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RESEARCH ARTICLE

AIn Vitro Assessment Of Aloe Vera Leaf Extracts As Natural Alternatives To Antibiotics Against Pathogenic Bacteria E. Coli, S. Aureus, and P. Aeruginosa

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ABSTRACT

Traditionally used in medicine, Aloe vera's components have shown proven anti-infective effects. It has been used for its healing, anti-inflammatory, anti-infective, anti-allergic, and antiviral benefits. This study investigates the antibacterial potential of Aloe vera polyphenols, particularly aloin, against Gram-positive and Gram-negative bacteria, aiming to explore their potential as natural alternatives to antibiotics. It aimed to extract and characterize polyphenols, particularly aloin and other phenolic compounds to maximize yield and purity. The antibacterial activity of these polyphenols was then evaluated in both pure and diluted forms. The antibacterial efficacy was assessed using the disk diffusion method and minimum inhibitory concentration (MIC) assays. The results demonstrated significant antibacterial effects against E. coli and S. aureus, with the highest inhibition zones observed for 50% diluted aloin (11.5 ± 2.35 mm) and pure aloin (13.08± 1.16 mm), respectively. In contrast, P. aeruginosa exhibited resistance to all Aloe vera extracts tested. The antibiotic ampicillin showed greater potency across all bacterial strains, indicating the extracts were less effective by comparison. These findings suggest that Aloe vera polyphenols, particularly aloin, possess potential antibacterial properties against S. aureus and E. coli, offering promising alternatives to synthetic antibiotics. Given the increasing concern over antibiotic resistance, this research highlights the potential role of Aloe vera polyphenols in developing natural antibacterial agents. Further investigation into their mechanisms of action, optimal formulations, and clinical applications is warranted. Nevertheless, caution is advised, as the use of whole Aloe vera leaves may pose toxicity risks

INTRODUCTION

Traditional medicine has been practised for centuries, serving as a fundamental component of healthcare in many cultures (Bouhalla et al., 2024; Setyowati et al., 2024). Among the numerous medicinal plants, *Aloe vera* (*Aloe barbadensis Miller*) stands out for its long history of therapeutic use and health benefits. Often referred to as the "miracle plant" or "silent healer," *Aloe vera* has been highly valued by ancient physicians for its healing properties. Despite its cactus-like appearance, *Aloe vera* belongs to the lily family and includes over 400 species cultivated worldwide. However, *Aloe barbadensis*, known as "True Aloe," has garnered the most attention for its medicinal applications (Mehta, 2017; Assaf et al., 2024).

Rich in bioactive compounds, *Aloe vera* contains vitamins, minerals, enzymes, sugars, phenolic compounds, lignin, saponins, sterols, and amino acids, contributing to its diverse pharmacological properties. Its medicinal properties have long been recognized in traditional medicine for a wide range of therapeutic benefits, with well-documented antibacterial effects being one of the most significant. Notably, *Aloe vera* contains an array of bioactive compounds, such as polyphenols, vitamins, enzymes, saponins, and polysaccharides, which contribute to its pharmacological activities, particularly in combating bacterial infections (Jain et al., 2016; Rahman et al., 2024).

In the case of *Aloe vera*, scientific research in recent years has focused on validating its traditional antibacterial claims and exploring the mechanisms underlying its potential. Several in vitro studies have confirmed *Aloe vera*'s effectiveness against a broad range of bacterial pathogens. For instance, Jain et al. (2016) demonstrated the gel's antibacterial activity against oral pathogens, suggesting its potential for treating infections in dental contexts. Further research by Majid et al. (2024) explored the antibacterial efficacy of *Aloe vera* peel extracts against *Streptococcus mutans* and *Porphyromonas gingivalis*, pathogens commonly associated with dental caries and periodontal diseases, reinforcing its potential as a natural antimicrobial agent in dental care.

Moreover, Haque et al. (2019) investigated the antibacterial effects of *Aloe vera* gel on clinically important bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. Their study revealed significant inhibition of bacterial growth, particularly against Gram-positive *S. aureus* and Gram-negative *E. coli*, underscoring the gel's broad-spectrum antibacterial capabilities. Similarly, Gharibi et al. (2015) reported the antibacterial effects of *Aloe vera* extracts on both human and animal bacterial pathogens, further supporting the plant's potential in clinical and veterinary applications.

