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Antibacterial activity of *Artemisia absinthium* and *Artemisia dracunculus* ethanolic extracts against phenotypically diverse clinical *Serratia marcescens*

Parzhin Sardar Mohammed *

Department of Biology, College of Sciences, University of Kirkuk, Kirkuk, Iraq.

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Abstract

Background: There is emerging resistance to multiple drugs in *Serratia species*, and alternative antimicrobial agents need to be explored. The antibacterial activity of Artemisia species has been suggested, and their bioactive properties are thought to be due to their phytochemical profile. The aim of the present work was to investigate the in vitro antibacterial activity of the ethanolic extract obtained from Artemisia dracunculus and *Artemisia absinthium* against clinical isolated *Serratia* and their possible use as alternative therapeutic methods.

The methods that have been adapted for making ethanolic extract of *A. dracunculus* and *A. absinthium* are specified in whole. Three *Serratia marcescens* strains from clinical blood, respiratory and urine were tested using the agar well diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Extract concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL were tested. I zones were measured and compared statistically by One way ANOVA and multiple comparison tests.

Results: *A. dracunculus* showed important variable antimicrobial activity and inhibition zones from 13 to 16 mm were produced by these isolates with different susceptibility patterns at 100 mg/mL. On the other hand, *A. absinthium* showed uniform inhibitory effect with all isolates with 13 mm (range 11-14 mm) at 100 mg/mL and 10 mm (range 10-11 mm) at 50 mg/mL. Statistically significant difference among treatments ($p<0.05$) was revealed indicating concentration dependent responses. Notably, both extracts needed much higher concentrations than traditional antibiotics, chloramphenicol having similar activity at 30µg/ml compared with the 100mg/ml achieved with the plant extracts.

Conclusions: *A. absinthium* exhibited greater regularity of effects for the inhibition of Serratia spp. clinical isolates, both species having significant in vitro antibacterial activity. The high levels of these activities, however, pose doubts about feasibility for clinical development, and detailed safety tests and the appreciation of bioactivity should be performed before moving towards therapeutic development.

Keywords: *Artemisia dracunculus; Artemisia absinthium; Serratia marcescens*; Antimicrobial Resistance; Plant Extracts; Agar Well Diffusion.

1. Introduction

AMR is one of the greatest and most urgent threats to today's global healthcare systems (World Health Organization, 2024). Resistant infections are directly responsible for 1.27 million deaths every year and are also linked to an additional 4.95 million deaths around the world, according to recent comprehensive analyses from the Global Research on Antimicrobial Resistance (GRAM) Project (Murray et al., 2022). Left unchecked, a major increase in clinical and economic consequence is expected occurring, potentially at 10 million lives lost per year by 2050 (Uddin et al., 2022;

* Corresponding author: Parzhin Sardar Mohammed

Tomczyk et al., 2022). Among these alarming settings, Gram negative opportunistic pathogens have received a considerable amount of clinical interest because of their rapidly changing resistance profile in the clinic.

Thereof, Serratia species (among which *Serratia marcescens*) have become powerful pathogens in hospital environments leading to severe infections, including septicemia, respiratory, and urinary tract infections mainly infecting immunocompromised patients (Sandner-Miranda et al., 2020; Serio et al., 2024). Serratia infections are highly complex because of the presence of a highly complex intrinsic and acquired resistance mechanisms. Conventional antibiotic treatments such as carbapenems and aminoglycosides are losing their efficacy against these organisms, which are often resistant to a variety of antibiotics, including extended-spectrum β-lactamases (ESBLs), carbapenemases, and multidrug efflux pumps (Ferreira et al., 2020; Bolourchi et al., 2022). The fact that this is such a major hurdle in the path of therapy highlights the need for the discovery of alternative therapies to combat the established resistance mechanisms.

