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(RESEARCH ARTICLE)

Impact of *Artemisia dracunculus* Alcoholic Extract on Different Strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*: An Experimental Study

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Abstract

Background: There is a growing problem with staphylococcal infections that are resistant to antibiotics, and we need to investigate other antibiotics that will be of benefit. The rich phytochemical composition of *Artemisia dracunculus* (tarragon) has shown that it has good antimicrobial properties.

Methods: employed for testing the antibacterial activity of *A. dracunculus* alcoholic extract was agar well diffusion method using four strains of *Staphylococcus epidermidis* and seven strains of *S. aureus*. The diameters of the inhibition zones were measured with serial dilutions of 6.25 mg/mL to 100 mg/mL.

Results: Inhibition zone of all *bacterial isolates were measurable at 100mg/mL (12–20mm for both species). S. aureus isolates were more sensitive than S. epidermidis* isolates at the medium concentrations (25–50 mg/mL). We were able to see that the concentration is important for the antibacterial activity and that there are great differences in susceptibility of each isolate.

Conclusion: The alcoholic extract of *A. dracunculus* can effectively kill the *Staphylococcus* bacteria and thus it can be used as a natural antimicrobial agent. More research is needed on minimum inhibitory concentrations and finding bioactive compounds.

Keywords: *Artemisia Dracunculus*; Antibacterial Activity; *Staphylococcus aureus*; *Staphylococcus epidermidis*; Plant Extract; Antimicrobial Resistance

1. Introduction

Multidrug-resistant bacterial pathogens are one of the greatest challenges in today's medicine. Staphylococcal infections are a big cause of illness and death around the world [1,2]. *Staphylococcus aureus* and *S. epidermidis* are opportunistic pathogens that can lead to a variety of infections in a healthcare environment including skin infections as well as life-threatening septicemia [3,4]. The growing number of methicillin-resistant *S. aureus* (MRSA) and coagulase-negative staphylococci (CoNS) has made it necessary to quickly look into other treatment options [5].

Medicinal plants have been a major source of novel antibacterial chemicals for a long time. Natural sources make up about 70% of the antibiotics we use today [6,7]. There are more than 400 species of the genus Artemisia around the world. People are very interested in it since it has numerous pharmacological benefits, such as being an antioxidant, an anti-inflammatory, and an antibacterial [8,9]. *Artemisia dracunculus L.* (tarragon), a perennial herb native to Central Asia and widely cultivated in temperate regions, contains a variety of bioactive chemicals, such as flavonoids, phenolic acids, terpenoids, and essential oils [10]. Chlorogenic acid, caffeic acid, rutin, and other monoterpenes are some of the chemicals that earlier investigations of *A. dracunculus* have shown that might be able to kill germs [11]. People think that Artemisia species fight bacteria in a multitude of ways, include tearing down cell membranes, blocking essential enzymes, and mucking up t

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the process of making bacterial DNA [12]. Still, there aren't many studies that look at how well *A. dracunculus* works against clinically important staphylococcal isolates. The purpose of this investigation was to see how effectively A. *dracunculus* alcoholic extract performs against different strains of *S. aureus* and *S. epidermidis* at different levels of concentration. This adds to the scientific proof that it could be used to treat staph infections.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

Fresh aerial parts of *A. dracunculus* were gathered at the flowering period and botanically identified by botanist experts. The plant material was shade dried, finely ground and extracted with ethanol by maceration technique. The crude extract was concentrated with rotary evaporator and stored in 4°C till further use.

2.2. Bacterial Strains and Culture Conditions

Four clinical isolates of *S epidermidis* and 7 isolates of *S aureus* were isolated from clinical samples. Bacterial identification was carried out by the standard biochemical tests and stored at 4°C on nutrient agar.

2.3. Antibacterial Assay

The anti-bacterial activity was determined by the agar well diffusion method as described by Clinical and Laboratory Standards Institute (CLSI, 2021) [13] with some modifications as described by Balouiri et al (2016) [14]. A series of dilutions of the extract (6.25, 12.5, 25, 50 and 100 mg/mL) were prepared following the method described by Hammer et al., 1999 [15] using dimethyl sulfoxide (DMSO). Mueller-Hinton plates were made as per the instructions and left to set. The bacterial suspensions were adjusted to 0.5 McFarland turbidity standard ($\approx 1.5 \times$ 10^8 CFU/mL) in accordance with standard procedures [16].