In addition to traditional applications, *Aloe vera* has been explored in modern biomedical and veterinary fields as a green alternative to conventional antibiotics. Khan et al. (2022) emphasized its utility in poultry production, where *Aloe vera* served as a natural antimicrobial agent in place of antibiotics, addressing concerns related to antibiotic resistance and the sustainability of animal husbandry. Adzitey et al. (2019) demonstrated similar findings in their study on the gastrointestinal pathogens of guinea fowls, showing that *Aloe vera* gel was effective against *E. coli* and *Salmonella enterica*, two major pathogens associated with foodborne illnesses in poultry.

Innovative approaches to enhance the antibacterial properties of *Aloe vera* have also been explored. For instance, Zhang et al. (2010) developed silver nanoparticles using *Aloe vera* in a green synthesis method, which significantly increased its antibacterial potency. The combination of silver nanoparticles and *Aloe vera* gel exhibited synergistic antibacterial effects, offering a promising strategy for developing advanced antimicrobial materials.

In dental care, *Aloe vera* has been utilized for its antibacterial and tissue regenerative properties. Bazvand et al. (2014) compared the antibacterial effects of *Aloe vera* with other natural materials and standard antibiotics against *Enterococcus faecalis*, a bacterium commonly implicated in root canal infections. Similarly, Namazi et al. (2023) integrated *Aloe vera* into biodegradable nanofibers for use in endodontic disinfection and immunomodulation, demonstrating its multifunctional potential.

In wound care, Yusman and Karim (2024) highlighted *Aloe vera*'s efficacy in treating Gram-negative bacterial infections in wound healing, particularly for its antibacterial and anti-inflammatory properties. Khurshid et al. (2024) further explored the synergistic effects of *Aloe vera* combined with cinnamon oil, showing enhanced antibacterial activity in wound healing applications. Tawfic et al. (2023) explored the comparative antibacterial efficacy of *Aloe vera* gel versus diode laser treatment in managing deep carious lesions, highlighting its potential as a non-invasive treatment in dental procedures. Furthermore, Al-Shaibani et al. (2023) investigated the role of *Aloe vera* gel in tooth

remineralization and its inhibitory effects against *Enterococcus faecalis*, confirming its value in endodontic treatments.

Finally, a systematic review and meta-analysis conducted by Tariq et al. (2023) compared the antibacterial efficacy of *Aloe vera* against *Enterococcus faecalis* with other intracanal medicaments, reinforcing the growing consensus on its therapeutic potential in dental practice. Collectively, these studies illustrate the growing scientific interest in *Aloe vera* as a natural, sustainable alternative to synthetic antibiotics. Its broad-spectrum antibacterial properties, minimal toxicity, and potential for synergistic combinations make it a promising candidate for applications in clinical and veterinary medicine.

The primary objective of this study is to extract and characterize polyphenols, particularly aloin and other phenolic compounds, from *Aloe vera* latex using the Arnnok method (Arnnok et al., 2012), a widely recognized approach for optimizing polyphenol extraction. These compounds are known for their potent bioactive properties, and the extraction will focus on maximizing yield and purity. Subsequently, this study aims to evaluate the antibacterial activity of the extracted polyphenols, both in their pure form and at diluting concentrations, against clinically relevant Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*).

The antibacterial efficacy will be assessed using standardized microbiological techniques, including the disk diffusion method and minimum inhibitory concentration (MIC) assays, to provide a quantitative measure of the antibacterial potential of these polyphenols. Given the rising concern over antibiotic resistance, this study seeks to determine whether *Aloe vera* polyphenols, particularly aloin, can serve as effective natural antibacterial agents. By exploring their effects on both Gram-positive and Gram-negative bacteria, this research aims to contribute to the development of alternative therapeutic strategies, offering sustainable solutions to bacterial infections and mitigating the overuse of synthetic antibiotics.

MATERIAL AND METHODS

1. Plant Material Collection

Fresh *Aloe vera* (*Aloe barbadensis Miller*) leaves were harvested from mature plants and immediately processed to preserve their bioactive compounds. The leaves were thoroughly washed to remove any contaminants, and the outer green layer was carefully peeled away to access the inner juice, latex, and gel components, which were then separated for extraction.

