aureus* (VRSA) strains [9].

2. Materials and Methods (Preliminaries and Basic Concepts)

2.1. Sample collection, drying, and grinding

Retama raetam leaves and flowers were collected in April–July 2024 from the Al'assabiea area of Libya (Figure 1). Plant samples were thoroughly cleaned with tap water and dried in an oven at 40°C. Using a blender, the dried leaves and flowers were first crunched into a fine powder and then stored in a glass bottle at 4ºC in refrigerator until used.

Figure (1): *R. raetam* plant in its natural habitat.

2.2. Preparation of extract and phytochemical tests

In all, 50-g powdered leaf extract was dissolved in 500 mL of distilled water and agitated for 4 h with a hot plate magnetic stirrer at 50°C with a rotation of 3,000 rpm. The extract was filtered after 4 h using filter paper Whitman No. 1 and placed in a an oven for 3 days at 40°C. Then, the powdered extract was collected and stored in a tightly closed container at suitable conditions until use.

The qualitative phytochemical screening was performed according to the method described by Shaikh and Patil [10].

2.3. Stock solution preparation

The solution was prepared by dissolving 2 g of the extract in 3 mL of dimethyl sulfoxide (DMSO), and distilled water was added to prepare a total of 10 mL solution to yield a concentration of 200 mg/mL. The stock was stored at 4°C until used.

2.4. Medicinal productive yield

The productive yield of the extract was calculated by utilizing the mass of dry plant used as well as the mass of the extracted material after adding solvents and performing the evaporation process by using the following equation:

$$\text{Percentage yield} = \frac{A}{A0} \times 100,$$

where A is the mass of crude extract obtained after drying, and A0 is mass of the leaves used for extraction [12].

2.5. Activation of bacterial isolates

About 20-µL MRSA added in a test tube containing 4-mL nutrient broth and incubated for 24 h at 37°C. The McFarland factor was used to measure the bacterial concentration to make it 10^6 CFU/µL.

2.6. Antibacterial assay

Antibacterial disc diffusion assay

Experimental disc diffusion assay was used to evaluate the antibacterial efficacy of plant extract [11]. Briefly, using a sterile cotton swab, two bacterial inoculates were streaked across the Mueller–Hinton agar (MHA) petri dish's surface. Sterile 6-mm filter paper discs (Whitman No. 1; Germany) were pre-wetted with aliquots of test extracts, and the petri dish was subsequently incubated for 24 h at 37°C and observed for clear zones, measured in millimeters with a ruler.

2.7. Determination of MIC and MBC

The MICs and MBCs were defined as described by the Clinical and Laboratory Standards Institute (CLSI) [12]. The MICs and MBCs of aqueous extract of MRSA were

carried out in a 96-well microtiter plate with a two-fold consecutive standard stock microdilution method and bacterial concentration inoculum of approximately 10^6 CFU/mL. In all, 50 µL of aqueous extract of 2% stock solution (200 mg/mL) was mixed and diluted two-fold with testing bacteria in nutrient broth (NB, 50 µL). (DMSO) served as a negative growth control, whereas (ceftriaxone) served as a positive control. On the other hand, microtiter plate was incubated aerobically at 37°C for 24 h.

3. Results and Discussion

3.1. Productive yield

In this study, the effects of aqueous extract of *R. raetam* on MRSA was evaluated, with a focus on how seasonal timings of plant collection affect the efficacy of this extract. After the completion of the process of aqueous extraction, only 5.2 g was extracted (Figure 2).

3.2. Screening of phytochemically active ingredients

Qualitative phytochemical tests, such as the Foam test, Killer–Killiani test, Salkowaski test, alkaline reagent test, Benedict's test, ferric chloride test, and Biuret's test, were carried out, as shown in Table 1. The qualitative phytochemical screening utilizing targeted assays provided a rapid, cost-effective, and crucial initial metabolic profile of *R. raetam* extract. The significant benefit of employing these set of tests lies in their ability to generate efficiently a holistic preliminary fingerprint of major phytoconstituent classes present. This comprehensive screening revealed the concurrent presence of pharmacologically relevant groups, including phenolics (intense ferric chloride reaction), flavonoids (positive alkaline reagent test), and saponins (persistent foam test), while indicating the absence of detectable cardiac glycosides (negative Killer–Killiani test), triterpenoids (Salkowaski test), and proteins (Biuret's test). This immediate, multifaceted insight into the extract's chemical diversity is invaluable because it directly indicates the interpretation of subsequent biological assays as antioxidant or antimicrobial results linked to phenolics/flavonoids and strategically guides to the selection of more sophisticated, resource-intensive quantitative or chromatographic analyses for identification and isolation of specific compounds.

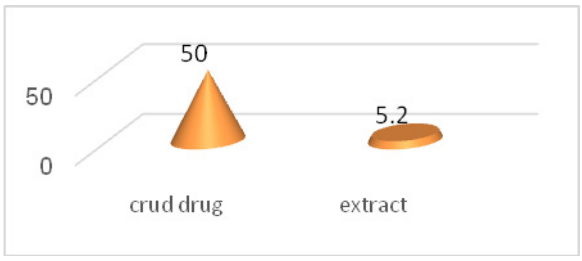

Figure (2): Yield of plant extract.

Table (1): Phytochemical screening tests.

Test	indication	Result
Foam	Saponins	+
Killer–Kiliani	Glycosides	-
Salkowaski	Triterpenoids	-
Benedict's	Reducing sugars	+
Ferric chloride	Tannins and phenols	+
Biuret's	Proteins	-
Alkaline reagent	Flavonoids	+

Note. + (plus) means available; - (minus) means not available.