Standardized bacterial suspension was spread uniformly on the entire surface of the agar-plates by using sterile cotton swabs and inoculated with 100 µL [17]. Sterile cork borers (6 mm in diameter) were used to make wells as previously described by Valgas et al. [18].(2007) The respective extract concentrations were added to each well, dimethyl sulfoxide (DMSO) was used as the negative control, and 50 µL was added to each well. Pre-diffusion of the plates was performed for a 30-minute time at room temperature followed by incubation at 37°C for 24 hours (CLSI, 2020). Diameters of the inhibition zones were determined with a digital caliper with an accuracy of 1 mm as per standard procedure [18]. Each experiment was repeated three times for reproducibility.

2.4. Statistical Analysis

All experiments repeated three times. All data were presented as mean ± standard deviation (SD). One-way ANOVA and Tukey's post hoc analysis were performed to compare the inhibition zones among different concentrations for each bacterial species and independent samples t-tests were performed to compare the mean inhibition zone between *S. aureus* and *S. epidermidis* at the same concentrations. A P value < 0.05 was chosen as the criterion of statistical significance. Descriptive statistics were used to analyse the data and graphical presentation set up to show the concentration-response relationships.

3. Results

The antibacterial activity of the extract from *A. dracunculus* against the clinical isolates of *S. epidermidis* and *S. aureus* are summarized in tables 1 and 2, respectively. Data are reported as individual inhibition zone diameters, and mean ± SD for each concentration. The *S. epidermidis* isolates showed measurable inhibition only at higher concentrations, as seen in Table 1, there was a sharp drop below 50 mg/mL. However, the activity of *S. aureus* isolates (Table 2) was more persistent at intermediate concentrations, particularly for those isolates that showed measurable activity at a concentration as low as 12.5 mg/mL

3.1. Antibacterial Activity Against *Staphylococcus epidermidis*

The antibacterial activity of *A. dracunculus* extract against *S. epidermidis* isolates showed a clear concentration dependent activity (Table 1). All four isolates had measurable inhibition zone ranging from 12 mm to 20 mm at the

maximum tested concentration (100 mg/mL) with isolate 3 giving the largest inhibition zone (20 mm) and isolate 1 giving the smallest inhibition zone (12 mm).

Table 1 The antibacterial activity of *Artemisia dracunculus* extract against *Staphylococcus epidermidis* isolates (inhibition zone diameter in mm)

Concentration (mg/mL)	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Mean ± SD
100.0	12	18	20	18	17.0 ± 3.46
50.0	11	0	16	0	6.75 ± 8.06
25.0	9	0	11	0	5.0 ± 5.83
12.5	0	0	0	0	0.0 ± 0.0
6.25	0	0	0	0	0.0 ± 0.0
0.0	0	0	0	0	0.0 ± 0.0

Isolates 1 and 3 were only resistant to antibacterial activity at 50 mg/mL with zones of 11 mm and 16 mm respectively, while isolates 2 and 4 were completely resistant. This trend was maintained at 25 mg/mL, with isolates 1 and 3 showing decreased, but measurable, inhibition zones of 9 mm and 11 mm, respectively. No antibacterial activity was detected with concentrations of 12.5 mg/mL and lower for any of the isolates tested.

Concentrations-dependent inhibition patterns of *Staphylococcus epidermidis* isolates against *Artemisia dracunculus* alcoholic extract. As shown in the figure 1, the concentration of the extracts was decreased and the inhibition zone diameter decreased progressively with the decrease in concentration. The sustained activity was seen at intermediate concentrations (25 to 50 mg/mL) by the isolates 1 and 3, whereas the activity was complete till 100 mg/mL by the isolates 2 and 4. Each error bar is the standard deviation of the triplicates.