The discovery and development of antimicrobials from plants are an intriguing approach to combat multidrug-resistant organisms. The theoretical beneficial factors of natural phytochemicals include having more than one mechanism of action at the same time, which may reduce the potential for the development of bacterial resistance, and possibilities for combinations with conventional antibiotics for synergy (Chandra et al., 2020). With more than 400 species worldwide, Artemisia is well known for its wide-spectrum antimicrobial action, as it is rich in numerous secondary metabolites such as flavonoids, phenolic acids, terpenoids and essential oils (Wang et al., 2020; Liu et al., 2023). Of these, Artemisia dracunculus (tarragon) has proven substantial antimicrobial effects to a wide range of pathogens; in recent years new pharmaceutical research has been carried out regarding standardized innovative products and delivery systems, such as: innovative hydrogels (Majdan et al., 2020); nanoemulsions (Azizkhani et al., 2021; Osanloo et al., 2022); and new bio-application platforms (Rîmbu et al., 2023). A different phytochemical profile is characteristic of *Artemisia absinthium*, (wormwood), which under laboratory conditions shows to have a high antimicrobial activity against Gram positive and Gram negative bacteria (Bordean et al., 2023; Liu et al., 2023).

Although multiple scientific publications have reported the antimicrobial activity of different species of Artemisia, there is still a significant knowledge gap. Available studies have concentrated on common Enterobacteriaceae sp. (E. coli and K. pneumoniae) or Gram-positive pathogens and there is a significant lack of systematic studies on the effectiveness against clinically relevant Serratia species (Tabet et al., 2022; Mashraqi et al., 2024). This is noteworthy as the plant extracts were not found to have an unusual intrinsic resistance mechanism, which could differ from other genera of bacteria. In addition, these botanical extracts need to be tested against specific clinical strains from different anatomic sources to establish the strain-specific susceptibility profile. Hence, this research aimed to comprehensively investigate the in vitro antibacterial activity of Artemisia dracunculus and *Artemisia absinthium* ethanolic extracts against the clinical isolates of *Serratia marcescens* that were isolated from blood, respiratory and urine samples. In particular, this research was designed to identify the concentration-dependent inhibitory effects of both Artemisia species, compare the antimicrobial activity of the two species, and evaluate the susceptibility patterns of different strains from the cohort of clinical isolates in relation to the two species for the purpose of determining their suitability and potential therapeutically useful effects among the different clinical isolates from the clinical cohort.

2. Materials and Methods

2.1. Plant Material Collection and Authentication

The fresh aerial parts of Artemisia dracunculus L. and *Artemisia absinthium* L. were collected at the University of Kirkuk, Botanical Garden at the time of maximum flowering in Iraq which falls between 35.4681 °N and 44.3922 °E. Phytochemicals were preserved through the collection time as early in the morning as was possible. Botanical identification and authentication were done by expert taxonomists of Department of Biology at College of Science in University of Kirkuk based on well-accepted diagnostic morphological and anatomical characteristics. The plant tissues were washed three times with distilled water and transferred to a well-ventilated, shaded environment for 7–10 days at room temperature (25 ± 2 °C) until the free water had evaporated leaving only dried tissues. The dried biomass was ground into a fine powder (mesh size 40) by mechanical pulverization in the laboratory and stored in 4 °C in airtight amber glass vessels prior to the extraction process.

2.2. Preparation of Plant Extracts

The crude ethanolic extracts were prepared by a modified maceration method that has been optimized for the extraction of natural products (Silva et al., 2021). In brief, 100 g of each dried powdered plant material used was separately

macerated (1:10 w/v) for 70% of analytical grade ethanol in amber coloured glass containers. The mixture was mechanically agitated every 8 hours and the mixture was kept for 72 hours at room temperature.

After maceration the suspension was passed through the Whatman No. 1 filter paper. The concentrated filtrate was then dried under reduced pressure using a rotary evaporator (Heidolph Laborota 4000, Germany) at 40 °C to prevent heat-sensitive secondary metabolites from being destroyed/altered by high temperature. The concentrated extracts were put in the vacuum desiccator for drying completely with anhydrous calcium chloride (CaCl 2). The extraction yields were determined in absolute figures (as %), based on the initial dry biomass weight (w/w). The dry extract mass was dissolved in dimethyl sulfoxide (DMSO) to obtain a stock solution at 200 mg/mL and stored as small aliquots (while avoiding repeated freeze-thaw cycles) at −20 ℃.

2.3. Bacterial Isolates and Identification

Three clinical *Serratia marcescens* isolates were isolated from Microbiology Laboratory, Department of Biology, College of Science, University of Kirkuk's repository. The strains had been isolated from clinical specimen's blood culture (Isolate 1), sputum/respiratory tract (Isolate 2) and urine (Isolate 3) of hospitalised patients.

