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Original Article

Comparative Antibacterial Activity of Aqueous and Alcoholic *Artemisia campestris* L. Extracts Against Clinical Uropathogens

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Abstract.

Background: *Artemisia campestris* L. is a traditional medicinal plant with documented antimicrobial properties, yet its activity against clinical uropathogens remains underexplored. **Objective:** This study compared the in vitro antibacterial activity of aqueous and alcoholic A. campestris extracts against clinical Gram-negative uropathogens. **Methods:** Aqueous and 70% ethanolic extracts were prepared by maceration and tested at 100%, 70%, and 50% concentrations using agar well diffusion against six clinical isolates (Escherichia coli n=3, *Klebsiella pneumoniae* n=2, Pseudomonas aeruginosa n=1). Six standard antibiotics served as positive controls. A three-way General Linear Model assessed solvent, concentration, and isolate effects. **Results:** Antibiotics demonstrated significantly greater activity (0.7–28.7 mm) than plant extracts (0–16.7 mm). The 70% aqueous extract showed the strongest extract-mediated inhibition, particularly against E. coli (16.7 ± 2.9 mm). The 50% alcoholic extract was inactive against all isolates. Concentration and solvent type significantly affected activity ($P < 0.001$). **Conclusion:** *A. campestris* extracts possess modest, concentration-dependent antibacterial activity against uropathogens, but remain less potent than conventional antibiotics. Further fractionation and compound isolation are warranted.

Keywords: *Artemisia campestris*, antibacterial activity, uropathogens, aqueous extract, alcoholic extract, agar well diffusion

Introduction

Artemisia campestris L., a perennial species of the Asteraceae family, is widely distributed across Eurasia, North Africa, and North America, and holds a longstanding role in traditional medicine. Its aerial parts and roots have been employed in folk remedies for diverse conditions including diabetes, skin disorders, hypertension, rheumatism, digestive complaints, obesity, and as anthelmintic and antivenom agents [1,2,3 and 4]. Within the genus *Artemisia*, comprising more than 400 species recognized for antimalarial, anti-inflammatory, antimicrobial, and anticancer properties, *A. campestris* has emerged as a promising source of pharmacologically active metabolites [5,6, and 7]

Ethnopharmacological surveys from North Africa and the Mediterranean highlight it is use in infusions and decoctions for hypertension, diabetes, digestive and respiratory disorders, dermatologic disorders, and even envenomation from scorpion stings and snake bites [4 and 8]. These traditional applications are supported by phytochemical investigations, which have identified phenolic compounds, flavonoids, dicaffeoylquinic acids, chlorogenic acid, vicenin-2, methoxylated flavones, phytosterols, sesquiterpene lactones, and terpenoids. Such components are associated with antioxidant, antimicrobial, and anti-inflammatory activities [1, 8 and 9]

Recent pharmacological studies have broadened our understanding of therapeutic profile of *A. campestris*, reporting antioxidant, anti-inflammatory, antihemolytic, antifungal, wound-healing, and cardiovascular effects [9, 10 and 11]. Extracts prepared with aqueous and methanolic solvents exhibit particularly strong antioxidant and anti-inflammatory properties, while cardiovascular investigations demonstrate antihypertensive, hypotensive, vasorelaxant, and vasodilatory effects, corroborating its traditional use in managing elevated blood pressure [3, 12]. Broader reviews of *Artemisia* species further emphasize antidiabetic, hepatoprotective, neuroprotective, anticancer, and enzyme-inhibitory activities, largely attributed to phenolic and terpenoid constituents [5, 7, 13].Taken together, *Artemisia campestris* L. represents a pharmacologically relevant medicinal plant with a robust ethnobotanical foundation and growing experimental support for antioxidant, anti-inflammatory, antimicrobial, cardiovascular, metabolic, and antiviral effects. Its chemically diverse profile positions it as a plausible candidate for nutraceutical and pharmaceutical development. However, further research is required to

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standardize extracts, identify bioactive constituents, and establish safety and clinical efficacy [14, 15, 16] Therefore, this study aimed to compare the in vitro antibacterial activity of aqueous and alcoholic extracts of *Artemisia campestris* L. against clinical uropathogenic isolates

Materials And Methods

Plant Material

Fresh aerial parts of *Artemisia* campestris L. were collected from the rural areas of Zawiya City, Libya, in April 2025.

