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Research Article

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Antibacterial Potential of Essential Oils of the Leaves of *Artemisia Annua* and *Aloe Vera* Against *Staphylococcus Aureus*

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Abstract

Artemisia annua and Aloe Vera have historically been used as medicinal plants through its extracts. Essential oils from Artemisia annua for decades have been used as mosquito repellants They have as well shown their potential to disrupt the biofilm formation of gram-positive pathogens; staphylococcus aureus being one of deadly pathogen of focus in this study. Thus, the study seeks to evaluate the activity of the essential oils present in both Artemisia annua and aloe vera against Staphylococcus aureus bacterium through extraction of essential oils obtained using steam distillation method. Antimicrobial effectiveness of the plant oils was assessed using the agar well diffusion test, and their Minimum Inhibitory Concentration (MIC) was determined by the serial dilution procedure. The study identifies that the essential oils of both Aloe Vera and Artemisia annua possess substantial antimicrobial activity against S. aureus. Artemisia annua essential oil demonstrated a zone of inhibition of 18.50 ± 0.50 mm at 100% concentration (MIC: 0.625 mg/ml), while Aloe vera essential oil – 13.40 ± 0.40 mm (MIC: 2.5 mg/ml). The results obtained indicate the high potential of these two plant oils as natural and affordable antibiotic substitutes for combating bacterial infections.

Keywords: Essential Oils, *Artemisia Annua*, Aloe Vera, *Staphylococcus Aureus*, Antibacterial Activity, MIC, Steam Distillation, Hydro-Distillation

1. Introduction

For decades, medicinal plants have remained as a rich source of antimicrobial Agents of Recent reports estimate that over 80% of the developing world's population is reliant on traditional and herbal medicine to address their health needs [1]. The biological activity of most medicinal plants is based on their constituent secondary metabolites that may broadly encompass chemical classes like, chromones, sesquiterpene lactone, flavonoids and essential oils. Essential oils are known for their unique fragrant properties but are also quite volatile and hydrophobic. Plant essential oils are often isolated from stems, leaves, shoots or roots using steam distillation. Apart from their fragrant odors, plant essential oils often exhibit a range of biological activities owing to due to the presence of ketones, esters, aldehydes, terpenes and other bioactive chemical compounds that may possess antimicrobial, antioxidant, anti-inflammatory and analgesic effects [2]. In addition to their widespread applications in pharmaceuticals, cosmetics and food preservation, essential oils are devoid of the toxicity associated with synthetic medicines and do not lead to development of resistance in microorganisms [3].

Artemisia annua, commonly known as sweet wormwood, is an annual aromatic herb belonging to the Asteraceae family. It is native to Asia but now widely distributed across temperate and tropical regions globally. The plant is characterized by erect stems, finely divided leaves and small yellow capitula [4]. It has been utilized in traditional Chinese medicine for over two millennia majorly for the treatment of fevers and malaria. Modern pharmacological research has validated its use as the primary source of artemisinin, an endoperoxide sesquiterpene lactone with potent antimalarial activity. Beyond antimalarial properties, the plant is also employed in folk medicine for the treatment of wounds, inflammation and infectious diseases [5-7]. The essential oil of *Artemisia annua* possesses bioactive compounds, such as Artemisia ketone, 1,8-cineole, camphor and α-pinene, that demonstrate remarkable antibacterial and antifungal activity including the inhibition of

Staphylococcus aureus [5,8].

Aloe vera is a succulent perennial plant belonging to the Asphodelaceae family, which is native to the Arabian Peninsula and nowadays is grown throughout the world. This plant is highly respected for a long time, being called "Plant of Immortality" by the Ancient Egyptians, and has been used in medicine by the Greek, Roman and Chinese civilizations for treatment of wounds, skin problems and digestive disorders [9]. In modern times, the plant is extensively used in cosmetic, pharmaceutical and food industries. Its notable antimicrobial properties make it a common ingredient in traditional remedies for infected wounds [10,11]. Apart from its famous moisturizing effect of the inner gel, the oil obtained from the outer leaf surface contains phenolic compounds, anthraquinones (aloin, emodin), and polysaccharides with documented Antimicrobial and anti-inflammatory effects [12]. It has been experimentally proved that *A. vera* extracts are able to inhibit the growth of *S. aureus* (Figure 1 and Figure 2) [10].

