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# Comparative Evaluation of Disc Diffusion and Volatilization Methods for Assessing the Antibacterial Activity of Essential oil of Libyan *Artemisia judaica* Against Pathogenic Bacteria

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## ABSTRACT

Background: Antimicrobial resistance (AMR) poses a serious global health challenge, demanding alternative therapeutic options. Essential oils (EOs) are complex, volatile mixtures with broad-spectrum antimicrobial properties and reduced likelihood of resistance development. *Artemisia judaica*, has been traditionally used for its medicinal effects, yet limited data exist regarding its antibacterial potential against clinically relevant pathogens under different assay conditions.

Objectives: This study aimed to evaluate and compare the antibacterial activity of *A. judaica* essential oil against *Staphylococcus aureus* and *Escherichia coli* using Disc diffusion (direct contact) and volatilization (vapour phase) assays.

Methods: Aerial parts of *A. judaica* collected from Al-Awaynat (Libya) were subjected to hydrodistillation via a Clevenger-type apparatus to obtain the EO. Antibacterial activity was assessed against *S. aureus* and *E.coli* following CLSI-adapted protocols. Disc diffusion and volatilization assays were employed, with levofloxacin serving as a positive control. Zones of inhibition (ZOIs) were measured, and mean values ± standard deviation were recorded.

Results: The EO yield was 0.8% ± 0.05 (v/w). In disc diffusion assays, the essential oil exhibited mean inhibition zones of 16.5 ± 2.1 mm for *S. aureus* and 11.7 ± 1.2 mm for *E. coli*. By contrast, volatilization assays showed minimal and uniform activity (8.0 ± 0.0 mm) for both strains, indicating limited vapour-phase antibacterial potential.

Conclusion: *A. judaica* essential oil demonstrates strong antibacterial activity in direct-contact assays, particularly against *S.aureus*, but negligible vapour-phase effects. These findings suggest its potential application in topical or direct-contact formulations rather than airborne disinfection, and highlight the importance of assay selection in EO bioactivity profiling.

**Keywords:** *Artemisia judaica*, essential oils, antimicrobial activity, disc diffusion, volatilization assay, *Staphylococcus aureus, E.coli.*

## 1. Introduction

Antimicrobial resistance (AMR) stands as one of the most formidable challenges to global public health in the 21st century. The World Health Organization (WHO) has consistently classified AMR as a top priority, issuing renewed calls to accelerate action against this silent pandemic, which is projected to cause millions of deaths annually if left unchecked [1]. The crisis is fueled by the indiscriminate use of conventional antibiotics in human medicine, veterinary practice, and agriculture, which exerts immense selective pressure on microbial

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populations. This has led to the rapid evolution and dissemination of resistance mechanisms across a broad spectrum of bacterial pathogens. The consequences are terrible, treatments for common infections are failing, routine surgical procedures and immunosuppressive therapies become high-risk undertakings, and healthcare costs rise due to prolonged illnesses and the need for more expensive, last-resort drugs.

The gravity of the situation is exemplified by the rise of multidrug-resistant (MDR) pathogens, which have developed sophisticated strategies to evade the action of antimicrobial agents. Prominent among these are Methicillin-resistant *Staphylococcus aureus* (MRSA) and MDR *E. coli*. MRSA, a Gram-positive bacterium, is notorious for its acquisition of the *mec A* gene, which confers resistance to all beta-lactam antibiotics. Beyond this, it employs efflux pumps to expel a range of other drug classes and can form robust biofilms that act as physical barriers, shielding embedded cells from both antibiotics and host immune responses. On the Gram-negative side, MDR *E. coli* poses a significant threat primarily due to its production of extended-spectrum beta-lactamases (ESBLs), enzymes that hydrolyze and inactivate a broad range of penicillin and cephalosporin antibiotics. Furthermore, *E. coli* utilizes a formidable double-membrane structure that is inherently less permeable to many drugs, and it enhances this intrinsic resistance with potent efflux pumps, such as the AcrAB-TolC system, which can export a diverse array of antimicrobials, including fluoroquinolones and tetracyclines **[2]**.