Preparation of Plant Extracts

The plant material was washed, shade-dried at room temperature (25–30°C), ground into a fine powder, and stored in airtight containers until use. Aqueous and 70% (v/v) ethanolic extracts were prepared separately by macerating **75 g** of powder in **375 mL** of solvent (1:5, w/v) for 48 h at room temperature on a tube roller mixer (20 rpm). Extracts were filtered through muslin cloth and Whatman No. 1 filter paper, centrifuged at 4500 × g for 10 min, concentrated under reduced pressure using a rotary evaporator, and oven-dried at ≤45°C to constant weight. Dried extracts were stored in sterile amber containers at 4°C until use

Yield Data and Stock Solution Preparation

A total of 75 g of dried plant material was used for the preparation of each extract. The alcoholic extraction yielded 2.653 g of dried extract (yield: **3.54% w/w**), while the aqueous extraction yielded 6.142 g of dried extract (yield: **8.19% w/w**). The extraction yield was calculated as (dry extract weight / dry plant material weight) × 100.

The dried extracts were subsequently dissolved in 1% (v/v) DMSO to prepare stock solutions at a final concentration of 1000 mg/mL (100% w/v). Specifically, 2.653 mL of 1% DMSO was added to the alcoholic extract and 6.142 mL of 1% DMSO was added to the aqueous extract.

Preparation of Extract Solutions

Dried extracts were dissolved in 1% dimethyl sulfoxide (DMSO) to prepare stock solutions. Three concentrations (100%, 70%, and 50%) were tested. The final DMSO concentration was maintained at 1%, which served as the negative control.

Bacterial Isolates and Inoculum Preparation

Six clinical uropathogenic isolates were obtained from Al-Shark Laboratories (Tripoli, Libya): Escherichia coli (n = 3), *Klebsiella pneumoniae* (n = 2), and Pseudomonas aeruginosa (n = 1). Species identification was confirmed using the Phoenix automated identification system (Becton Dickinson, USA). Isolates were stored at −20°C in Tryptic Soy Broth with 20% glycerol and subcultured twice on Tryptic Soy Agar (TSA) prior to testing. Bacterial suspensions were adjusted to 0.5 McFarland standard (≈1.5 × 10^8 CFU/mL) immediately before testing.

Antibacterial Activity

Antibacterial activity was evaluated using the agar well diffusion assay. Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions, and 6-mm wells were filled with 50 µL of each extract concentration. Plates were incubated at 37°C for 24 h, after which inhibition zones (mm) were measured. Experiments were performed in triplicate. Ciprofloxacin (CIP), ceftriaxone (CTR), meropenem (MER), gentamicin (GEN), amikacin (AK), and ceftazidime (CAZ) were used as positive controls, while 1% DMSO served as the negative control. The six antibiotics were selected to cover both Gram-positive and Gram-negative spectra: gentamicin and amikacin (aminoglycosides, broad-spectrum), meropenem (carbapenem, broad-spectrum), ceftriaxone and ceftazidime (third-generation cephalosporins), and ciprofloxacin (fluoroquinolone). Antibiotic susceptibility was interpreted according to CLSI guidelines where applicable; however, no formal breakpoint classification was applied to the plant extracts due to the lack of standardized criteria for natural product screening.