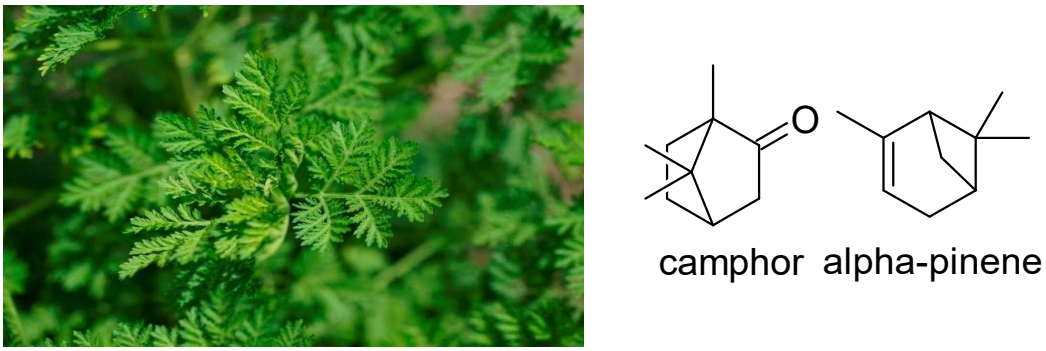
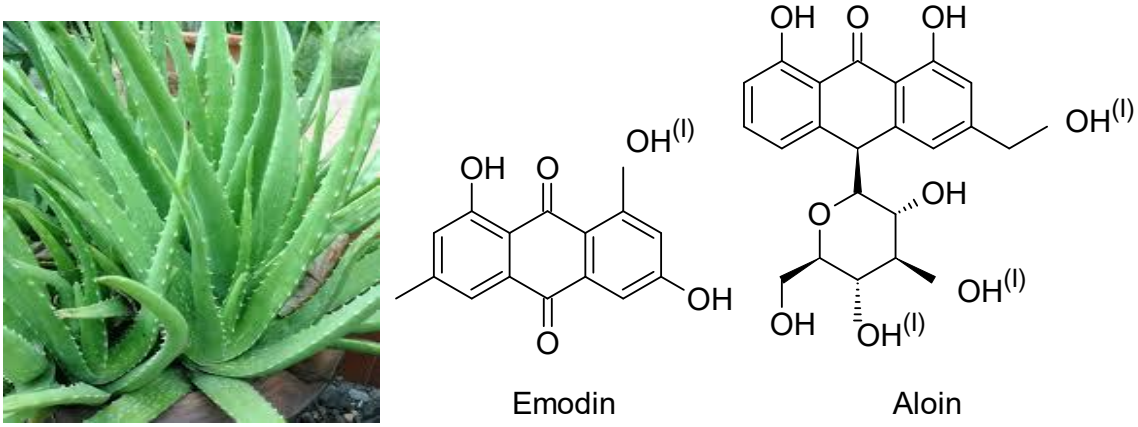


Figure 1: *Artemisia Annua* and Some of its Compounds



(Pictures captured using techno spark 50 camera and chemical structures drawn using chemdraw version 15)

