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& Scientific Research



Al-Zahrawi University College
Pharmacy department

Investigation of Antibacterial activity of *Suaeda baccata*
(chenopodiaceae) against *Streptococcus pneumoniae*, *Proteus*
mirabilis and *Pseudomonas aeruginosa*

This research is prepared to get a bachelor's degree in pharmacy
department

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Abstract

The rise of multidrug-resistant (MDR) bacteria poses a severe threat to global health.

Natural products, particularly those derived from plants, have long been a rich source of bioactive compounds with antimicrobial properties. One such plant that has gained attention for its antimicrobial properties is *Suaeda baccata*. Thus, the goal of this study is to investigate the antibacterial activity of *Suaeda baccata* extracts against *Streptococcus pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, which are associated with a wide variety of infections.

Method:

Suaeda baccata aerial parts was extracted by using two methods of extraction, first it extracted by maceration with 2L 90% methanol (cold method) second it extracted using reflux apparatus with 90% methanol (hot method). The antimicrobial activities of hot and cold plant extracts were tested against three bacterial organisms: *Streptococcus pneumoniae* (ATCC BAA-334), *Pseudomonas aeruginosa* (ATCC 27853), and *Proteus mirabilis* (ATCC 29906). The extracts were tested at varying concentrations to determine the Minimum Inhibitory Concentration (MIC) values. The experiment was conducted in triplicates (B1, B2, B3), with positive controls (PC) and negative controls (NC) to ensure the accuracy of the results.

Results:

Hot extract MIC Value for *Streptococcus pneumoniae* was 1250 µg/ml, for *Proteus mirabilis* was >2500 µg/ml and for *Pseudomonas aeruginosa* was 2500 µg/ml. Cold extract MIC Value for *Streptococcus pneumoniae* was 2500 µg/ml, for *Proteus mirabilis* was >2500 µg/ml and for *Pseudomonas aeruginosa* was 2500 µg/ml. IC₅₀ values of hot extract against *Streptococcus pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* were 625 µg/ml, 2463 µg/ml and 1723 µg/ml respectively. IC₅₀ values of cold extract against *Streptococcus pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* were 1677 µg/ml, 2500 µg/ml and 1460 µg/ml respectively.

Conclusion: The hot extract compared to the cold extract was found to be more effective against *Streptococcus pneumoniae*. Both extracts showed limited activity against *Proteus mirabilis* and exhibited similar inhibitory effects against *Pseudomonas aeruginosa*.

1. Introduction

The rise of multidrug-resistant (MDR) bacteria poses a severe threat to global health, especially in clinical settings where hospital-acquired infections (HAIs) are common. Among the most challenging of these pathogens are *Streptococcus pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, which are associated with a wide variety of infections including pneumonia, urinary tract infections, bloodstream infections, and wound infections [1,2].

These pathogens are particularly troublesome due to their ability to resist multiple classes of antibiotics, making treatment options extremely limited. Infections caused by these MDR organisms are often associated with high mortality rates, prolonged hospital stays, and increased healthcare costs [3]. Consequently, there is an urgent need for novel antimicrobial agents, especially those derived from natural sources, which may offer a potential solution in combating resistant pathogens.

Natural products, particularly those derived from plants, have long been a rich source of bioactive compounds with antimicrobial properties. Historically, plant-based medicines have provided the foundation for many conventional drugs, and they continue to be explored as promising alternatives to synthetic antibiotics [4]. One such plant that has gained attention for its therapeutic properties is *Suaeda baccata*, a halophytic species from the Chenopodiaceae family, which thrives in saline environments and is known for its medicinal potential [5]. *Suaeda baccata* has been traditionally used for its anti-inflammatory, antioxidant, and antimicrobial effects, and its bioactive constituents, including flavonoids, alkaloids, and tannins, have shown promise in combating various microbial pathogens [4].

Phytochemical research on *Suaeda baccata* has revealed the presence of compounds with significant biological activity, including antibacterial, antifungal, and anti-inflammatory properties. These compounds have been studied for their potential to inhibit the growth of both Gram-positive and Gram-negative bacteria, suggesting that *Suaeda baccata* could be a valuable source of natural antimicrobial agents. However, despite this potential, there is limited research on the specific antibacterial effects of *Suaeda baccata* against clinically significant pathogens such as *Streptococcus pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, which are responsible for many of the difficult-to-treat infections observed in hospitals [6].