Minimum Inhibitory Concentration (MIC)

Broth microdilution was performed in Mueller–Hinton broth. Two-fold serial dilutions of each extract were prepared, starting from 50% (500 mg/mL) and descending to lower concentrations. A standardized inoculum (0.5 McFarland) was added to each well. Plates were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration preventing visible growth. The MIC was 50% (500 mg/mL) for both extracts against all isolates; all lower dilutions showed no inhibition. Experiments were performed in triplicate.

Statistical Analysis

One-way ANOVA compared extract concentrations within each solvent type, and antibiotic activity across isolates. Normality and homogeneity of variance were assessed using Shapiro–Wilk and Levene's tests, respectively. Tukey's HSD post-hoc test was used for pairwise comparisons where appropriate. A General Linear Model evaluated combined effects of solvent, concentration, and isolate. Analyses were performed using IBM SPSS v25.0. Significance was set at $P < 0.05$. Data are mean ± SD.

Results.

Antibacterial Activity of *Artemisia* Extracts and Standard Antibiotics

The antibacterial activity of aqueous and alcoholic *Artemisia* extracts at 100%, 70%, and 50% concentrations was evaluated against six clinical Gram-negative bacterial isolates and compared with six standard antibiotics. Overall, the antibiotics showed much stronger activity than the plant extracts, with inhibition zones ranging from 0.7 to 28.7 mm, whereas the extracts ranged from 0 to 16.7 mm (Figure 1). The negative control (1% DMSO) produced no inhibition zones (0 mm) against all tested isolates, confirming that

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the solvent itself did not contribute to the observed antibacterial activity.

Among the extracts, the 70% aqueous extract showed the highest activity, with the largest inhibition zone against *Escherichia coli* isolate 2 (16.7 ± 2.9 mm). The 100% aqueous extract also showed moderate activity, with the highest response against *Klebsiella pneumoniae* isolate 2 (13.3 ± 1.5 mm). In contrast, the 50% aqueous and 50% alcoholic extracts showed little or no activity against most isolates.

Regarding the antibiotics, meropenem and ciprofloxacin showed the greatest activity, with inhibition zones reaching 28.7 mm. In contrast, *K. pneumoniae* isolate 1 was resistant to meropenem, ciprofloxacin, amikacin, and ceftazidime, while *P. aeruginosa* showed high susceptibility to gentamicin, meropenem, and ciprofloxacin (Figure 1)

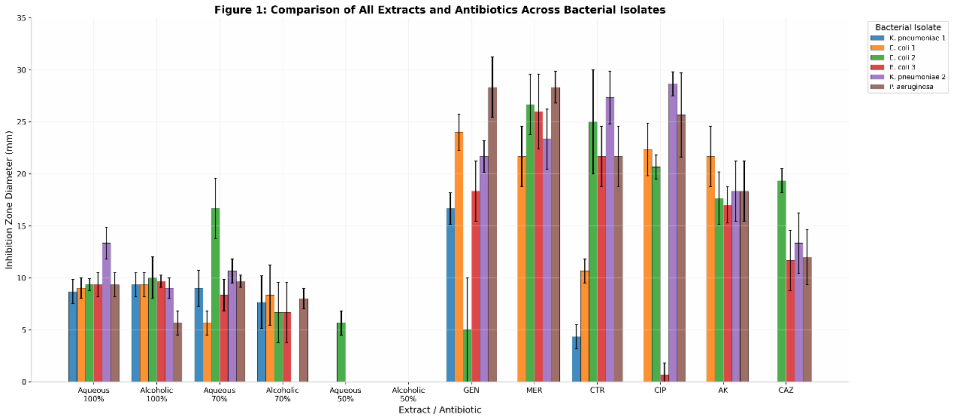


Figure 1. Comparative antibacterial activity of *Artemisia campestris* extracts and standard antibiotics against clinical bacterial isolates. Grouped bar chart showing mean inhibition zone diameters (mm ± SD) of aqueous and alcoholic extracts at 100%, 70%, and 50% concentrations, together with six standard antibiotics (GEN, MER, CTR, CIP, AK, and CAZ) against six clinical isolates.. Values are mean ± SD (n = 3)

Effect of Extraction Solvent and Concentration

Comparison of aqueous and alcoholic extracts showed that antibacterial activity was influenced by both solvent type and concentration (Figure 2). In general, aqueous extracts produced slightly larger inhibition zones than alcoholic extracts at the same concentration, although this varied among isolates.