In this context, the search for alternative and complementary antimicrobial agents has intensified, with natural products offering a rich reservoir of chemical diversity. Essential oils (EOs), complex volatile and lipophilic mixtures synthesized by aromatic plants as secondary metabolites, have re-emerged as promising candidates. These oils are typically composed of terpenes (monoterpenes and sesquiterpenes), phenolics, and aromatic compounds, which collectively contribute to their broad-spectrum biological activities [3]. The antimicrobial efficacy of EOs is attributed not to a single mechanism but to a multi-targeted approach, a feature that may crucially hinder the development of resistance [4]. Their lipophilic nature allows them to partition into and disrupt the integrity of bacterial cell membranes, leading to increased permeability, leakage of vital cellular constituents, and impairment of energy generation. Beyond membrane disruption, EOs can interfere with key enzymatic systems, and quench quorum-sensing signals, thereby inhibiting the formation of recalcitrant biofilms and the expression of virulence factors [3]. This polypharmacological profile stands in contrast to the single-target action of many conventional antibiotics, making EOs a compelling area of investigation.

The genus *Artemisia* (family *Asteraceae*), with its global distribution and rich history in traditional medicine, represents a particularly valuable source of bioactive essential oils. Numerous species, including *A. judaica* and *A. herba-alba*, are indigenous to the Mediterranean region and have been used for centuries in folk remedies to treat a variety of ailments, including wounds, fevers, and gastrointestinal infections [5, 6]. The chemical composition of *Artemisia* oils is highly variable, influenced by factors such as geographic origin, harvest time, and plant part used, leading to distinct chemotypes. Regional chemotypes from North Africa and the Middle East are frequently reported to be rich in oxygenated monoterpenes and sesquiterpenes, with compounds like camphor, 1,8-cineole, thujone, and artemisia ketone being prominent [5, 6]. A growing body of scientific evidence from these regions supports their traditional use, demonstrating significant *in vitro* antibacterial activity for various *Artemisia* essential oils against a panel of pathogens, including MDR strains [6, 7].

However, the reported antimicrobial potency of an essential oil is profoundly influenced by the methodological approach used for its assessment. The choice of assay can dramatically alter the outcome and interpretation of results, a critical consideration often overlooked in comparative studies. The most commonly employed techniques include broth microdilution, which determines the Minimum Inhibitory Concentration (MIC) in a liquid medium, and agar diffusion methods (disc or well diffusion). These methods measure the activity of the oil in its liquid phase through direct contact with the bacterial lawn [8]. While invaluable for initial screening, they may not fully capture the bioactivity of highly volatile compounds. In contrast, the volatilization method (also known as the vapour-phase or aerial diffusion assay) specifically evaluates the efficacy of the gaseous phase of the EO within a sealed environment [8, 9]. This distinction is not merely technical

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but has profound practical implications. The vapour-phase activity is directly relevant for potential applications in aerial disinfection (in hospitals or food storage facilities), in treating respiratory tract infections via inhalation, and in understanding the ecological roles of plant volatiles.

Research on other well-characterized essential oils, such as those from thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*), has revealed that antibacterial activity can differ significantly between the liquid and vapour phases. Generally, vapour-phase treatments have demonstrated potent inhibitory effects against Gram-positive bacteria like *S. aureus* and *Bacillus subtilis* [10, 11]. The relatively simple cell wall structure of Gram-positives, comprising a thick peptidoglycan layer, appears to be more readily penetrated by volatile compounds. Conversely, Gram-negative bacteria, such as *E. coli*, with their complex outer membrane containing lipopolysaccharides (LPS) that acts as a formidable permeability barrier, often exhibit reduced susceptibility to both liquid and vapour-phase EOs, though the vapours can sometimes show enhanced activity against some strains by potentially bypassing certain efflux mechanisms [10]. Despite the extensive pharmacological investigations into the *Artemisia* genus, comprehensive and comparative data on the vapour-phase antibacterial activity of its essential oils remain scarce. This gap in knowledge is particularly pronounced for specific Mediterranean chemotypes like *A. judaica*, whose full antimicrobial potential may be underestimated if assessed by liquid-phase assays alone.