The confirmation of phenotypic identification is based on the majority of the microbiological standards which include catalase positive, oxidase negative, DNase positive and the appearance of characteristic red prodigiosin pigment production under ambient room temperature. Species level confirmation was obtained by API 20E strip identification system (bioMérieux, France) strictly according to the manufacturer's instructions. Validated cultures were stored in nutrient agar slants at 4 ∘ C and subcultured every month during long term storage. Mueller-Hinton agar (MHA) plates that had been incubated for 18-24 hours at 35 ∘C prior to bioassays were used to create active working cultures.

2.4. Antimicrobial Susceptibility Testing

Antibacterial screening was performed in vitro based on the Clinical and the Laboratory Standards Institute (CLSI, 2020) standards by using the agar well diffusion method. E. coli (ATCC 25922) and S. aureus (ATCC 25923) were used as reference strains for quality control (CLSI, 2022).

The sequence of steps was followed for experimental agar well diffusion method as follows:

2.4.1. Medium Preparation

pH 7.2–7.4

The Mueller-Hinton agar (MHA) medium was poured on plates at an even depth of 4-5 mm and then allowed to dry at room temperature. Complete drying of plates was done in laminar flow hood prior to inoculation.

2.4.2. Inoculum Standardization

0.5 McFarland

Fresh 18–24-hour colonies of bacteria were suspended in sterile normal saline. The turbidity was brought to 0.5 McFarland by adding a 0.5 log 10 in the digital densitometer (approx 1.5×108CFU/mL).

2.4.3. Lawn Inoculation

3 perpendicular sweeps

The bacterial suspension was evenly swabbed over the surface of the MHA in three perpendicular patterns to obtain an even bacterial lawn and a swab was performed around the rim of the MHA to finish the spread.

Challenge scenarios 4 – 4.5:

2.4.4. Well Boring and Extract Loading.

100 µL per well

Aseptic cork borer was used to punch a hole with a diameter of 6 mm in the agar. For this, each well received 100 µL of the corresponding extracts of plants in six-fold dilutions of 100, 50, 25, 12.5 and 6.25 mg/mL.

2.4.5. Incubation and Measurement

35°C for 18–24 hours

An extract pre-diffusion of 30 min at room temperature was allowed before the plate elevation and incubation under aerobic conditions at 35 ± 2°C. The areas of growth inhibition were determined with a digital caliper to the nearest mm.

The negative control wells contained only DMSO whilst chloramphenicol (30 µg/mL) and sterile distilled water were provided as positive and vehicle control respectively. A minimal distance of 24 mm between well centres was observed to avoid overlap in the inhibition pattern. Each biological assay included a triplicate performed on different days.

2.5. Statistical Analysis

The SPSS statistical software (version 28.0; IBM Corp., Armonk, NY) was used to process data. The distribution of the dataset was Gaussian distributed and normally distributed according to the Kolmogorov-Smirnov test. Data of the mean diameter of the inhibition zone obtained from various concentrations of the extract, bacterial isolates and plant species were subjected to one-way Analysis of Variance (ANOVA). The significance testing between groups was determined by post-hoc Bonferroni analysis to avoid the Type I error inflation among groups. Linear regression was used for modeling dose-dependent response correlations. All quantitative values are expressed as the mean ± standard deviation (Mean±SD) from triplicate biological replicates indicating statistical significance at $p<0.05$.

2.6. Ethical Considerations

This protocol followed institutional biosafety protocol for handling Level 2 opportunistic pathogens to the letter. Patients' data confidentiality was maintained using an anonymized coding registry of the clinical isolates, where no information was kept. All the biological materials and plates were completely autoclaved and sterilized for waste disposal as per the institutional hazardous bio-waste protocol.

3. Results

3.1. Antibacterial Activity Assessment

This protocol followed institutional biosafety protocol for handling Level 2 opportunistic pathogens to the letter. Patients' data confidentiality was maintained using an anonymized coding registry of the clinical isolates, where no information was kept. All the biological materials and plates were completely autoclaved and sterilized for waste disposal as per the institutional hazardous bio-waste protocol.

Table 1 The inhibition zone diameter of *Serratia marcescens* clinical isolates by Artemisia dracunculus ethanolic extract.