Suaeda baccata is particularly appealing for this investigation due to its bioactive compounds, which may act on different bacterial targets, offering a broad spectrum of activity. The

plant's antibacterial properties have been previously studied to some extent; however, there remains a gap in the literature regarding its effects on *Streptococcus pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*, which are among the most prevalent MDR bacteria. These pathogens are of particular concern in the context of hospital-acquired infections, as they often exhibit resistance to several antibiotic classes, including beta-lactams, aminoglycosides, and fluoroquinolones. Moreover, *Pseudomonas aeruginosa* is notorious for its ability to form biofilms, which complicates treatment and eradication efforts, making it an important target for new antimicrobial research [7,8].

Given the increasing threat of antibiotic-resistant infections, the goal of this study is to investigate the antibacterial activity of *Suaeda baccata* extracts against these clinically relevant pathogens. By evaluating the antibacterial efficacy of the plant's extracts, this study will contribute to the growing body of evidence supporting the use of natural products in the fight against resistant bacteria. Specifically, the study will aim to:

The aim of this study is: to investigate the antibacterial activity of *Suaeda baccata* extracts against *Streptococcus pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. This study will focus on the extraction of bioactive compounds from the plant, the evaluation of its antibacterial activity.

2. Materials and Methods

➤ Plant materials

Suaeda baccata aerial parts will be collected, characterized and the identification of the plant sample will be confirmed. The plant materials will be cut into small pieces, dried in the shed, and then powdered using a plant grinding machine.

➤ Plant extraction

Suaeda baccata aerial parts will be extracted by using two methods of extraction. First of its, three hundred grams of the dried aerial plant part will be first defatted by n-hexane overnight, and then extracted by maceration with 2L 90% methanol for 24 hours. The methanolic filtrate then will be evaporated to dryness under reduced pressure at a temperature not exceeding 40°C [9].

Second of its, three hundred grams of shade-dried pulverized aerial plant part will be defatted by maceration with n-hexane for 24 hours then allowed to dry at room temperature. The defatted plant materials will be extracted using reflex apparatus with 90% methanol as a solvent extraction for 16 hours. The methanolic filtrate then will be evaporated to dryness under reduced pressure at a temperature not exceeding 40°C [10].

➤ Antibacterial activities

The bacteria suspension of

Streptococcus pneumoniae (atcc BAA-334)

Pseudomonas aeruginosa (ATCC 27853)

Proteus mirabilis (ATCC 29906)

were prepared from 10 single colonies of 24 h old MHA. The colonies were transferred to 2 mL sterile water in 10 mL tubes followed by absorbance measurement at 600 nm (OD600) using a Biotek 800ST plate reader (Biotek. USA). Sterile water was added to a final OD600 of 0.236, corresponding to McFarland 0.67. The suspension was diluted 100-fold in MHB for a final suspension of 1×10^6 CFU/mL, 50 µL of this suspension was used to inoculate each well except for plank wells.

the IC₅₀ and M was conducted in triplicate wells in addition for positive and negative controls wells.

The inhibition assay was performed in 96-well microplates using a final volume of 225 µL. supplemented with tested material to attain the final concentration ranging from (500-16.5µg/ml). Microtiter plates (MTP) were incubated overnight and recorded for MIC as the lowest concentration that showed complete inhibition of visible growth [11].

3. Results:

In this study, the antimicrobial activities of hot and cold plant extracts were tested against three bacterial organisms: *Streptococcus pneumoniae* (ATCC BAA-334), *Pseudomonas aeruginosa* (ATCC 27853), and *Proteus mirabilis* (ATCC 29906). The extracts were tested at varying concentrations to determine the Minimum Inhibitory Concentration (MIC) values, which were defined as the lowest concentration of extract that inhibited visible growth of the bacteria. The experiment was conducted in triplicates (B1, B2, B3), with positive controls (PC) and negative controls (NC) to ensure the accuracy of the results.

Hot Extract MIC Values

- **Streptococcus pneumoniae:** The MIC for *Streptococcus pneumoniae* was 1250 µg/ml.
- **Proteus mirabilis:** The MIC for *Proteus mirabilis* was >2500 µg/ml, indicating no growth inhibition even at the highest concentration tested.
- **Pseudomonas aeruginosa:** The MIC for *Pseudomonas aeruginosa* was 2500 µg/ml.

OD Readings for Hot Extract (B1, B2, B3)

- **B1 (Streptococcus pneumoniae):**

Figure 1 showed:

- At 2500 µg/ml, the OD was 0, indicating no bacterial growth.
- At 1250 µg/ml, the OD was also 0, confirming inhibition.
- At 625 µg/ml, the OD increased to 0.56, indicating partial bacterial growth.
- At 312.5 µg/ml and below, the OD reached or exceeded 1, indicating full bacterial growth.