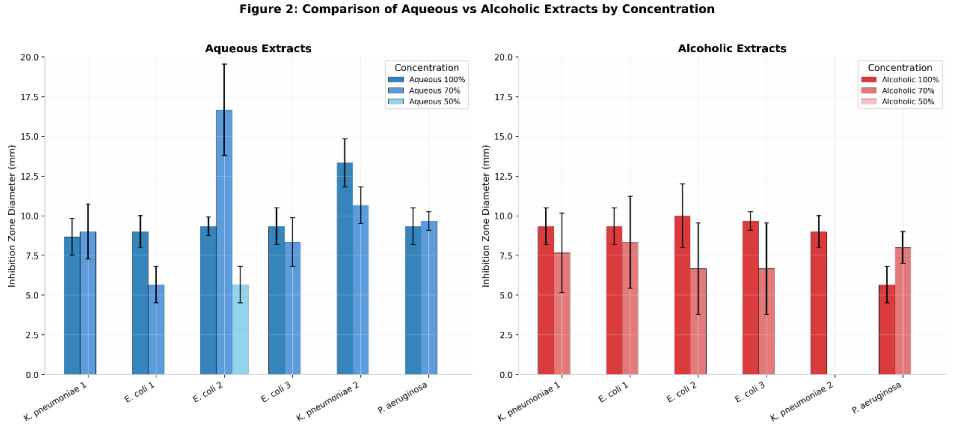


Figure 2. Effect of extraction solvent and concentration on antibacterial activity of *Artemisia campestris* extracts. Two-panel grouped bar chart comparing aqueous (left) and alcoholic (right) extracts at 100%, 70%, and 50% concentrations. Bars represent mean inhibition zone diameter ± SD (n = 3).

One-way ANOVA revealed that extract concentration had a highly significant effect on inhibition zone diameter for both aqueous and alcoholic extracts ($P < 0.001$). Tukey's HSD post-hoc analysis showed that the 100% and 70% aqueous extracts produced significantly larger inhibition zones than the 50% aqueous extract ($P < 0.05$), while no significant difference was detected between 100% and 70% aqueous concentrations. Similarly, for alcoholic extracts, the 100% concentration significantly outperformed both 70% and 50% concentrations ($P < 0.05$), and the 50% alcoholic extract showed no detectable activity against any isolate.

General Linear Model analysis confirmed that extract concentration ($P < 0.001$), extraction solvent ($P < 0.001$), and the solvent × concentration interaction ($P = 0.021$) significantly affected inhibition zone diameter. In contrast, bacterial isolate alone and the remaining interaction terms were not significant ($P > 0.05$).

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Overall Antibacterial Activity Profiles

The heat map provides an integrated view of the antibacterial activity of all extracts and antibiotics across the six isolates (Figure 3). The standard antibiotics consistently produced stronger inhibition than the plant extracts, with meropenem and ciprofloxacin showing the highest overall activity.Among the plant extracts, the 70% aqueous extract showed the strongest activity, while both 50% extracts showed negligible or no inhibition. The heat map also revealed clear differences in susceptibility among isolates. For example, K. pneumoniae isolate 1 showed a multidrug-resistant pattern, whereas P. aeruginosa remained susceptible to several antibiotics.