Therefore, to address this research gap and provide a more universal evaluation of its antibacterial profile, we conducted a comparative study. This work aimed to evaluate the antibacterial activity of *A. judaica* essential oil against two clinically relevant and structurally distinct pathogens—the Gram-positive *S. aureus* and the Gram-negative *E. coli*—using two complementary methodologies: the standard disc diffusion assay, which measures direct contact activity, and the volatilization method, which specifically assesses vapour-phase efficacy. This direct, side-by-side comparison seeks to elucidate the critical influence of the testing method on the observed antibacterial outcomes, to determine whether the vapor phase of *A. judaica* oil holds distinct advantages for certain bacterial types, and ultimately, to contribute to application-oriented understanding of its potential in developing novel strategies to combat resistant bacterial pathogens.

## 2. Materials and Methods

### 2.1 Plant material:

Aerial parts (stems & leaves) of *A. judaica* were collected in July 2025 from Al-Awaynat region, Southern of Libya. The species was identified in National Herbarium, Department of Botany, Faculty of Science, Tripoli University, Libya.

### 2.2 Essential oil extraction:

The extraction method was described by Suwaydan & Altraiki (2025)[12] with some modification. dried plant material (aerial parts)of *A. Judaica* (about 100 grams) were cut to small pieces and subjected to essential oil extraction by hydro-distillation using Clevenger-type apparatus for 3hrs in triplicate. The percentage yield of essential oil was calculated by using:

$$\text{Yield (\%)} = (\text{EO (mL)}) / (\text{dried plant (g)}) \times 100$$

The extracted Essential oil was dried over anhydrous sodium sulphate and stored in well-sealed umber vials and kept in refrigerator at 4°C until further uses

### 2.3 Microorganisms and culture condition:

The experimental work was conducted using two bacterial strains, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 8739, which were generously supplied by the Centre for Diseases Control in Tripoli, Libya. Stock cultures were preserved on Mueller-Hinton agar (MHA) slants. To ensure viability and purity, bacteria were sub-cultured onto fresh MHA plates 24 hours before each assay. All manipulations were carried out under aseptic conditions to avoid external contamination.

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### 2.4 Disc diffusion assay:

The antibacterial activity of *A. judaica* essential oil was evaluated using the disc diffusion method, as per the Clinical and Laboratory Standards Institute (CLSI) guidelines for testing essential oils [8,9]. Overnight bacterial cultures were suspended in sterile saline and adjusted to a 0.5 McFarland standard turbidity, equivalent to approximately $1.5 \times 10^8$ CFU/mL. For the assay, sterile filter paper discs (6 mm in diameter) were impregnated with 3 µL of the essential oil. These prepared discs were then aseptically placed onto the surface of Mueller-Hinton agar (MHA) plates that had been pre-inoculated with the standardized bacterial suspensions. Levofloxacin discs (5 µg) were used as a positive control. All plates were incubated at 37°C for 24 hours under aerobic conditions. Following incubation, the zones of inhibition (ZOIs) were measured in millimetres (mm), with the diameter of the clear zone around the disc indicating the level of antibacterial activity.

### 2.5 Volatilization assay:

The potential vapor-phase antibacterial effect of *A. judaica* oil was evaluated following the method of Inouye et al. [9]. Sterile Eppendorf lids containing 40 µL oil containing 40 µL of essential oil were attached to the underside of the Petri dish lid. MHA plates previously inoculated with standardized bacterial suspensions were used as the test surface. Plates were carefully sealed with parafilm to prevent vapour escape and incubated at 37°C for 24 hours without direct contact between the oil and the agar surface. Following incubation, ZOIs were measured in mm as described above to assess the antibacterial efficacy of the oil vapour.