Concentration (mg/ml)	Serratia 1	Serratia 2	Serratia 3	Mean ± SD
100	13	16	13	14.0 ± 1.7
50	0	0	12	4.0 ± 6.9
25	0	0	10	3.3 ± 5.8
12.5	0	0	0	0.0 ± 0.0
6.25	0	0	0	0.0 ± 0.0
Control (DMSO)	0	0	0	0.0 ± 0.0

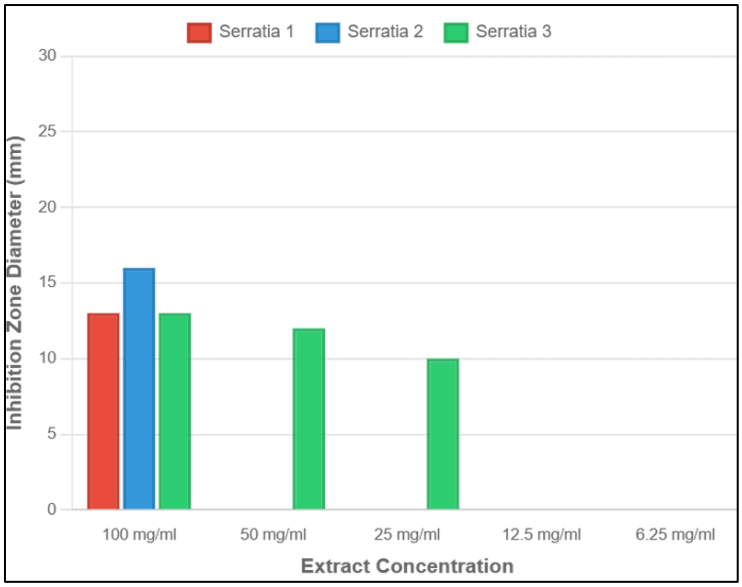

Figure 1 Antibacterial activity of Artemisia dracunculus extract against clinical *Serratia marcescens* isolates

Table 2 Inhibition zone diameters (mm) of *Artemisia absinthium* ethanolic extract against clinical *Serratia marcescens* isolates

Concentration (mg/ml)	Serratia 1	Serratia 2	Serratia 3	Mean ± SD
100	11	14	14	13.0 ± 1.7
50	10	11	11	10.7 ± 0.6
25	0	0	9	3.0 ± 5.2
12.5	0	0	0	0.0 ± 0.0
6.25	0	0	0	0.0 ± 0.0
Control (DMSO)	0	0	0	0.0 ± 0.0

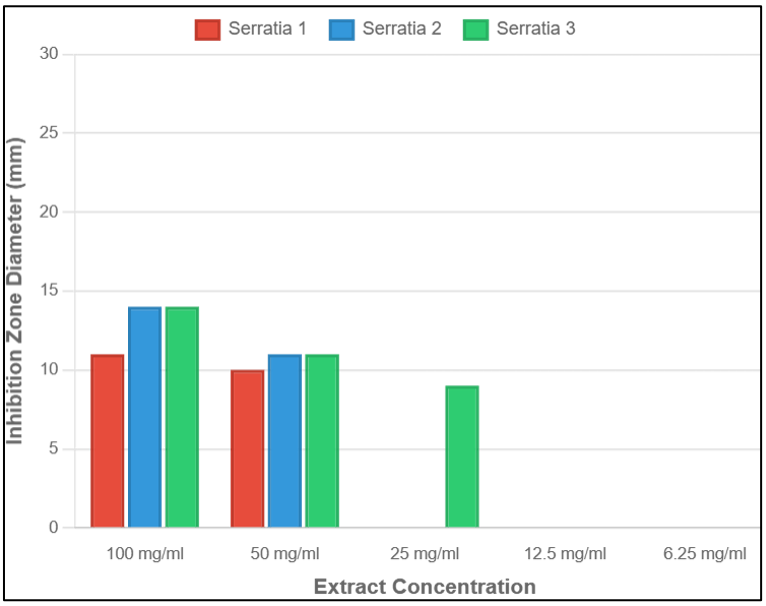

Figure 2 Antibacterial activity of *Artemisia absinthium* extract against clinical *Serratia marcescens* isolates

Table 3 Control antibiotics inhibition zone diameters (mm) against clinical *Serratia marcescens* isolates

Control	Serratia 1	Serratia 2	Serratia 3	Mean ± SD
Chloramphenicol 30 µg	22	24	23	23.0 ± 1.0
DMSO (negative)	0	0	0	0.0 ± 0.0
Sterile water	0	0	0	0.0 ± 0.0