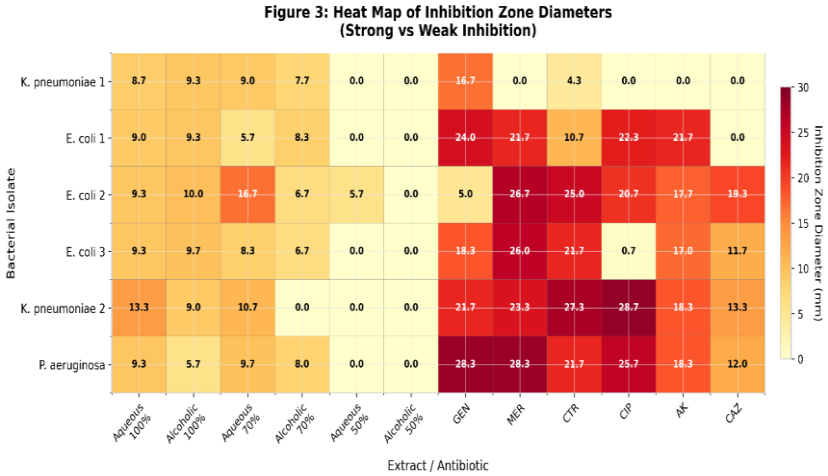


Figure 3. Heat map summarizing antibacterial activity of *Artemisia campestris* extracts and standard antibiotics. Mean inhibition zone diameters (mm) of aqueous and alcoholic extracts (100%, 70%, 50%) and six standard antibiotics against six bacterial isolates. Colour intensity reflects activity, with darker red indicating stronger inhibition. Values are mean ± SD (n = 3)

To better illustrate isolate-specific response patterns, radar charts were also prepared for each bacterial isolate (Figure 4). These charts show a compact susceptibility profile for each isolate and highlight the multidrug-resistant behavior of K. pneumoniae isolate 1, the strong response of E. coli isolate 2 to the 70% aqueous extract, and the broad susceptibility of P. aeruginosa to selected antibiotics and extracts. This figure complements the bar charts and heat map by showing the overall response pattern more clearly.

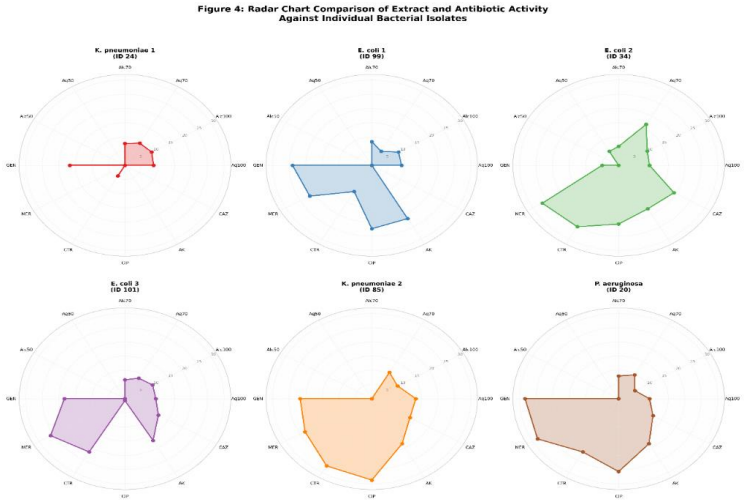


Figure 4. Radar chart of antibacterial activity against individual clinical bacterial isolates. Radar charts showing the inhibition profiles of aqueous and alcoholic *Artemisia campestris* extracts (100%, 70%, and 50%) and six standard antibiotics for each bacterial isolate. Larger shaded areas indicate greater antibacterial activity, while smaller profiles indicate reduced susceptibility or multidrug resistance. Values are mean ± SD (n = 3).

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Minimum Inhibitory Concentration

Broth microdilution assays, initiated at 50% (500 mg/mL) and serially diluted, revealed that the crude aqueous and alcoholic extracts inhibited visible bacterial growth only at the starting concentration of 50% (500 mg/mL). All lower dilutions (25%, 12.5%, 6.25%, etc.) failed to prevent growth after 24 h incubation. Thus, the MIC for both extract types against all tested isolates was determined as 50% (500 mg/mL).