Figure 2: Aloe Vera and its Compounds

Staphylococcus aureus is a Gram-positive, facultative anaerobic bacterium that commonly colonizes the mild skin and soft tissue to life threatening infectious such as endocarditis, bacteremia, sepsis etc. of healthy individuals [13]. The emergence of Methicillin-Resistant *S. aureus* (MRSA) has been classified by the World Health Organization (WHO) as a high-priority pathogen requiring for an urgent research into alternative antibiotics that may help in prevention or disruption of its formation [14]. Given the limitations of conventional antibiotics and the growing demand for natural therapeutic agents, this study focused on extracting essential oils from *Artemisia annua* and *Aloe vera* and evaluating their antibacterial potential against *S. aureus*. The combination of these two plant oils may also exhibit synergistic effects, offering a promising approach to antibiotic-resistant infections. The findings of this study will serve as baseline data for the development of novel pharmaceutical formulations such as topical ointments, creams or disinfectants. Additionally, the potential synergistic effect between both oils could lead to more potent combination therapies.

2. Genesis of the Discussion

The global escalation of antibiotic resistance represents one of the most critical threats to public health in the 21st century. The ability of pathogenic bacteria to evade conventional therapeutic agents has necessitated the exploration of novel antimicrobial compounds from natural sources [14]. Medicinal plants have served as a reservoir of

bioactive molecules for centuries, offering a promising avenue for drug discovery. The Pathogen: Staphylococcus aureus Morphology and Pathogenicity: *Staphylococcus aureus* is a Gram-positive, catalase-positive, facultative anaerobic bacterium. It typically colonizes the skin and mucous membranes of approximately 20–30% of healthy humans [13]. As an opportunistic pathogen, it is implicated in a wide spectrum of infections from superficial skin abscesses and wound infections to severe systemic conditions such as bacteremia, endocarditis and pneumonia.

2.1. The Crisis of Antimicrobial Resistance: The clinical management of S. aureus infections has become increasingly challenging due to the emergence and rapid dissemination of multi-drug-resistant strains. Following the widespread use of penicillin, resistant strains emerged rapidly. This was followed by the emergence of MRSA, which is currently resistant to nearly all β-lactam antibiotics [15]. More recently, strains with reduced susceptibility or complete resistance to vancomycin have also been reported. Consequently, the World Health Organization has categorized MRSA as a high-priority pathogen requiring urgent research into alternative therapeutic strategies [14]. Antibacterial Activity and Mechanism of Action.

2.2. Efficacy of *Artemisia Annua*: Numerous scientific investigations have confirmed the broad-spectrum antimicrobial activity of *A. annua. Ziaei* et al., reported that the essential oil of *A. annua* exhibited potent inhibitory effects against *S. aureus*, with MIC values as low as 0.02 mg/ml [5]. The oil has also demonstrated efficacy against MRSA strains, suggesting its potential in combating resistant infections [16]. The mechanism of action involves the lipophilic nature of essential oils, which allows them to penetrate the bacterial cell wall and membrane. This leads to increased permeability, leakage of cellular contents, disruption of ion gradients and ultimately cell death [2].

2.3. Efficacy of Aloe Vera: Reynolds and Dweck, reviewed that Aloe vera extracts are effective against various Gram-positive bacteria. Specific studies have demonstrated that methanol extracts produce significant zones of inhibition against S. aureus [12]. The activity is primarily attributed to anthraquinones such as emodin, which interfere with nucleic acid synthesis and cell wall function [10]. In the present work, we investigate the antimicrobial resistance-mitigating potential of essential oil extracts of *Artemisia annua* and *Aloe vera* against *Staphylococcus aureus.*

3. Methodology

3.1. Materials and Equipments

Distilled water, round bottomed flask, separating funnel, heat source, complete retort stands, beakers, *Artemisia annua* leaves and aloe vera leaves.

3.2. Collection of Plant Material

Fresh leaves of *Artemisia annua* and Aloe vera were collected from outskirts of chepkoilelel Eldoret, Kenya. Collection was conducted during morning hours to ensure maximum potency of the phytochemicals present in the plant tissue.

3.3. Preparation of Plant Material

3.3.1. For *Artemisia Annua*

The fresh leaves were thoroughly washed under running tap water to remove dust, soil and debris, then rinsed with distilled water. Excess water was drained, and the leaves were chopped into small pieces to increase surface area for extraction. The leaves were used fresh, without drying, to preserve volatile constituents.