2. Extraction of Polyphenols from Aloe Vera

The extraction procedure was adapted from the methods of Arnnok et al. (2012), Yin et al. (2023) and Anshori et al. (2024). First, 100 g of fresh aloe vera was thoroughly cleaned, cut into small pieces, and ground using a mechanical grinder. The ground plant material was then mixed with 125 mL of 99% pure methanol and left under continual agitation for 24 hours at room temperature in a dark environment to prevent degradation of light-sensitive compounds.

After the agitation period, the resulting mixture was filtered using Whatman No. 4 filter paper to separate the liquid phase of the solid residue. The residue was then transferred to a new vessel and mixed with 125 mL of 99% ethyl acetate. This mixture was again stirred for 30 minutes and subsequently filtered through Whatman No. 4 filter paper.

The methanol and ethyl acetate filtrates were combined, and the solvent was evaporated at 45°C using a rotary evaporator to obtain the dried *aloe vera* extract. This crude extract was then stored under controlled conditions for further analysis.

The whole Aloe vera leaf, including latex and gel, was homogenized and macerated in 80% ethanol at room temperature for 72 hours. The mixture was filtered, and the solvent was evaporated under

reduced pressure to obtain the crude extract, which was then diluted to 50% with sterile distilled water for antibacterial testing. Aloin was isolated from the latex through maceration in 95% ethanol over 72 hours, followed by purification using column chromatography (Ioannou et al., 2024). The purified aloin was dried and diluted to a 50% concentration for testing. Phenolic compounds were extracted from the latex by maceration in 80% ethanol, with subsequent filtration and solvent evaporation yielding pure polyphenols, which were also diluted to 50% with sterile distilled water for antibacterial evaluation.

The inhibition zones of ampicillin and amoxicillin/clavulanic acid served as benchmarks to evaluate the relative potency of the *Aloe vera* extracts. This comparison aimed to determine whether *Aloe vera* polyphenols, particularly aloin and phenolic compounds, could offer a natural alternative to comparable or superior antibacterial effects.

3. Bacterial Strains and Culture Conditions

Three bacterial strains were used to evaluate the antibacterial activity of Aloe vera extracts: *Pseudomonas aeruginosa* (Gram-negative), *Escherichia coli* (Gram-negative), and *Staphylococcus aureus* (Gram-positive).

These strains were obtained from certified culture collections and maintained under sterile conditions. Prior to testing, each strain was cultured in Mueller-Hinton agar and incubated at 37°C for 24 hours to ensure optimal bacterial growth and viability, following the standardized protocol (Bauer et al., 1966; Mehrish et al., 2022; Mariselvam et al., 2023; Nsofor et al., 2023).

4. Antibacterial Activity Assay

The antibacterial properties of Aloe vera extracts were evaluated using the agar well diffusion method where bacterial suspensions were adjusted to a 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/mL). Extract solutions (whole leaf extract, 50% diluted extract, pure aloin, 50% aloin, pure phenolic compounds, and 50% phenolic compounds) were prepared in sterile distilled water. Ampicillin (10 µg) and amoxicillin/clavulanic acid (20/10 µg) served as standard antibiotics. Agar plates were prepared with wells (6 mm diameter), into which 100 µL of each extract and antibiotic solution was added. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured. Each experiment was conducted in triplicate, with the average inhibition zone diameters recorded for analysis.

5. Statistical Analysis

All data were expressed as mean values ± standard deviation, and each assay was performed in triplicate to ensure reproducibility. Statistical comparisons between the antibacterial effects of the extracts and the standard antibiotics were conducted using ANOVA as described in . A p-value of less than 0.05 was considered statistically significant for determining differences between the groups (Zineb et al., 2021).

RESULTS AND DISCUSSION

Preliminary Test

To assess the potential development of resistance in the pathogenic strains under study, a preliminary sensitivity test was conducted. The bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were tested for their susceptibility to two widely prescribed antibiotics: ampicillin and amoxicillin-clavulanic acid. Sensitivity to the was evaluated based on the diameter of inhibition zones, following the classification system proposed by Ponce et al. (2003). Strains were considered resistant or non-sensitive if the inhibition zone measured less than 8 mm. Sensitivity was classified as mild if the inhibition zone ranged between 9 and 14 mm, while a zone of

15 to 19 mm indicated high sensitivity. Strains were deemed extremely sensitive if the inhibition zone exceeded 20 mm.