There was some inter-isolate variation in the susceptibility pattern of *S. epidermidis* strains, which may be associated with variation in cell wall composition or other resistance mechanisms. Strains 2 and 4 showed a clear resistance break point below 100 mg/mL, suggestive of threshold dependent resistance mechanisms

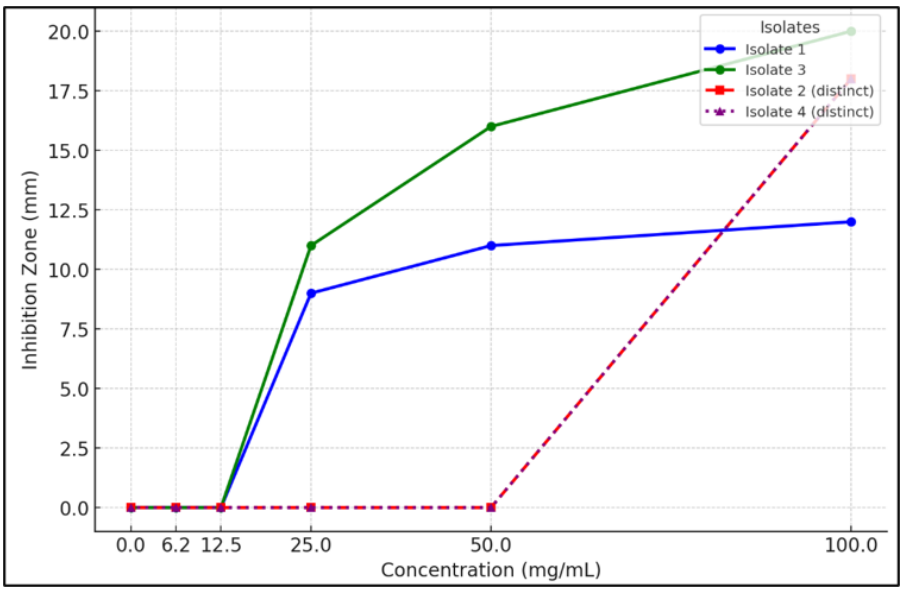

Figure 1 Inhibition patterns of *Staphylococcus epidermidis* isolates at different concentrations

3.2. Antibacterial Activity Against *Staphylococcus aureus*

The *S. aureus* isolates had a variety of, but generally more lasting, antibacterial susceptibility than *S. epidermidis* (Table 2). In the case of the seven isolates all of them had the same inhibition zone of 100 mm with isolate 6 having largest zone of 20 mm and isolate 7 having smallest zone of 12 mm.

Table 2 The data of the antibacterial activity of *Artemisia dracunculus* extract against *Staphylococcus aureus* isolates (inhibition zone diameter in mm)

Concentration (mg/mL)	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Mean ± SD
100.0	15	14	17	15	20	12	16	15.57 ± 2.51
50.0	14	15	16	11	15	10	0	11.57 ± 5.56
25.0	10	0	13	10	11	0	0	6.29 ± 5.96
12.5	10	0	11	0	10	0	0	4.43 ± 5.53
6.25	0	0	0	0	0	0	0	0.0 ± 0.0
0.0	0	0	0	0	0	0	0	0.0 ± 0.0

Antibacterial activity (Zones 10 to 16mm) was found with six out of seven isolates at 50mg/mL and complete resistance was observed for isolate 8. Four isolates (1, 3, 5, 6) showed measurable inhibition zone at 25 mg/mL but the *S epidermidis* isolates did not. This demonstrated the high sensitivity of these four isolates. Three isolates (1, 3 and 6) remained susceptible to the antibacterial agents even at 12.5 mg/mL with a zone of 10-11 mm.

Figure 2. Inhibition patterns of *S. aureus* isolates in the presence of *Artemisia dracunculus* alcoholic extract and as a function of concentration. The variable but generally greater inhibition compared to S. epidermidis is shown graphically. Isolates 1, 3 and 6 exhibited persistent growth at lower concentrations (12.5 mg/mL) and isolate 8 was completely resistant at concentrations below 100 mg/mL. Error bars are standard deviation of triplicate measurements.