3.3.2. For Aloe Vera

The outer green rind was carefully separated and the leaves were cut. were then washed thoroughly under running tap water and rinsed with distilled water. Later placed upright in a container and allowed to drain for **15–20 minutes** to remove the yellowish latex sap, which may cause irritation. The spiky margins and outer green rind were peeled off with a sharp knife. Then a clear, transparent inner gel was collected, chopped into small pieces and used immediately for extraction.

3.4. Extraction of Essential Oils

3.4.1. Extraction of Artemisia Annua Oil Steam Distillation Method

The **500g** of freshly chopped leaves were placed in a 2-litre round-bottom flask with **1,000 ml** of distilled water. The mixture was heated and boiled for 4 hours. Steam carried the volatile components upward into the condenser, where they condensed into a liquid mixture. The essential oil separated and floated on top of the water in the graduated receiver. The oil was collected, dried over anhydrous sodium sulfate (Na_2SO_4) to remove traces of water, and stored in a dark amber glass bottle at 4°C until use.

3.4.2. Extraction of Aloe Vera Oil Steam Distillation Method

500 g of freshly prepared Aloe vera gel were placed in a round-bottom flask. Distilled water was added, and the apparatus was assembled. Heating was applied and distillation carried out continuously for 4 hours. The steam vaporized the active aromatic compounds, which were condensed back into liquid form. The collected oil (0.7 ml, amber-coloured) was separated, dried and stored in a sterile amber bottle.

3.4.3. Calculation of Percentage Yield

The yield of the extracted oil was calculated using the formula:

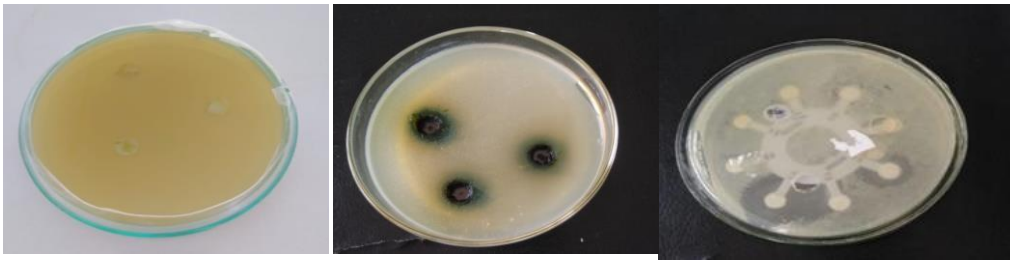
Plant	Fresh Sample Weight (g)	Oil Obtained (ml)	Percentage Yield (%)
	500	1.8	0.36
Aloe vera	500	0.7	0.14
Percentage yield (%) = [Volume of oil obtained (ml) / Weight of fresh sample (g)] × 100%			

3.4.4. Agar Well Diffusion Method

Mueller-Hinton Agar (MHA) plates were prepared by pouring 20 ml of molten media into sterile petri dishes and allowed to solidify. A standardized bacterial suspension was uniformly swabbed onto the agar surface using a sterile cotton swab. Wells of 6 mm diameter were bored into the agar using a sterile cork borer. The following concentrations of essential oils were prepared using DMSO as solvent:

- 100% (pure oil)
- 50% (1:1 v/v with DMSO)
- 25% (1:3 v/v with DMSO)

0.1 ml of each concentration was introduced into the respective wells. With the application of Controls; Positive control: Standard antibiotic disc ciprofloxacin (5 µg) placed on the agar surface. Negative control: Pure DMSO added to one well. Plates were allowed to stand for 30 minutes at room temperature for diffusion, then incubated at 37°C for 24 hours. After incubation, the diameter of the zone of inhibition (ZOI) was measured in millimeters (mm) using a ruler.



a). *Artemisia annua* b). Aloe vera c). Positive control

3.4.5. Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an extract that prevents visible growth of the bacteria. Serial dilutions of the essential oils were prepared to obtain concentrations ranging from 10 mg/ml to 0.156 mg/ml. Each dilution was tested against a standardized bacterial suspension. The MIC was recorded as the lowest concentration showing a clear zone of inhibition.