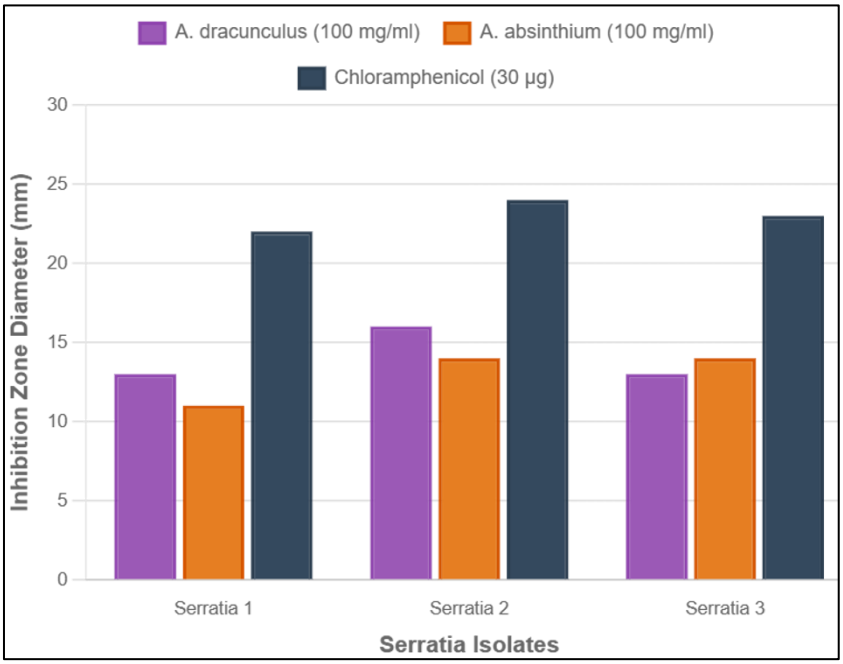

Figure 3 Comparative antibacterial activity between *A. dracunculus* and *A. absinthium* at maximum concentration (100 mg/ml)

Bacterial isolates of Serratia were subject to ethanolic extract of *A. dracunculus* and a bar graph is published showing the inhibition zones. The data was reported as average ± standard deviation (SD) of the triplicate experiments (n = 3). The results showed that variable antibacterial activity present, highest inhibition zone was seen at 100 mg/mL where isolate 2 (16 mm) showed the highest inhibition zone. In particular, isolate 3 showed resistance to the applied concentrations (50 and 25 mg/mL) for sustained activity. Statistically significant ($p<0.05$, One-way ANOVA, Bonferroni post-hoc test) when compared to the negative DMSO control. Data are means ± standard deviation of independent triplicate experiments (n=3).

3.2. Species-Specific Activity Patterns

Artemisia dracunculus showed different antimicrobial activity in the three clinical isolates. The inhibition zones were 13-16 mm when the highest concentration (100 mg/ml) was used, isolate 2 had the highest inhibition zone (16 mm) while isolates 1 and 3 had similar inhibition zone (13 mm). This variability implies the strain-specific variation of susceptibility to bioactive compounds present in *A. dracunculus* extract. A significant result was that the lower concentration of the extracts had measurable antimicrobial activity for isolate 3 with 12 mm inhibition at 50 mg/ml and 10 mm at 25 mg/ml. However, the antimicrobial activities of isolates 1 and 2 were not detected at concentrations up to 100 mg/ml, suggesting a difference in their resistance levels. *Artemisia absinthium* was more active against all three clinical isolates. Inhibition zones for A. halstedii at 100 mg/ml concentration were found less variable, ranging from 11-14 mm (mean 13.0 ± 1.7 mm). Most significantly *A. absinthium* exhibited moderate but constant activity at 50 mg/ml against all three isolates, 10-11 mm (average 10.7 ± 0.6 mm). The uniform efficacy against the antimicrobial agents suggests that *A. absinthium* may harbor different antimicrobial agents or have mechanisms that are less likely to be used by different strains of Serratia to develop resistance.