The differential response of the *S. aureus* isolates indicates variations in the susceptibility of *S. aureus* strains to bioactive compounds in *A. dracunculus* extract. Isolate 6 has the highest sensitivity of all the isolates tested, maintaining measurable inhibition zones in all concentrations except for 6.25 mg/mL.

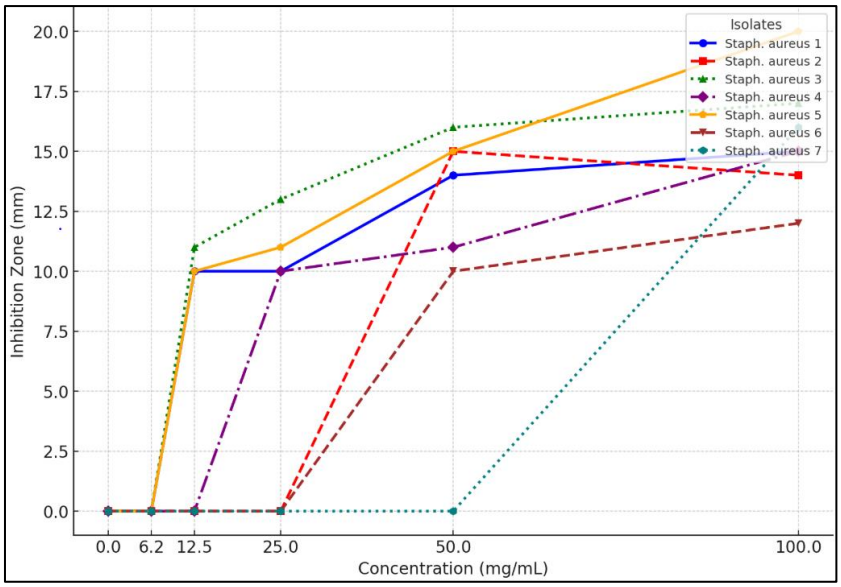

Figure 2 Inhibition patterns of *Staphylococcus aureus* isolates at different concentrations

3.3. Comparative Analysis of Species Susceptibility

The relative inhibition patterns showed that the susceptibility of extracts was species dependent (Figures 3 & 4). In most cases, the relative inhibition percentage of *S. aureus* isolates remained higher than *S. epidermidis* isolates at intermediate concentrations, indicating higher sensitivity towards bioactive compounds of *A. dracunculus* extract.