Observed MIC values:

- *Artemisia annua*: **0.625 mg/ml**
- Aloe vera:
- Aloe vera: **2.5 mg/ml**

3.5. Experimental Design and Data Analysis

The experiment was performed in **triplicate (n = 3)** to ensure accuracy and reproducibility. Results are presented as **mean ± standard deviation (SD).**

4. Results and Discussion

4.1. Physical Characteristics and Percentage Yield

Table 1 below presents the physical characteristics and percentage yield of essential oils obtained from both plant species.

Parameter	Artemisia annua Oil	Aloe vera Oil
Colour	Pale yellow to green-yellow	Pale yellow to amber
Odour	Strong, aromatic — characteristic complex smell	Mild, slightly spicy
Consistency	Mobile, thin liquid	Slightly viscous
Weight of fresh sample (g)	500	500
Volume of oil obtained (ml)	1.8	0.7
Percentage yield (%)	0.36	0.14

Table 1: Physical Characteristics and Percentage Yield of Essential Oils

4.1.1. Discussion on Yield and Characteristics

For *Artemisia annua*: The percentage yield of 0.36% falls within the range reported in previous studies (0.2%–1.2%), which varies depending on geographical location, climate and harvest stage [17]. The greenish-yellow colour and strong aroma are attributed to the presence of volatile terpenoids and ketones particularly Artemisia ketone, 1,8-cineole and camphor which are the major constituents responsible for the oil's biological activity [8]. The relatively high yield observed in this study indicates that fresh leaves are an excellent source of essential oil, as drying may cause some loss of volatile compounds.

For Aloe vera: The yield of 0.14% was lower than that of *A. annua*. This is expected because Aloe vera leaves are composed of approximately 95–98% water, with the essential oil fraction present in smaller quantities, primarily in the green rind rather than the clear gel [15]. Despite the low yield, the steam distillation process successfully extracted the volatile aromatic compounds. The amber colour and distinctive odour suggest the presence of active compounds such as phenolic and anthraquinone derivatives, confirming that steam distillation is a viable extraction method for Aloe vera [11].

4.2. Antibacterial Activity Assay

The antibacterial potential of the essential oils was evaluated using the agar well diffusion method against *Staphylococcus aureus*. The diameters of the zone of inhibition (ZOI) were measured in millimeters (mm). Results are presented in Table 2.

Sample	Concentration	Zone of Inhibition (mm) Mean ± SD	Activity Level
	100% 50% 25%	18.50 ± 0.50 14.20 ± 0.30 10.00 ± 0.20	High Moderate Moderate
Aloe vera oil	100% 50% 25%	13.40 ± 0.40 10.10 ± 0.30 7.10 ± 0.10	Moderate Moderate Low
Positive control (Ciprofloxacin)	Standard	25.00 ± 0.00	Very High
Negative control (DMSO)	—	0.00 ± 0.00	No Activity
Activity Scale: >15 mm = High; 10–14 mm = Moderate; <9 mm = Low			