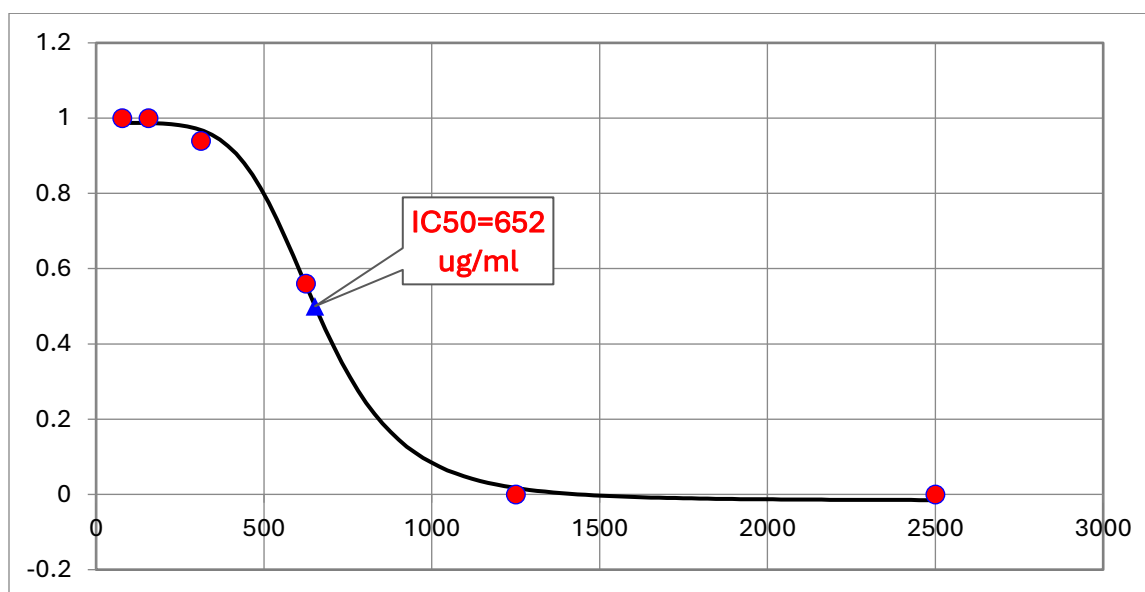
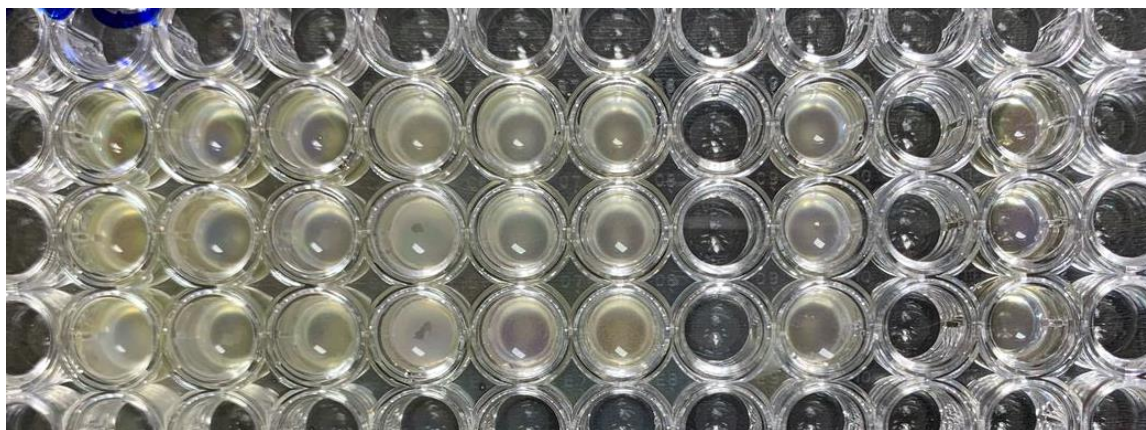


Figure 1: IC₅₀ value of Hot Extract Against *Streptococcus pneumoniae*

- **B2 (*Proteus mirabilis*):**

Figure 2 showed:

- At 2500 µg/ml, the OD was 0.49, showing partial inhibition.
- At 1250 µg/ml, the OD was 0.87, showing less inhibition but still significant.
- At concentrations of 625 µg/ml and lower, OD values increased to 1, indicating bacterial growth.