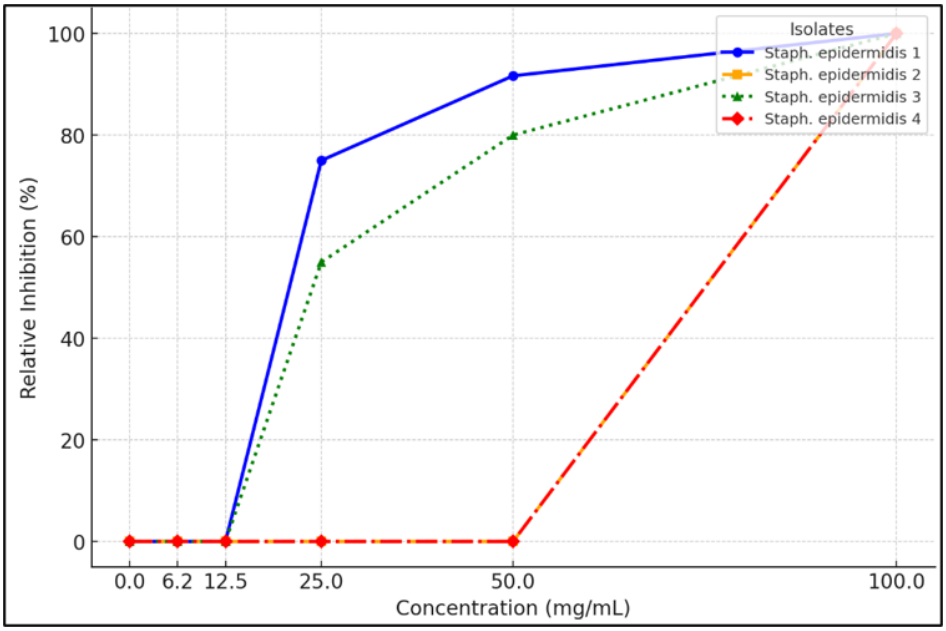

Figure 3 Antibacterial activity of *Staphylococcus epidermidis* isolates against various concentrations of Artemisia dracunculus extract (inhibition %). Data is shown as mean ± SD of 3 replicates and are expressed as a percentage of the maximum inhibition for each isolate (set at 100%). The graph shows that relative inhibition decreased sharply at concentrations < 100 mg/mL, and that the two isolates (2 and 4) were completely resistant, whereas the other two (1 and 3) had significant activity down to 25 mg/mL.

A comparison of Figures 3 and 4 shows that there are basic differences in species susceptibility patterns. *S. epidermidis* isolates exhibited "all or nothing" response pattern with distinct activity changes whereas *S. aureus* isolates exhibited dose-dependent responses. This differential response could be due to differences in cell envelope architectures, efflux pump activity, or metabolic pathways being targeted by the bioactive compounds of the *A. dracunculus* extract in these species.

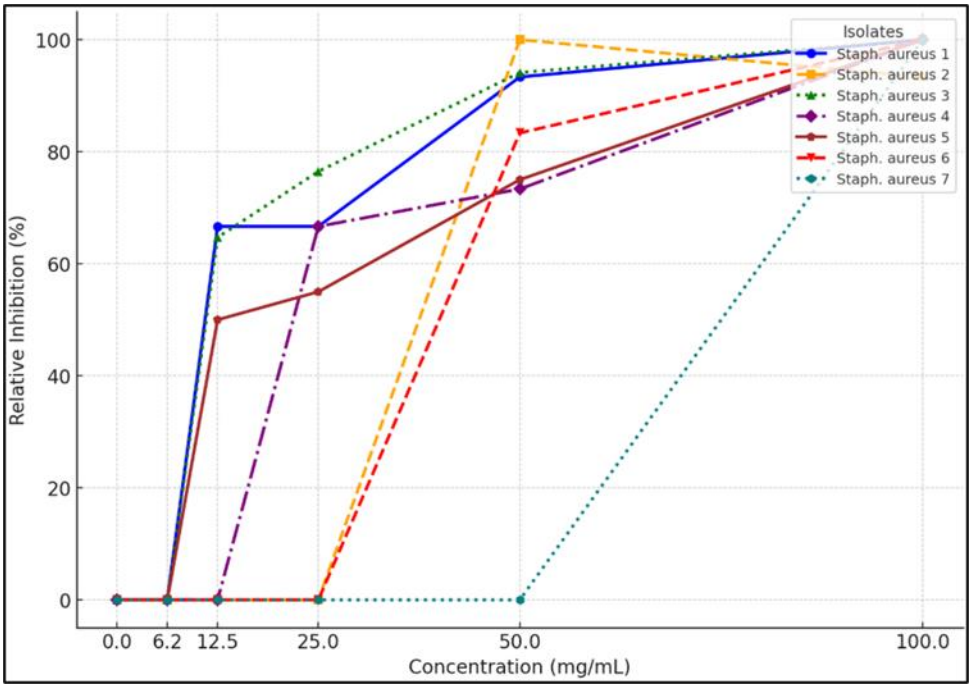

Figure 4 Relative inhibition percentages of *Staphylococcus aureus* isolates at different concentrations of *Artemisia dracunculus* extract