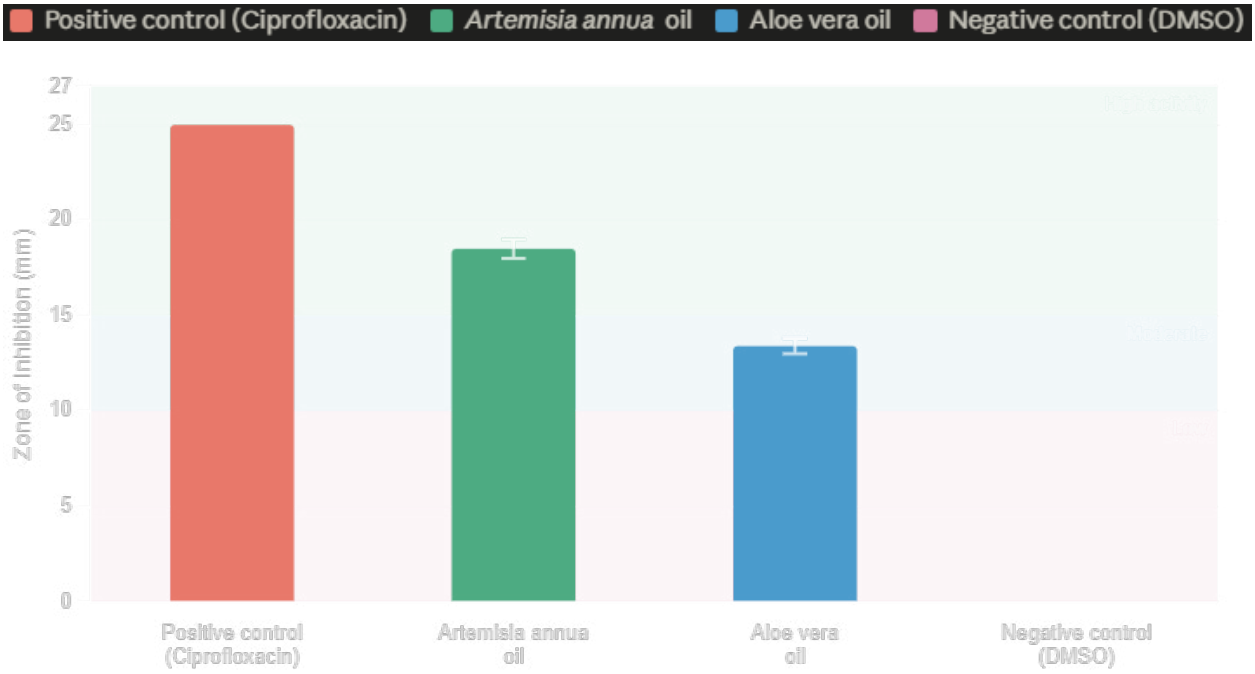


Table 2: Zone of Inhibition (mm) for Essential Oils Against *S. Aureus*

4.2.1. Discussion on Antibacterial Activity

Efficacy of Artemisia annua oil: The results clearly demonstrate that *Artemisia annua* essential oil exhibited significant inhibitory activity against *S. aureus*. At 100% concentration, the oil produced a maximum zone of inhibition of 18.50 ± 0.50 mm, classified as high activity.

This high activity is attributed to the rich chemical composition of the oil particularly oxygenated monoterpenes such as 1,8-cineole, camphor and α-pinene [17]. According to Bakkali et al., these lipophilic compounds act by disrupting the integrity of the bacterial cell membrane and cell wall, leading to increased permeability, leakage of cellular contents and ultimately cell death [4]. The findings are consistent with those of who observed strong antibacterial activity against Gram-positive bacteria. The low standard deviation values confirm that results were consistent and reproducible [8].

Efficacy of Aloe vera oil: Aloe vera oil also showed measurable antibacterial activity, producing a zone of inhibition of 13.40 ±

0.40 mm at full concentration classified as moderate activity. The inhibitory effect is primarily attributed to anthraquinones such as aloin, emodin and chrysophanol, which interfere with bacterial cell wall synthesis [11]. These findings support the traditional use of Aloe vera for treating wounds and preventing infections.

Dose dependent relationship: A clear dose-response relationship was observed. As the concentration of the essential oil decreased, the diameter of the zone of inhibition also decreased. For example, *A. annua* dropped from 18.50 mm at 100% to 10.00 mm at 25%. This indicates that higher concentrations contain greater amounts of active ingredients, resulting in more effective bacterial inhibition.