Statistical Analysis of Antibiotic Susceptibility

Antibiotic susceptibility was analysed separately using a two-way General Linear Model, with bacterial isolate and antibiotic type as fixed factors. Both factors significantly affected inhibition zone diameter ($P < 0.001$). In addition, the bacterial isolate × antibiotic interaction was significant ($P < 0.001$), showing that antibiotic activity differed according to the bacterial isolate..

Discussion

Rresent findings show that *Artemisia* extracts inhibited the tested Gram-negative isolates with variable potency, and that activity declined sharply at lower concentrations. This pattern agrees with multiple plant-extract studies showing that inhibition zones increase as extract concentration increases and may disappear at low concentrations [17 and 18].

The strongest extract response in the present study came from the 70% aqueous extract, while the 50% alcoholic extract was completely inactive. That concentration effect is consistent with *Artemisia* reports showing minimal activity with weaker solvent systems such as 40% ethanol and stronger activity with 70% or 90% ethanol [19]. It also agrees with studies showing that activity can begin only above a threshold concentration, such as 150 µg/mL for ethanol extracts of several *Artemisia* species [20]

Regarding the solvent effect, the findings of the present study were clear but not absolute. Overall, the aqueous extract performed better at the tested concentrations and produced the highest single inhibition zone, whereas the alcoholic extract was more uniform but weaker and showed no effect at 50% concentration. This pattern matches one *A. herba-alba* study in which the aqueous extract revealed considerably higher antibacterial activity than the ethanolic extract and further contained higher levels of phenolics, flavonoids, and tannins [21]. It also fits reports that solvent type significantly affects the extraction of antimicrobial phytochemicals across *Artemisia* species [24, 25 and 26].

At the same time, the literature shows that the "best" solvent is not universal across *Artemisia*. Several studies found stronger activity in methanolic or ethanolic extracts than in aqueous extracts [22, 25 and 26] Others reported no activity for water extracts [22], while some found only modest aqueous or methanolic effects against *E. coli* [27 and 28]. This mixed evidence suggests that the superiority of aqueous versus alcoholic extraction depends on species, plant part, geographic origin, and the phytochemicals enriched by the solvent rather than on solvent class alone [26 and 28].

Isolate-level variation is also consistent with prior work. In this dataset, some *E. coli* and *Klebsiella pneumoniae* isolates responded clearly, whereas others were less susceptible, and Pseudomonas aeruginosa showed only modest inhibition. Similar heterogeneity appears across the literature: some *Artemisia* extracts inhibit both Gram-positive and Gram-negative organisms [19, 24], whereas others show selectivity, including weak or absent effects against *E. coli* or *Klebsiella* [27 and 29]. Gram-positive bacteria also tend to be more susceptible overall in some studies, likely because of cell-wall differences [23 and 24].

Comparison with the antibiotics in this study dataset shows that the plant extracts demonstrated lower antibacterial activity than GEN, MER, and CIP for the majority isolates. This finding is inconsistent with many comparative studies in which ciprofloxacin and gentamicin **ex**hibited greater antibacterial activity or lower MICs than crude plant extracts [18 and 30]. These findings are especially consistent with work showing that plant extracts can have appreciable but modest broad-spectrum activity while still remaining less potent than standard antibiotics on a concentration basis [30].

Nevertheless, plant extracts are not necessarily therapeutically irrelevant when they exhibit lower in vitro activity than antibiotics. Some studies have reported that medicinal plant extracts matched or exceeded the activity of selected antibiotics against particular isolates or resistant strains [31–33]. Others demonstrated that combining plant extracts with antibiotics can produce synergistic effects, resulting in larger inhibition zones and lower MIC values [19]. Therefore, the findings of the present study are better interpreted as evidence of lead antimicrobial activity rather than proof that crude extracts can outperform conventional antibiotics.