The inhibition zones for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* against ampicillin and amoxicillin-clavulanic acid are shown in the table1.

Table 1: inhibition zones and sensitivity of the studied bacterial strains to ampicillin and amoxicillin-clavulanic acid.

Antibiotic	Strain	Inhibition Zone (mm)	Sensitivity
Ampicillin	*E. coli*	18.16 ± 3.97b	++
	P. aeruginosa	11.5 ± 3.17a	+
	S. aureus	17.5 ± 2.71c	++
Amoxicillin	*E. coli*	10.75 ± 2.49d	+
	P. aeruginosa	9.08 ± 3.26a	+
	S. aureus	8.5 ± 2.50d	-

Sensitive (+), Very sensitive (++), Extremely sensitive (+++) and Resistant (-)

The sensitivity of the bacterial strains to the antibiotics ampicillin and amoxicillin-clavulanic acid shows varying degrees of effectiveness. For ampicillin, *E. coli* and *S. aureus* were classified as "very sensitive" (++) with inhibition zones of 18.16 mm and 17.5 mm, respectively, indicating a good level of efficacy for this antibiotic against these strains. However, *P. aeruginosa* exhibited a lower inhibition zone of 11.5 mm, classifying it as "sensitive" (+), but suggesting reduced susceptibility compared to the other two strains.

For amoxicillin-clavulanic acid, the results showed less pronounced antibacterial activity. *E. coli* and *P. aeruginosa* displayed smaller inhibition zones of 10.75 mm and 9.08 mm, respectively, classifying them as "sensitive" (+). However, *S. aureus* showed resistance to this antibiotic, with an inhibition zone of only 8.5 mm, below the threshold for sensitivity.

These results indicate that ampicillin demonstrates stronger antibacterial activity than amoxicillin-clavulanic acid, especially against *S. aureus*, which was resistant to the latter. The lower effectiveness of amoxicillin-clavulanic acid, particularly against *S. aureus*, may suggest an emerging resistance, highlighting the need for careful consideration when using this antibiotic for clinical treatments. Moreover, *P. aeruginosa*'s limited susceptibility to both antibiotics points to the inherent resistance mechanisms often seen in this pathogen, reinforcing the challenge of treating infections caused by this strain.

Antibacterial activity of *aloe vera* extracts compared to antibiotics

Escherichia coli

In comparison, ampicillin exhibited the most potent antibacterial activity against *Escherichia coli*, with an inhibition zone of 18.16 ± 3.973 mm, categorizing *E. coli* as "very sensitive" (++). This demonstrates ampicillin's continued effectiveness in treating *E. coli* infections, outperforming *Aloe vera* extracts and amoxicillin, which displayed moderate activity with an inhibition zone of 9.92 ± 1.165 mm (sensitive, +). The reduced efficacy of amoxicillin, compared to ampicillin, may indicate either emerging resistance in *E. coli* or a narrower antibacterial spectrum for this antibiotic (Table 2).

Table 2: Inhibition Zones and Sensitivity of *Escherichia coli* to *Aloe vera* Extracts and antibiotics (Ampicillin and Amoxicillin).

Treatments	inhibition zone (mm)	sensitivity
crude extract (CE)	10.25 ± 1.545b	(+)
50% diluted crude extract (DCE)	10.92 ± 2.109b	(+)
aloin (A)	9.75 ± 1.913b	(+)
50% diluted aloin (DA)	11.5 ± 2.355b	(+)
latex phenols (LP)	9.42 ± 1.443b	(+)
50% diluted latex phenols (DLP)	10.92 ± 1.24b	(+)
ampicillin (Amp)	18.16 ± 3.973a	(++)
amoxicillin (Amox)	9.92 ± 1.165b	(+)

CE: Crude extract; DCE :50% Diluted Crude Extract; A: Aloin; DA: 50% Diluted Aloin; LP: Latex Phenols; DLP: 50% Diluted Latex Phenols; Amp: Ampicillin; Amox: Amoxicillin; Sensitive (+), Very sensitive (++), Extremely sensitive (+++) and Resistant (-).