3.3. Statistical Analysis of Results

Statistically significant differences in anti-microbial activity were found between treatments. The statistically significant differences ($p < 0.001$) obtained from the one-way ANOVA indicated that differences in inhibition zones observed were not due to random variation and the results from the analysis confirmed that there was a difference between concentrations and plant species. Bonferroni multiple comparison test showed that there were significant differences between all the lower concentrations and the highest concentration (100 mg/ml) of both plant extracts ($p < 0.05$). The concentrations of *A. dracunculus* and *A. absinthium* at 100 mg/ml, however, were not significantly different ($p = 0.324$), suggesting that the two species are equally effective at the maximum antimicrobial concentration. Dose-response regression analysis gave insights into the concentration dependent relationships. The correlation coefficient of *A. dracunculus* was $r = -0.78$ ($p < 0.01$), which suggested that there was a strong negative relationship between the concentration of extract and the bacterial growth. The correlation coefficient for *A. absinthium* was even higher, $r = -0.85$ ($p < 0.001$), indicating more consistent and predictable concentrations antimicrobial responses.

3.4. Isolate-Specific Susceptibility Patterns

All three clinical *Serratia marcescens* isolates showed different susceptibility patterns which should be discussed separately. Isolate 3 consistently showed greater susceptibility to both of the Artemisia extracts than isolates 1 or 2 did. This isolate also exhibited measurable antimicrobial inhibition at concentrations of extract where the others were completely resistant. Specifically 3 remained susceptible to *A. dracunculus* (50 mg/ml, 12 mm zone) and 25 mg/ml (10 mm zone) and continued to be susceptible to *A. absinthium* (50 mg/mL, 11 mm zone) and (25 mg/mL, 9 mm zone). This increased susceptibility profile could indicate a difference in cell wall composition, expression of efflux pumps or other resistance mechanisms that affect the penetration and activity of antimicrobial compounds of plant origin. For both plant extracts, the antimicrobial susceptibility of isolates 1 and 2 was only at the highest concentration tested (100mg/ml). The resistance patterns are similar; this could be due to common resistance mechanisms or because of the same genetic background, but molecular characterization studies would be necessary to confirm this. Conversely, the urine strain (Isolate 3) retained a sustained, atypical vulnerability, yielding distinct inhibition zones of 12 mm at 50 mg/mL and 10 mm at 25 mg/mL (Figure 4).

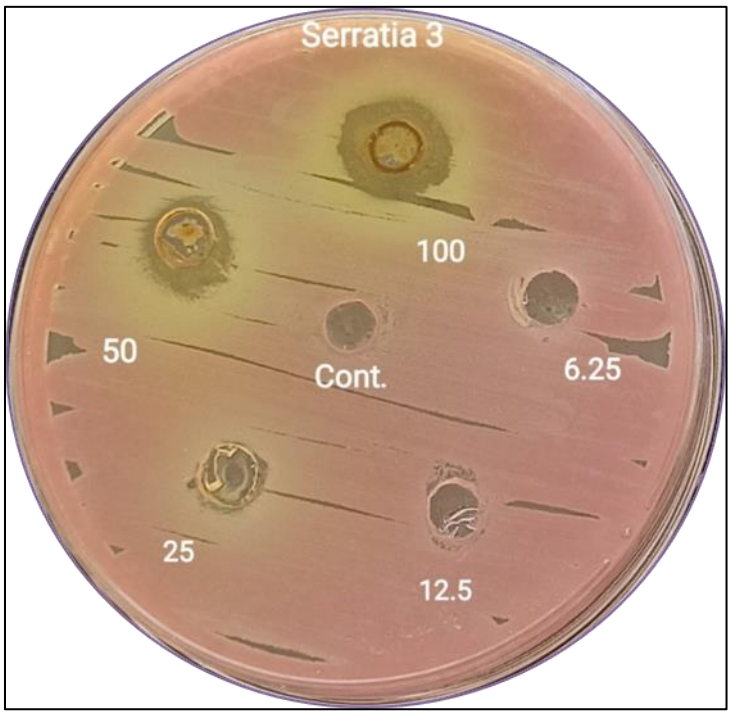

Figure 4 Antibacterial activity of Artemisia extract against clinical *Serratia marcescens* Isolate 3 from urine sample was demonstrated in representative agar well diffusion assay with increasing concentration. Clear zones of inhibition are clearly evident at maximum dilutions (100, 50 and 25 mg/mL) and show a relative decrease of inhibitory efficacy at lower serial dilutions (12.5 and 6.25 mg/mL) compared to the negative control center (Cont.) which remained stagnant