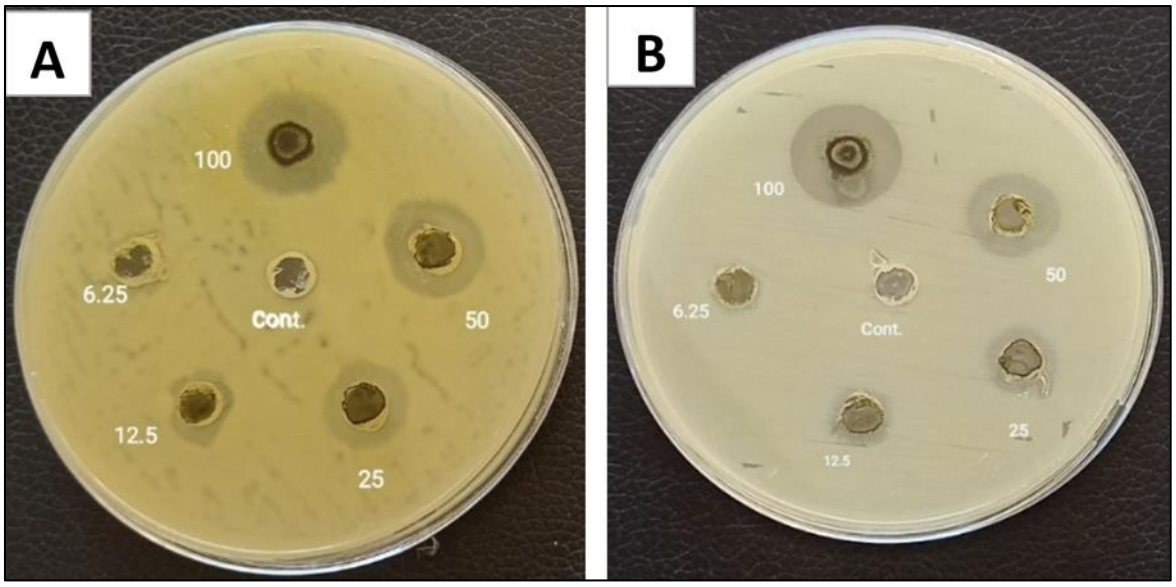

Figure 5 A. *Staphylococcus aureus* isolate 3 B. *Staphylococcus aureus* isolate 6 Concentration (mg/mL) 100.0, 50.0, 25.0, 12.5, 6.25, 0.0; Cont. = Control

4. Discussion

These inhibitory zones were significantly different between the different concentrations of the two bacteria (ANOVA, $P<0.05$). Comparative analysis also revealed that *S. aureus* had significantly higher levels of inhibition at intermediate concentrations (25–50 mg/mL) than *S. epidermidis* (t-test, $p < 0.05$). The results indicate that *S. aureus* is more susceptible to *A. dracunculus* than other species, and that *A. dracunculus* may be a better inhibitor of *S. aureus*.

4.1. Concentration-Dependent Antibacterial Activity

The present study further shows that alcoholic extract of *A. dracunculus* has concentration dependent potent antibacterial activity against both *S. aureus* and *S. epidermidis* isolates. The antimicrobial activities of Artemisia species have been consistently reported, with a dose-dependent effect [19;20], which is consistent with these results. The observed inhibition zones ranged from 12-20 mm at 100 mg/mL as compared to the results of Sharopov et al., 2015[21] which were obtained for *A. dracunculus* essential oil against various bacterial pathogens, indicating that both volatile and non-volatile compounds play a role in the antimicrobial activity.

As shown in Table 1, the sensitivity of the *S. epidermidis* isolates were different with the highest sensitivity at 20 mm inhibition zone at 100 mg/mL for isolate 3 and the lowest sensitivity at 12 mm at 100 mg/mL for isolate 1. The activity of *S. epidermidis* was very sensitive to the concentration, with only isolates 1 and 3 being active at concentrations below 100 mg/mL (see Figure 1). This binary response pattern indicates that it has a threshold-dependant mechanism of action.

In contrast, Table 2 shows that the *S. aureus* isolates had a more consistent level of antibacterial susceptibility throughout the concentration range. This increased resilience is shown in figure 2, where 6/7 isolates were active at 50 mg/mL and four isolates were able to produce inhibition zones at 25 mg/mL. Notably, isolates 1, 3 and 6 retained measurable zones (10-11 mm) at the highest concentration (12.5 mg/mL) suggesting a better sensitivity than *S. epidermidis*.

4.2. Species-Specific Susceptibility Patterns

Comparative analysis of the two figures (Figures 3 and 4) shows basic differences in the relative inhibition pattern of the two species of staphylococci. As shown in Figure 3, isolates of *S. epidermidis* have an "all-or-nothing" type response with activity lost rapidly at concentrations below 100 mg/mL; isolate 2 and 4 were completely resistant at all concentrations except the highest tested. Figure 4 shows, by contrast, a gradual, dose-dependent decrease in *S. aureus* susceptibility, with some isolates still showing 25-50% maximum inhibition at intermediate doses.