The moderate performance of *Aloe vera* extracts in this study is consistent with findings by Arsene et al. (2022), who reported that *Aloe vera* aqueous extracts exhibit antibacterial activity against resistant Gram-negative bacteria, including *E. coli*, albeit at lower efficacy than synthetic antibiotics. Similarly, Parnomo and Pohan (2021) demonstrated that *Aloe vera* extracts showed inhibitory effects on *E. coli* growth, although less potent than conventional antibiotics, suggesting Aloe vera's potential as a supplementary antibacterial agent. Najafi et al. (2022) further confirmed Aloe vera's antibacterial activity, noting its effectiveness against *E. coli* alongside other natural agents like green tea extracts, indicating its broad-spectrum antibacterial properties.

Despite not matching the high potency of ampicillin, *Aloe vera* extracts consistently demonstrated antibacterial activity, indicating their potential as an alternative or complementary treatments, especially in situations where antibiotic resistance is a concern or where natural remedies are preferred. Harini et al. (2024) found that *Aloe vera* extract could be bioinspired to synthesize antibacterial agents such as calcium magnesium aluminate nanoparticles, suggesting that *Aloe vera* might enhance antibacterial activity when combined with other compounds. This aligns with the possibility of using *Aloe vera* in combination therapies to improve antibacterial efficacy.

The observed antibacterial activity of aloin, a key component of *Aloe vera* extracts, suggests that optimizing extraction methods and concentrations could enhance Aloe vera's efficacy against *E. coli*. Sraboni et al. (2024) highlighted that medicinal plants, including Aloe vera, possess antibacterial properties that can be beneficial in various applications, reinforcing the idea that *Aloe vera* extracts could serve as a natural antibacterial option, particularly in the context of growing antibiotic resistance. Therefore, exploring *Aloe vera*'s synergistic effects with conventional antibiotics could reduce dosage requirements, minimize side effects, and limit resistance development, making it a promising candidate for alternative therapeutic strategies against bacterial infections.

Pseudomonas aeruginosa

The study demonstrates that different *Aloe vera* extracts and components exhibit varying degrees of antibacterial activity against *Pseudomonas aeruginosa* (Figure 1). The raw *Aloe vera* extract showed a notable inhibition zone of 7.25±3.793 mm, suggesting significant antibacterial effectiveness, comparable to the phenolic component of latex pure (7.5±4.964 mm) and relatively close to the standard antibiotic (ampicillin/amoxicillin-clavulanic acid), which had the highest activity at 10.75±4.393 mm.

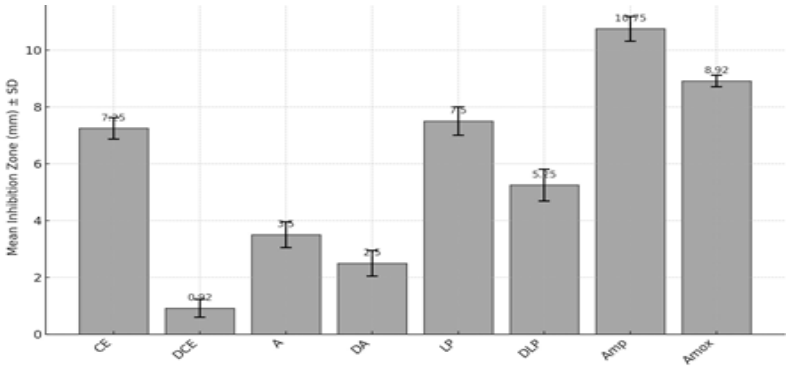

Figure 1: The antimicrobial activity of various extracts against *P. aeruginosa* compared to antibiotics.