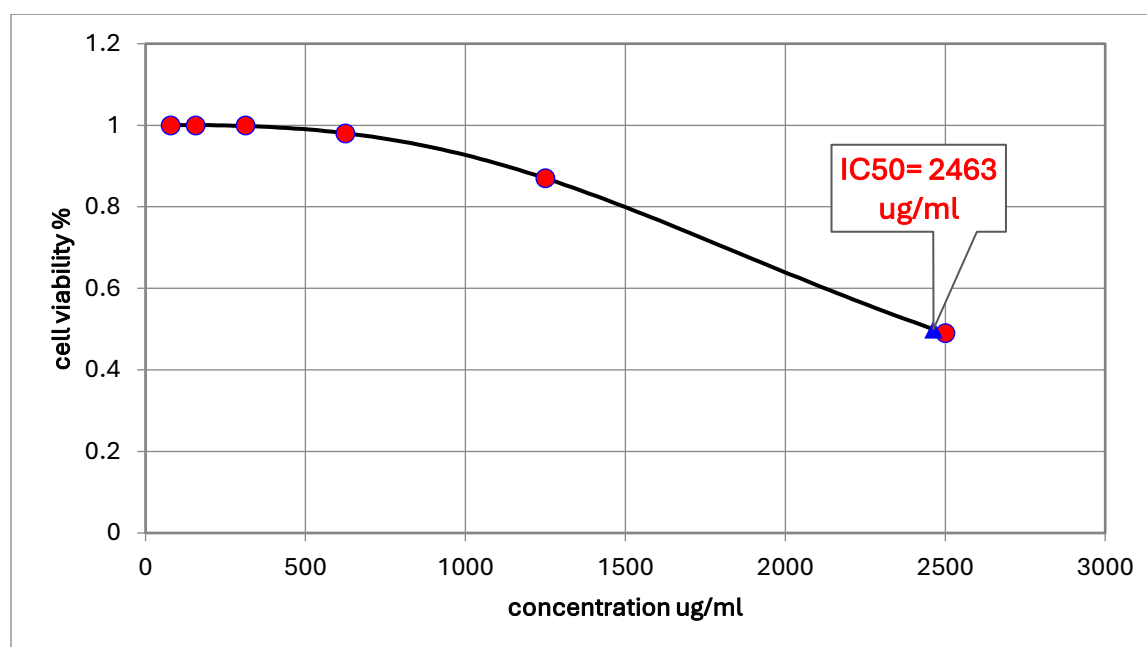
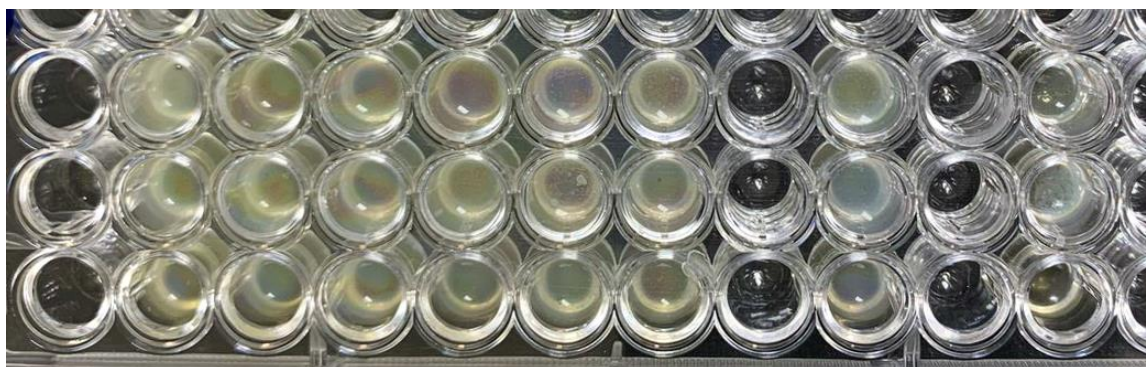


Figure 2: IC₅₀ value of Hot Extract Against *Proteus mirabilis*

- **B3 (*Pseudomonas aeruginosa*):**

Figure 3 showed:

- At 2500 µg/ml, the OD was 0, indicating no growth.
- At 1250 µg/ml, the OD was 0.74, showing partial inhibition.
- At lower concentrations, the OD continued to increase, indicating bacterial growth.