The mean inhibition zones at 100 mg/mL were similar in both species (*S. epidermidis*: 17.0 ± 3.5 mm and *S. aureus*: 15.6 ± 2.6 mm (Tables 1 and 2)), but *S. aureus* exhibited greater activity at lower concentrations. This difference in susceptibility can be explained by the differences in the cell wall structure and efflux pump mechanism between the species [22;23].

4.3. Inter-Isolate Variability and Clinical Implications

There is good inter-isolate variation observed in both Tables 1 and 2, which is due to the natural genetic differences found within staphylococcal populations. The variation in the inhibition zones of the *S epidermidis* isolates (12-20 mm) and the *S aureus* isolates (12-20 mm) at 100 mg/mL was 67% and 67% respectively. But there was a significant difference in the ability to persist in activity among the different species isolates, with *S. aureus* isolates 1, 3, and 6 activities persisting at 12.5 mg/mL whereas all *S. epidermidis* isolates were not inhibited at < 25 mg/mL.

The relative inhibition data shown in Figures 3 and 4 allow standardisation to be achieved when comparing across different isolates according to their baseline sensitivity differences. This analysis showed that some of the *S. aureus* isolates (1, 3, 6) are still retaining 50-60% of their maximum inhibitory potential at concentrations as low as 25 mg/mL which may prove to be therapeutically useful at clinically attainable concentrations.

4.4. Phytochemical Basis of Antibacterial Activity

The antibacterial activity of *A. dracunculus* may be attributed to the abundant phytochemical constituents such as phenolic compounds, flavonoids and terpenoids [10;11]. The antimicrobial activity of chlorogenic acid, which is the main phenolic compound of *A. dracunculus*, has been extensively investigated and is reported to cause disruption of bacterial cell membranes and inhibit certain enzymes [24] that are important for the bacteria's survival.

Derivatives of flavonoids, rutin and quercetin found in *A. dracunculus* have antimicrobial activity mediated by different mechanisms such as inhibition of DNA gyrase, disruption of the energy metabolism and cytoplasmic membrane function [25]. The antibacterial effect of these different phytochemical classes may be synergistic contributing to the overall effect observed in our study [26].

Previous studies have shown *A. dracunculus* essential oil to have significant antimicrobial activities, with the components of essential oils such as estragole, ocimene and limonene exhibiting notable antimicrobial effects [27;28].

The main mechanism of action of these volatile compounds is through increased permeability of bacterial cell membranes leading to cell death [29;30].

4.5. Comparative Analysis with Existing Literature

Our results corroborate the results of several recent studies of the antimicrobial activity of Artemisia species. In the same method, Rustaiee et al. (2011) obtained similar inhibition zones (10-18 mm) in the case of *A. dracunculus* extract against *S. aureus* [31]. In the same way, [19] and [10] have shown that *A. dracunculus* possesses strong antibacterial properties against gram-positive bacteria and its MIC value has varied from 15.6 to 62.5 mg/mL.

A. dracunculus has moderate to strong antibacterial activity when compared to other Artemisia species. However, the antimicrobial activity of various Artemisia species has been found to be different [9;19] which indicate that the antimicrobial activity can differ widely depending on geographical origin, the extraction method and the phytochemical composition [8,10].

4.6. Mechanisms of Action and Resistance Considerations

In general, the multi-target approach of plant-derived antimicrobials limits the potential for rapid development of resistance to them, as compared to single-target synthetic antibiotics [32]. Because the extract of *A. dracunculus* is a complex mixture of bioactive compounds, they probably work in various ways at once, which makes it hard for bacteria to build up resistance to them [33].

Mechanisms of action include: (1) disruption of cell membrane integrity (interaction with phospholipid bilayers), (2) inhibition of essential enzymes (important to bacterial metabolism and/or DNA replication), (3)[34;35] interference with quorum sensing and biofilm formation, (4) production of ROS and subsequent oxidative stress[36;37].