CE: Crude extract; DCE :50% Diluted Crude Extract; A: Aloin; DA: 50% Diluted Aloin; LP: Latex Phenols; DLP: 50% Diluted Latex Phenols; Amp: Ampicillin; Amox

This indicates that the raw extract has a substantial antibacterial effect. However, the diluted extracts (50%) exhibited a marked reduction in antibacterial activity, with the raw extract at 50% concentration showing only 0.917±3.175 mm of inhibition, indicating that dilution significantly reduces efficacy. Similarly, pure aloin had moderate activity (3.5±4.503 mm), but its effectiveness decreased when diluted (2.5±4.523 mm), suggesting that aloin is less potent compared to other components. The phenolic component of latex in its pure form displayed considerable antibacterial action, but its efficacy also diminished when diluted. The antibiotic control, however, proved to be the most effective, indicating a higher susceptibility of *P. aeruginosa* to conventional antibiotics compared to *Aloe vera* extracts.

In comparison, other studies have reported similar antibacterial effects of *Aloe vera*. For instance, Danish et al. (2020) found that *Aloe vera* plant extracts possess significant antibacterial activity, supporting the idea that *Aloe vera* has potential therapeutic properties. Pawar et al. (2005) reported that *Aloe vera* leaf gel extracts exhibited antibacterial activity against *Staphylococcus aureus*, indicating a broad spectrum of antibacterial properties, which aligns with the current study's findings against *P. aeruginosa*. Moreover, Zhou et al. (2017) demonstrated that *Aloe vera* could be used for green synthesis of silver nanoparticles, enhancing the antibacterial activity and durability when applied to textiles, showcasing *Aloe vera*'s versatility as an antibacterial agent. Iqbal and Ahmed (2021) also highlighted the antibacterial efficacy of *Aloe barbadensis Mill.* extracts against various pathogens, reinforcing the potential use of *Aloe vera* in antibacterial applications.

The findings suggest that while *Aloe vera* extracts, especially in their pure forms, possess antibacterial properties, they are less potent than conventional antibiotics, which may limit their standalone therapeutic application against *P. aeruginosa*. This could be due to the concentration and presence of bioactive compounds like aloin and phenolic components, which play a critical role in antibacterial efficacy. The diminished effect upon dilution indicates that the concentration of active compounds is essential for optimal antibacterial activity. Further research could explore the synergistic effects of combining *Aloe vera* extracts with other antibacterial agents to enhance efficacy, as suggested by its potential in green synthesis applications, as observed by Zhou et al. (2017).

Staphylococcus aureus

The results demonstrate significant variations in antibacterial activity of different *Aloe vera* extracts and components against *Staphylococcus aureus* (Figure 2).

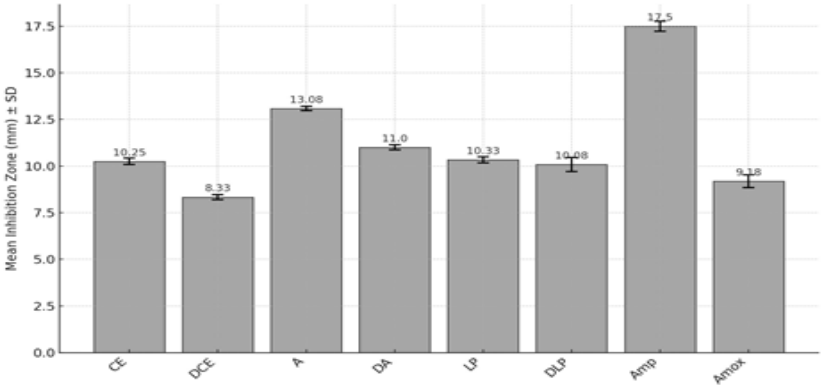

Figure 2: The antimicrobial activity, compared to antibiotics, of various extracts against *Staphylococcus aureus*

CE: Crude extract; DCE :50% Diluted Crude Extract; A: Aloin; DA: 50% Diluted Aloin; LP: Latex Phenols; DLP: 50% Diluted Latex Phenols; Amp: Ampicillin; Amox

Aloin in its pure form exhibited a notable antibacterial effect with an inhibition zone of 13.083±1.165 mm, which is the highest among all *Aloe vera*-derived treatments, indicating that aloin is a key active compound with substantial antibacterial properties. However, it remains less potent compared to the conventional antibiotic, ampicillin/amoxicillin-clavulanic acid, which recorded the highest activity (17.5±2.714 mm). The raw *Aloe vera* extract also displayed considerable activity (10.25±1.685 mm), showing comparable efficacy to the phenolic component of latex pure (10.333±1.671 mm) and its diluted form at 50% (10.083±3.801 mm).