### 2.6 Data analysis:

All experiments were carried out in duplicate and repeated on two independent experiments to ensure reproducibility and reliability of results. The mean ZOI values and corresponding standard deviations (SDs) were calculated. Where appropriate, comparisons between the essential oil and the positive control (levofloxacin) were performed to determine relative antibacterial potency.

## 3. Results

### 3.1 Extractive yield of *A. judaica* essential oil

The percentage yields of **0.8% ±0.05 (V/W)** of the essential oils of *A. judaica* aerial parts were obtainedby the hydro- distillation method of the essential oil extraction.

### 3.2 Direct-contact Antibacterial Activity

The disc diffusion assay revealed that *Artemisia judaica* essential oil exhibited differential inhibitory effects against the tested bacterial strains. Against *Staphylococcus aureus*, the essential oil produced a mean inhibition zone of 16.5 ± 2.1 mm, which was considerably lower than the potent activity of the levofloxacin positive control (29.3 ± 0.6 mm). A similar trend was observed for *Escherichia coli*, where the essential oil yielded a mean zone of 11.7 ± 1.2 mm, again significantly less than the levofloxacin control (31.3 ± 2.1 mm). These results demonstrate the direct-contact antibacterial activity of *A. judaica* oil, with a more pronounced effect against the Gram-positive *S. aureus* compared to the Gram-negative *E. coli*, though its efficacy against both was substantially lower than that of the conventional antibiotic.

### 3.3 Vapour-phase Antibacterial Activity

The volatilization method was used to assess the antibacterial activity of *A. judaica* oil in the vapour phase. The results demonstrated uniform inhibition zones of 8.0 ± 0.0 mm for both bacterial species (Table 1). These minimal and non-variable ZOIs indicate only negligible antibacterial activity for the oil's volatile components under the tested conditions.

Collectively, the data indicate that *A. judaica* essential oil exhibits notable contact-dependent antibacterial effects, particularly against *S. aureus*, whereas its activity in the gaseous phase is insignificant, limiting its potential for airborne disinfection applications.

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**Table 1. Zone of inhibition (mm) of *A. judaica* oil against test microorganisms.**

| Organism | Agar diffusion (Mean ± SD) | Disc volatilization (Mean ± SD) | Levofloxacin disc (Mean ± SD) |
|---|---|---|---|
| *Staphylococcus aureus* | 16.5 ± 2.1 | 8.0 ± 0.0 | 29.3 ± 0.6 |
| *Escherichia coli* | 11.7 ± 1.2 | 8.0 ± 0.0 | 31.3± 2.1 |