Activity seems to be concentration dependent, with possible bactericidal activity at higher concentrations and bacteriostatic activity at lower concentrations. This is a good thing because it allows flexibility in therapeutic uses depending on the severity and site of infection [38].

4.7. Limitations and Future Research Directions

This study has contributed to the knowledge of the antibacterial activity of *A. dracunculus*, however some limitations should be noted. Although the agar well diffusion method is a routine technique for initial screening, it may not give a true estimate of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values needed for clinical use. Testing should be repeated in broth microdilution assays to obtain accurate MICs as recommended by the Clinical and Laboratory Standards Institute (CLSI) [13; 16] in the future.

Since all of this study's experiments were conducted without positive controls (standard antibiotics), it is not possible to make a direct comparison with the effectiveness of the antibacterial activity of *A. dracunculus* extract compared to conventional antibiotics. Further, the study was performed in laboratory-grown bacterial cultures which may not reflect the behaviour of bacteria in complex in vivo environments.

4.8. Clinical and Therapeutic Implications

The anti-bacterial effect of *A. dracunculus* extract has been shown to be effective especially against the antibiotic-resistant staphylococcal strains, which may be useful in the treatment of skin and soft tissue infections, wound infection and other localized staphylococcal infections. Due to its traditional use and natural occurrence as a culinary herb, an *A. dracunculus* has a favorable safety profile but requires full-scale toxicological evaluation before clinical applications.

The activity found to be concentration dependent in this study suggests that formulation strategies should aim to provide and maintain therapeutic concentrations at infection sites*. A. dracunculus*-based antimicrobial products might be most useful initially as topical products, such as creams, ointments and wound dressings.

Recommendations

The results of this present study suggest the following recommendations for future studies:

- Comprehensive MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) determination: Perform extensive MIC and MBC testing according to standardised broth microdilution methods to determine accurate concentration thresholds for bacteriostatic and bactericidal activity.

- Bioactive Compound Identification: Conduct detailed phytochemical analysis and bioactivity-guided fractionation to isolate and identify the compounds that possess antibacterial activity.
- Synergistic Studies: Explore the potential synergism between *A. dracunculus* extract and conventional antibiotic treatment to maximise drug effectiveness and reduce the likelihood of resistance.
- In Vivo Efficacy Studies: Do animal model studies to assess the therapeutic efficacy, pharmacokinetics and safety *of A. dracunculus* extract for staphylococcal infections.
- Resistance Studies: Conduct serial passage experiments to evaluate the potential for resistance to the extract of *A. dracunculus* and conventional antibiotics.
- Clinical Isolate Testing: Include clinically relevant antibiotic-resistant isolates (e.g., MRSA and vancomycin-resistant staphylococci).
- Formulation Development: Formulation Development of topical formulations including *A. dracunculus* extract for possible clinical use, optimization.
  Cytotoxicity Assessment: Conduct comprehensive cytotoxicity studies to establish therapeutic windows and safety margins for human applications.

5. Conclusion

The result of this study shows that alcoholic extract of *A. dracunculus* is effective in inhibiting both *S. aureus* and *S. epidermidis* in a concentration dependent manner. The increased sensitivity of *S. aureus* isolates at intermediate concentrations, together with the multi-target mode of action of the plant-derived antimicrobials, suggests the possible use of *A. dracunculus* as a natural antimicrobial therapeutic agent for *S. aureus* infections.

The observed inter-isolate variability emphasizes the importance of comprehensive susceptibility testing and highlights the need for personalized antimicrobial approaches. The relatively high maintained activity of some of the isolates at low concentrations of the compounds shows that there are potential therapeutic possibilities, especially for topical applications in the treatment of local infections.

To demonstrate the clinical potential of these in vitro results, further studies targeting the identification of bioactive compounds as well as the determination of MIC values and in vivo efficacy studies are necessary. Traditional medicine is therefore playing a significant role in challenging the global issue of antimicrobial resistance, and the combination of traditional medicinal knowledge with modern scientific techniques is still providing valuable opportunities.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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