Dilution appeared to significantly reduce the antibacterial effect of *Aloe vera* extracts. For instance, the raw extract at 50% concentration showed a lower inhibition zone (8.333±1.303 mm), and aloin at 50% still exhibited relatively strong activity but decreased to 11±1.348 mm. This trend is consistent with earlier findings against *Pseudomonas aeruginosa* in these results, suggesting a concentration-dependent efficacy of *Aloe vera* extracts against bacterial strains.

These findings align with the results reported by Danish et al. (2020), who demonstrated that *Aloe vera* extracts possess notable antibacterial properties against various pathogens, supporting the potential therapeutic applications of *Aloe vera*. Pawar et al. (2005) also observed significant antibacterial activity of *Aloe vera* leaf gel extracts against *S. aureus*, which corresponds closely with the present observations, indicating the broad-spectrum antibacterial potential of *Aloe vera*. Moreover, Dewi and Marniza (2019) also confirmed the antibacterial activity of *Aloe vera* gel against *S. aureus*, reinforcing the consistency of these results across different research studies. Another study by Ehsani et al. (2013) compared the antibacterial activity of *Aloe vera* to propolis against *Enterococcus faecalis*, which demonstrated *Aloe vera*'s broader antibacterial effectiveness, suggesting that its potential application is not limited to *S. aureus* but may extend to other bacterial species.

In the context of these results, while *Aloe vera* extracts, particularly aloin, exhibit significant antibacterial activity against *S. aureus*, they are still less effective compared to conventional antibiotics, as indicated by the (+++) sensitivity rating of positive control. This suggests that, although *Aloe vera* possesses intrinsic antibacterial compounds, its extracts might not be sufficient as standalone treatments against infections caused by *S. aureus*. However, the substantial activity observed, especially in the pure forms, suggests potential applications of *Aloe vera* extracts as complementary agents to conventional antibiotics, potentially reducing antibiotic resistance issues. The consistent findings across various studies (Danish et al., 2020; Pawar et al., 2005; Dewi & Marniza, 2019; Ehsani et al., 2013) indicate that *Aloe vera* could be a valuable source of antibacterial agents. Further investigation is warranted to explore synergistic effects between *Aloe vera* extracts

and antibiotics or to enhance the antibacterial potency of *Aloe vera* through bioengineering or formulation techniques, as suggested by the growing body of literature on its diverse applications.

CONCLUSION

The present results confirm that *Aloe vera* extracts, particularly the polyphenolic components, exhibit notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with aloin identified as a key bioactive compound. The observed inhibition zones indicate that *Aloe vera* polyphenols, even in diluted forms, hold potential as natural antibacterial agents, offering a promising alternative or complementary approach to synthetic antibiotics.

Specifically, *E. coli* showed sensitivity to 50% diluted aloin (inhibition zone: 11.5 ± 2.355 mm), while *S. aureus* was most sensitive to pure aloin (13.083 ± 1.165 mm), suggesting the extracts' efficacy against both Gram-negative and Gram-positive bacteria. However, *Pseudomonas aeruginosa* demonstrated resistance to all *Aloe vera* extracts, highlighting a need for further investigation into its resistance mechanisms and the factors influencing the efficacy of *Aloe vera* compounds.

Compared to standard antibiotics like ampicillin, which displayed superior inhibition zones, *Aloe vera* extracts exhibited moderate antibacterial activity. This finding underscores their potential in addressing antibiotic resistance, particularly in cases where synthetic antibiotics are less effective. Although ampicillin remains the most potent treatment in this study, the antibacterial properties of Aloe vera, especially aloin, suggest potential integration into treatment regimens, either as complementary agents or in combination with conventional antibiotics to enhance effectiveness and reduce resistance development.

These findings highlight *Aloe vera* polyphenols as promising, sustainable alternatives in combating bacterial infections, especially for *E. coli* and *S. aureus*. Future research should focus on refining extraction methods to improve yield and purity, exploring synergistic effects with antibiotics, and conducting comprehensive in vivo studies to establish the clinical relevance of *Aloe vera* polyphenols in treating bacterial infections.

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