Comparison with controls: The standard antibiotic ciprofloxacin showed the highest activity (25.00 mm), as expected. However, the substantial activity of *A. annua* at 18.50 mm suggests it is a potent natural alternative with considerable therapeutic potential.

4.3. Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) represents the lowest concentration of an extract required to completely inhibit the visible growth of the target microorganism. Table 3 presents the MIC values for both plant oils.

Plant Extract	MIC Value (mg/ml)
Artemisia annua	0.625
Aloe vera	2.5

Table 3: MIC Values for Essential Oils Against *S. Aureus*

The MIC of 0.625 mg/ml for *A. annua* indicates that this oil is a highly potent antibacterial agent, requiring only a very small amount to exert its effect. This is consistent with findings by Politi et al. (2024), who reported MIC values of 0.5–1.0 mg/ml for similar essential oils against *S. aureus*.

For Aloe vera, the higher MIC of 2.5 mg/ml indicates that while effective, a greater concentration is needed to achieve the same level of inhibition compared to *A. annua*. Nevertheless, both values confirm the **bacteriostatic potential** of both oils.

5. Conclusion

Essential oils have been effectively obtained from the fresh leaves of *Artemisia annua* and Aloe vera through the process of steam distillation and the antibacterial properties against *S. aureus* have been determined. From the results of this experiment, the following conclusions are made:

Steam distillation was found to be an efficient technique for extraction of essential oils from fresh material of both plants. The yield obtained was 0.36% for Artemisia annua compared to 0.14% for Aloe vera. This is due to higher water content and less amount of volatile compounds in Aloe vera.

Essential oil from Artemisia annua was very effective, with ZOI of 18.50 ± 0.50 mm at 100% concentration level. It is thus a very potent oil against S. aureus. Aloe Vera was moderately effective with ZOI of 13.40 ± 0.40 mm, hence it is effective although it is not as potent as *Artemisia annua* [18,19].

The MIC for *A. annua* (**0.625 mg/ml**) confirms it is effective even at very low concentrations.

The MIC for Aloe vera (**2.5 mg/ml**) confirms bacteriostatic potential but requires higher concentrations to achieve complete inhibition.

Although ciprofloxacin showed the highest activity, *A. annua* essential oil demonstrated considerable natural potential, suggesting that these plant extracts **can serve as safe, effective and affordable alternatives** for treating infections caused by S. aureus, particularly in regions where antibiotic resistance is a growing concern.

Recommendations

I. Medical and Pharmaceutical Applications

- **Development of herbal medicines:** Efforts should be made to formulate these essential oils into pharmaceutical products such as topical ointments, creams, lotions or disinfectants for treating skin infections, wounds and burns.
- **Integration in healthcare:** Community health workers and herbal practitioners should be educated on the proper use and dosage of these plants to treat common bacterial infections effectively.

II. Further Research

- **Chemical profiling:** Advanced analytical techniques such as Gas Chromatography–Mass Spectrometry (GC-MS) should be employed to identify the specific chemical components responsible for the antibacterial activity observed in this study.
- **Activity against resistant strains:** Further studies should test these oils against clinically isolated resistant strains such as MRSA and Vancomycin-Resistant S. aureus (VRSA).
- Synergy studies: Studies are needed to analyze the synergy between the oils from A. annua and Aloe vera to see if they have a synergistic effect that increases their antibacterial activity.

III. Agricultural and Conservation Strategies

- Cultivation: Because of their medicinal properties, it is necessary to cultivate these plants in large numbers to make sure there is sufficient availability of their raw material for the drug manufacturing industries.
- Raising awareness: There must be awareness raised among

the public about the medicinal significance of these plants in order to avoid their over-exploitation and preserve them.

IV. Policy Implications: Governmental agencies and other health bodies should think of considering the natural plant products as authentic remedies, particularly in cases where conventional drugs are either expensive or unavailable.

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