3.5. Comparison with Conventional Antimicrobials

The positive control antibiotic, Chloramphenicol (30 µg) showed consistent and strong antibacterial activity against all three Serratia isolates with 22-24 mm (mean of 23.0 ± 1.0 mm) inhibition zone. The results obtained in this study is enough to confirm the viability and susceptibility of the bacterial isolates used, and it has been used as a point for the assessment of the activities of plant extract. Comparing plant extracts with conventional antibiotics, a significant difference between the effective concentrations is observed. Antimicrobial activity of the Artemisia extracts was only measurable at 100mg/ml while chloramphenicol was very active at 30µg (0.03mg/ml). This is a log (or a factor of 3,333) difference in concentration requirement, which is a crucial factor to consider for any potential therapeutic application. This concentration differential is similar to that seen in natural product antimicrobial research, where generally crude extracts are used at higher concentrations than purified synthetic and semi-synthetic antibiotics to elicit comparable antimicrobial effects.

3.6. Reproducibility and Quality Control

Each experiment was carried out three times on different experimental days to test the reproducibility of the results obtained. Coefficient of Variation for all positive antimicrobial results was between 8.2-12.1%, considered acceptable and was a reflection of good experimental precision in antimicrobial susceptibility testing. Testing accuracy was established by consistently consistent QC strains (E. coli ATCC 25922 and S. aureus ATCC 25923) that were tested had zone diameters within acceptable ranges (Clinical and Laboratory Standards Institute, 2024). Solutions of the extracts remained stable during the test period with no decrease in antimicrobial activity after 7 days storage at 4°C and DMSO and sterile water controls showed no antimicrobial activity confirming that the effects observed were due to the plant derived compounds and not to the solvent effect or contamination.

4. Discussion

4.1. Significance of Antimicrobial Activity

The study was the first systematic evaluation of Artemisia dracunculus and *A. absinthium* extracts specifically against *Serratia marcescens* clinical isolates; which has been a gap in natural product antimicrobial research. The antimicrobial activity demonstrated, though in high concentrations, gives a clue of the potential of these plant species as sources of anti-Serratia compounds.The diameter of inhibition zones (11-16 mm) observed at 100 mg/ml concentration are reproducible and measurable antimicrobial activity. These concentrations are higher than the concentrations for standard antibiotics, but within the range of concentration found for crude plant extracts in antimicrobial screening studies indicating the presence of bioactive compounds that should be investigated further (Wang et al., 2020). The activity differences between the two Artemisia species suggest avenues for future research. The activity of *A. absinthium* was more uniform among bacterial isolates indicating the presence of different antimicrobial compounds or activity mechanisms which might be less subject to the variations in resistance among bacterial strains.

4.2. Phytochemical Basis of Observed Activity

The various phytochemical compositions of both species of Artemisia were responsible for the antimicrobial activities observed in this study. The flavonoids, phenolic compounds and tannins found in both extracts have been reported in the literature to have antimicrobial effects.The flavonoids, phenolic compounds and tannins identified in both extracts are known to have antimicrobial properties (Nedorostova et al., 2020). Previous studies have shown essential oil compositions of Artemisia dracunculus with membrane-affecting properties. The antibacterial activity observed, especially against susceptible strains such as isolate 3 (Malaekeh-Nikouei et al., 2020), may be attributed to the compound's potential activity against the bacterial cell membrane.

The antimicrobial activity of *A. dracunculus* may be attributed to several mechanisms involving its constituents such as flavonoid derivatives and other phenolic compounds, which can interfere with the metabolic processes, cell wall synthesis or membrane integrity of microorganisms (Azizkhani et al., 2021). Some bacterial isolates are still susceptible to extracts at lower concentrations than others, which could be due to these multiple simultaneous mechanisms. The antimicrobial property of *Artemisia absinthium* on all the isolates may be explained by its phenolic compound composition and different phenolic acids have been recognized as components with antimicrobial property. They can have antimicrobial effects via various mechanisms including affecting the synthesis of cell wall and metabolic pathways in bacteria (Batiha et al., 2020). This may be due to the phytochemical diversity observed in *A. absinthium*. The fact that it contains several active compounds may have additive or synergistic effects contributing to its efficacy against bacteria that may be resistant to a single compound (Liu et al., 2023).