The antibacterial activity observed in the present study is likely attributable to bioactive secondary metabolites known to occur in *Artemisia* species, including phenolics, flavonoids, tannins, and terpenoids, as reported in previous phytochemical investigations [20 and 23]. However, the present study did not include phytochemical screening or compound identification due to limited access to chromatographic facilities (HPLC, GC-MS). Future studies should incorporate qualitative and quantitative phytochemical analysis to correlate specific compounds with antibacterial activity. Plant-based antibacterials often act by disrupting the bacterial cell membrane and interfering with protein synthesis or DNA replication, and *Artemisia* activity has been linked specifically to compounds such as chlorogenic acid, phenolic acids, and flavonoids that can damage bacterial cells [26]. Because solvent choice changes which

compounds are recovered, the solvent-dependent activity in this data is biologically plausible [23 and 24].

The MIC findings further support that the antibacterial activity of the *Artemisia* extracts was concentration-dependent. Broth microdilution assays, initiated at 50% (500 mg/mL) and serially diluted, confirmed that visible bacterial growth was inhibited at this starting concentration, while all lower two-fold dilutions failed to prevent growth. The MIC was therefore determined as 50% (500 mg/mL), indicating that the crude extracts possessed measurable antibacterial activity, but only at a relatively high concentration [20 and 27]. This agrees with previous studies showing that the inhibitory activity of medicinal plant extracts declines with serial dilution and that MIC values vary widely depending on the *Artemisia* species, extraction solvent, and bacterial strain tested [25 and 31]. The relatively high MIC observed here may reflect the use of crude extracts, in which active constituents are less concentrated than in optimized solvent fractions or purified components [17, 23, and 24].

Limitations

This study was limited to six Gram-negative isolates, restricting generalizability to other uropathogens. Phytochemical screening was not performed due to lack of chromatographic facilities. The agar well diffusion method provides qualitative rather than quantitative MIC/MBC data. Crude extracts were used, potentially diluting active constituents. Clinical efficacy cannot be inferred from in vitro results.

Conclusion

The present study demonstrates that *Artemisia* extracts possess measurable in vitro antibacterial activity against the tested bacterial isolates, and that this activity is strongly influenced by extract concentration and solvent system. Higher concentrations produced greater antibacterial effects in the agar diffusion assay, with the 70% aqueous extract showing the strongest overall response, while lower concentrations and the 50% alcoholic extract were less effective. MIC determination by broth microdilution, starting from 50% (500 mg/mL), confirmed this concentration-dependent pattern, as this was the lowest concentration that inhibited visible bacterial growth. The relatively high MIC value indicates that the antibacterial potency of the crude extracts was modest.Although the extracts showed detectable activity, their effects remained markedly lower than those of the most effective conventional antibiotics, particularly gentamicin, meropenem, and ciprofloxacin. These findings support the presence of antibacterial phytochemicals in *Artemisia*, but suggest that the crude extracts are not suitable as substitutes for standard antibiotics in their current form. Further studies should focus on solvent optimization, fractionation, identification of active compounds, MIC and MBC evaluation of purified fractions, and assessment of synergistic activity with conventional antibiotics to better define the therapeutic potential of *Artemisia*-derived antibacterial agents.

Ethics Statement

This study used archival, de-identified bacterial strains from a clinical microbiology laboratory in Tripoli, Libya. No human participants or animals were involved, and no patient-identifiable information was accessed. Formal ethical approval was not required.

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This research received no external funding.

Authors' Contributions

Huda R. Alghanma and Mahmoud B. Agena: conception, study design, and experiments. Mahmoud B. Agena: data processing, statistical analysis, interpretation, and manuscript drafting. All authors: critical revision, final approval, and accountability for the work.

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Conflict Of Interest

The authors declare no competing interests.

Data Availability

Data are available from the corresponding author upon reasonable request.

Abbreviations

- GEN = gentamicin
- MER = meropenem
- CTR = ceftriaxone
- CIP = ciprofloxacin
- AK = amikacin
- CAZ = ceftazidime

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