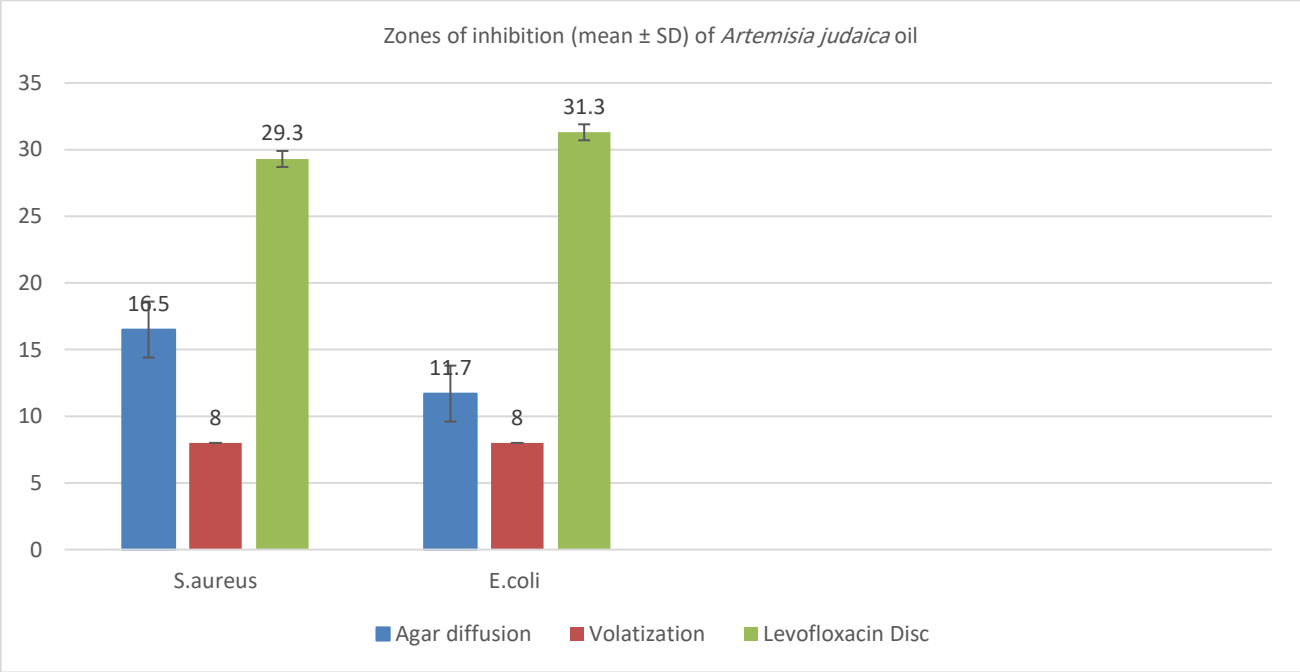


**Figure 1. Zones of inhibition (mean ± SD) of *A judaica* oil (agar diffusion, volatilization) compared with levofloxacin against tested bacteria**

## 4. Discussion

Our findings confirm that methodological context drives EO activity readouts. *A. judaica* oil showed robust direct contact antibacterial effects but negligible vapour phase effects under volatilization method. This aligns with reports that Gram negative bacteria often resist EO vapours due to outer membrane barriers and efflux [2,4]. Mediterranean *Artemisia* chemotypes (*A. herbaalba, A. judaica*) frequently contain camphor/1,8,cineole/thujone, regional studies from Algeria, Libya, and the Middle East report antibacterial activity, including against MDR strains, largely in direct contact assays [6,13]. Recent GC MS profiling of *A. judaica* from the Arabian Peninsula highlights oxygenated monoterpenes and supports bioactivity relevant to wound healing, consistent with our direct contact results[13].

Essential oils such as thyme and oregano oils that rich in phenolic monoterpenes (thymol, carvacrol)—often retain measurable vapour-phase activity, especially against Gram positive bacteria, and have shown efficacy in vapour contact on food and clinical isolates[9,10, 14]. Eucalyptus oils and novel formulations show antimicrobial and anti-inflammatory effects and are used mouth care [15]. Moreover, Inouye's foundational gaseous contact work demonstrated activity of several EO constituents against respiratory pathogens, providing mechanistic precedent for vapour testing [16]. However, assay parameters (medium depth, headspace geometry, sealing, inoculum) critically affect reproducibility. Synergy screens indicate that certain EOs can potentiate antibiotics [17], a strategy worth exploring for *Artemisia*. Future work should profile our *A. judaica* oil antibiotic synergy in planktonic and biofilm models.

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## 5. Conclusion

*Artemisia judaica* oil showed marked antibacterial activity in disc diffusion assays. However, its vapour-phase activity was minimal. These findings highlight its potential for topical or direct-contact applications rather than airborne disinfection and underscore the need for standardized vapour-phase testing protocols.

## ETHICS STATEMENT

Not Applicable

## CONFLICT OF INTEREST

*The authors declare no conflict of interest*

## AUTHOR CONTRIBUTIONS

Abdulkarem Tamer: Conceptualization, Investigation, writing original draft, Writing review & editing.
Abdulaziz Sh. Suwaydan: Methodology, writing original draft , Writing review & editing.
Amnah Galboun: Investigation, Resources.

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