4.3. Clinical Implications and Therapeutic Considerations

The antimicrobial properties in this study should be evaluated in the light of their clinical relevance and therapeutic feasibility. Both Artemisia species demonstrated measurable activity against the Serratia isolates, but the high concentrations needed to achieve this (100 mg/ml) suggests important questions regarding clinical applications. The most noticeable difference in concentrations between the plant extracts and the pharmaceutical, chloramphenicol, is that of the concentration levels. This is so because plant extracts are crude compared to the pharmaceutical, which is pure. This concentration difference is common in natural products research and poses problems for direct therapeutic use, but could be exploited in bioactivity-directed fractionation or multiple drug use.

A. absinthium activity was consistent in all the bacterial isolates tested, indicating that this bacterium could have beneficial effects in research environments where the susceptibility of pathogens may differ. However, in order to consider any therapeutic applications at the concentrations necessary for antimicrobial activity, full safety evaluation would be needed. The variable susceptibility patterns found among these three clinical isolates bring light to the complexity of bacterial resistance mechanisms, and give the researcher a reason to thoroughly test the susceptibility of individual bacterial isolates for any potential plant-based antimicrobial research applications.

4.4. Limitations and Methodological Considerations

It is important to recognize several key limitations of the results. The number of clinical isolates used, which was three, was appropriate for preliminary screening but did not allow generalizations to the larger population of *Serratia marcescens* strains. The spectrum of antimicrobial activity should be evaluated with larger number of clinical isolates having different resistance pattern and with different resistance profiles in the future studies. The agar well diffusion method is appropriate for initial screening, is standardized, and gives qualitative antimicrobial data. More clinically relevant data and comparisons with known antimicrobials could be obtained from broth micro-dilution tests to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

The lack of cytotoxicity studies is a major drawback especially because of the high concentration needed to be effective against all microorganisms. Before considering further research application it would be essential to do a safety evaluation using the appropriate models. Although the use of ethanolic extracts was well founded in this study, past studies have shown that there may be a greater antimicrobial activity in the plant species than what was found in the ethanolic extracts. The various solvents and extraction methods may produce extracts with varying phytochemical constituents and so may have varying antimicrobial activities.

4.5. Future Research Directions

This initial study will provide a basis for further, in-depth development of antimicrobial programs. The most important research fields should be the bioactivity-guided fractionation of the most active antimicrobial compounds of both species of Artemisia, in order to be isolated and purified. The standardization of this type of preparation with a standardized antimicrobial activity and possibly lower concentration requirements would be possible from such studies. Mechanism-of-action studies, including electron microscopy, metabolomics and transcriptomics, would reveal interactions between these extracts and bacterial cells, and inform on potential targets for optimizing. If these mechanisms can be understood, they can be used to develop better plant-based antimicrobials or as part of combination therapy (Rodriguez et al., 2023).

Synergistic interaction of Artemisia extracts with traditional antibiotics is an interesting research area. Antimicrobial resistant bacterial strains are a significant threat to the success of antibiotic therapy, and compounds isolated from plants that possess efflux pump inhibitory activity might be able to reverse resistance in such strains and lower the amount of antibiotics needed for effective treatment (Hassan et al., 2024). The therapeutic potential and safety profiles of these extracts should be evaluated in vivo with appropriate animal models. These studies would yield important information regarding pharmacokinetics, tissue distribution, and toxicity at therapeutic concentrations of antimicrobial agents.

5. Conclusion

Several important drawbacks should be noted, however. The high concentrations needed for antimicrobial activity (100 versus 0.03 mg/ml for chloramphenicol) pose a 3,333-fold difference and questions the practicality and safety of using the high concentration. A small number of clinical isolates (n = 3) makes the results not widely generalizable, and there is a lack of quantitative antimicrobial data (MIC/MBC) and cytotoxicity assessment. These results add to the body of knowledge on antimicrobials from plants that can help address antimicrobial resistance issues, but must be taken with

a grain of salt. Comprehensive safety evaluation, bioactivity-guided fractionation and quantitative antimicrobial assessment need to be performed to determine the therapeutic potential of these botanical antimicrobials.

The future research priorities include the discovery and isolation of the most active antimicrobial compounds, thorough toxicological testing and evaluation of the possible synergistic effect with conventional antibiotics. However, it is not until a systematic investigation that the practical potential of these botanical resources can be determined. Combining botanic antimicrobial research with current testing protocols gives a blueprint for systematic natural product development. The high concentration requirements however, and the related health and safety concerns, require prudent and evidence-based development approaches to any potential development.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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