3.3. Efficacy of aqueous extract collected in different seasons

The aqueous extract collected in April showed significant efficacy against MRSA with a zone of inhibition equal to 25 mm, while the aqueous extract collected in June showed an efficacy of only 9 mm and the aqueous extract collected in in July showed with no inhibition zone. Spring's moderate climatic conditions, such as balanced humidity and temperature, contribute to increasing the levels of active compounds, such as flavonoids, having antibacterial activity that explains notable effectiveness against MRSA.

3.4. MIC and MBC assays of aqueous extract against MRSA

The MIC values indicated the lowest concentration of the extract that inhibited visible growth, while the MBC values confirmed the minimum concentration required to destroy bacterial cells. Table 2 summarizes the MIC and MBC values observed in this study:

The results in Table 2 showed that the aqueous extract was effective against MRSA at concentrations of 200 mg/mL and 100 mg/mL, as no bacterial growth was observed at these concentrations. Hence, the concentration of 100 mg/mL was considered as the lowest concentration (MIC) that inhibited bacterial growth, whereas 200 mg/mL is the lowest concentration (MBC) that completely eradicated the bacteria. However, bacterial growth resumed with further dilution.

The present study demonstrated that the aqueous extract of *R. raetam* exhibited season-dependent antibacterial activity against MRSA, with spring (April)-harvested material showing maximum potent efficacy. The observed 25-mm inhibition zone and MIC (100 mg/mL) and MBC (200 mg/mL) values highlighted the potential of this plant as an antimicrobial substance. Notably, the effect of plant extract was due to flavonoids, and it appeared that the low temperature of April played a key role in increasing

Table (2): MIC and MBC assays against MRSA.

Concentration (mg/mL)	200	100	50	25	12.5	6.25	3.125
MRSA	-	-	+	+	+	+	+

the production of flavonoids, compared to June and July. This indicates that seasonal factors decisively affected the biosynthesis of biologically active compounds in *R. raetam* [13].

Qualitative phytochemical screening of the extract revealed the presence of bioactive substances, such as saponins, reducing sugars, tannins, phenols, and flavonoids with antimicrobial activities. Flavonoids particularly damage bacterial cell membranes and inhibit efflux pumps [14], the mechanism that may demonstrate the extract's activity against MRSA. Extraction inefficiency and limitations of seasonal biosynthesis may reflect the absence of glycosides and triterpenoids in *R. raetam* samples.

The effectiveness of the samples collected in April was due to climatic conditions of the spring season, having moderate temperature and balanced humidity, which induce production of secondary metabolities.

Conversely, increased temperature and scarcity of water availability in the summer season may stress the plant, reducing the production of secondary metabolites, which reflects a reduced bactericidal effect.

These findings were consistent with the results of previous studies on seasonal variation of medicinal plants. For instance, Saada *et al.* [8] determined that the phenolic content of *R. raetam* was maximum in the spring, with increased antioxidant activity—a trend mirrored in our antibacterial results. Similarly, other plants the family *Fabaceae*, such as *Glycyrrhiza glabra* (liquorice), demonstrated seasonal fluctuations in bioactive compounds [15]. This indicates the role of environmental stresses in modifying chemical profiles of medicinal plants.

The MIC and MBC values observed in the current study were comparable to those of other *R. raetam* extracts against MRSA, such as garlic (MIC: 125 mg/mL) and thyme (MIC: 50 mg/mL), but are lower than those of synthetic antibiotics. This suggests that although crude extracts may lack the efficacy of conventional drugs, they provide a basis for the development of synergistic formulations [15].

With the increasing resistance of bacteria to antibiotics, especially those causing hospital-acquired infections, this research is of great importance, as the plant may be an effective alternative, provided further testing is conducted on other bacteria types. However, the high MIC–MBC values indicate that crude extracts alone may not be sufficient for clinical use unless they work through a synergistic effect.

Instead, isolation of active principles, such as flavonoids or saponins, could yield derivatives that are more potent. Additionally, combining the extract with the existing antibiotics might enhance efficacy through synergistic interactions, a strategy successfully employed with other plant extracts.

This study has several limitations. Only aqueous extracts were tested; ethanol or methanol extracts might yield different results due to varying solubility of bioactive compounds. In addition, the phytochemical analysis was qualitative; quantitative profiling (e.g., high-performance liquid chromatography [HPLC]) is required to correlate specific compounds with antibacterial activity. The single geographic collection site limited the generalizability of results. Finally, *in vitro* results required validation of *in vivo* models to assess bioavailability and toxicity.

The future studies need to isolate and purify active molecules (e.g., flavonoids) to determine their individual and synergistic effects while also optimizing extraction methods by testing solvents of varying polarities and advanced techniques (e.g., ultrasound-assisted extraction) to enhance yield and potency. Concurrently, ecological studies must be conducted to investigate how soil composition, rainfall, and other abiotic factors influence *R. raetam*'s phytochemistry across different regions. In parallel, *in vivo* testing should be caused to evaluate the efficacy and safety of extracts in MRSA-infected animal models. Additionally, synergy trials are required to explore combinations with conventional antibiotics, aiming to reduce effective doses and prevent resistance development.

Conclusion

This work emphasized the potential of *R. raetam* as a season-dependent antimicrobial source and highlighted the importance of ecological factors in phytochemical efficacy. By addressing the outlined limitations, the findings could pave the way for integrating this plant into modern therapeutic strategies against multidrug-resistant pathogens.

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Conflict of interest

The authors confirmed that they had no motive other than those related to scientific research conducted in the present study.

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