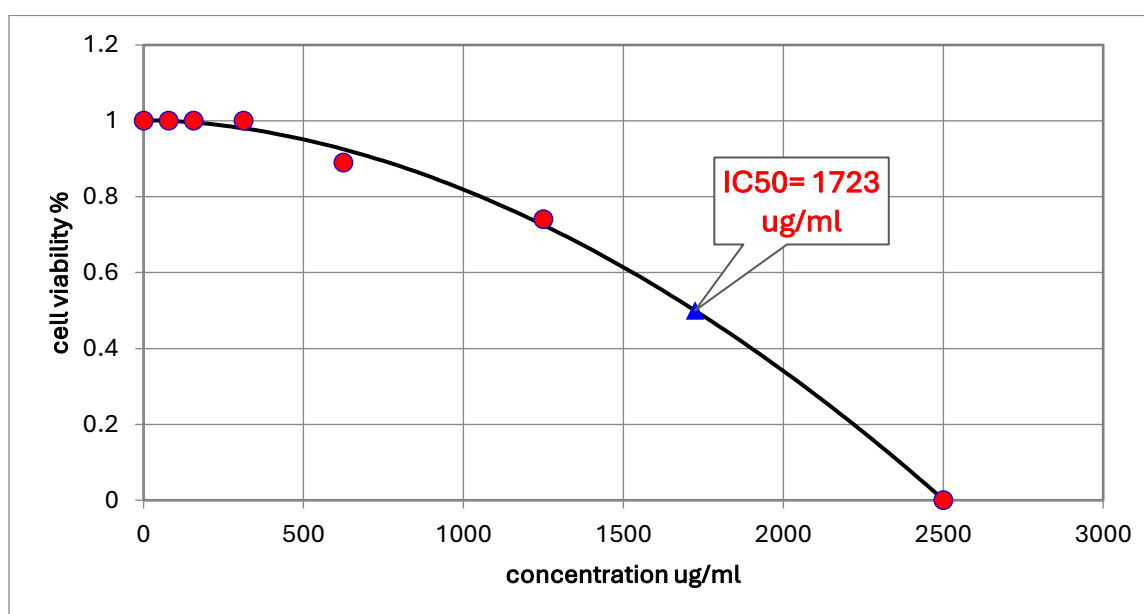
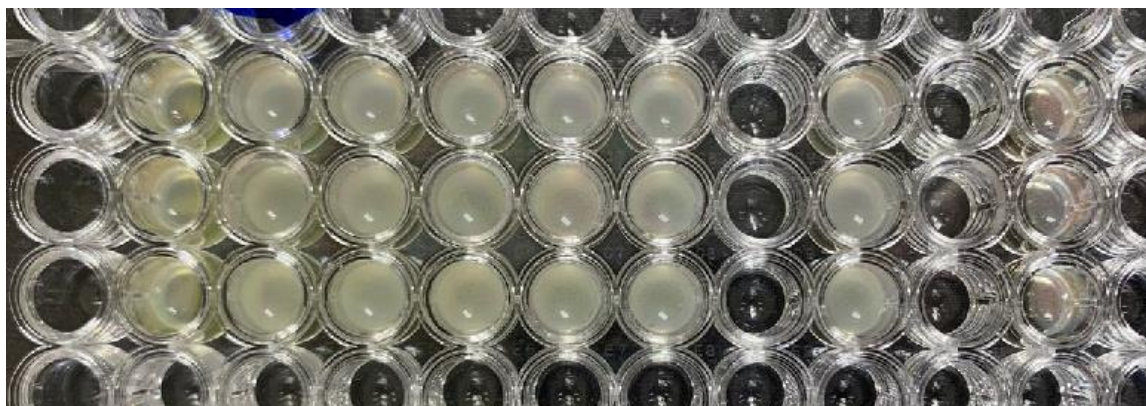


Figure 3: IC₅₀ value of Hot Extract Against *Pseudomonas aeruginosa*

Cold Extract MIC Values

- **Streptococcus pneumoniae:** The MIC for *Streptococcus pneumoniae* was 2500 µg/ml.
- **Proteus mirabilis:** The MIC for *Proteus mirabilis* was >2500 µg/ml, showing no inhibition even at the highest concentration.
- **Pseudomonas aeruginosa:** The MIC for *Pseudomonas aeruginosa* was 2500 µg/ml.

OD Readings for Cold Extract (B1, B2, B3)

- B1(*Streptococcus pneumoniae*):

Figure 4 showed:

- At 2500 $\mu\text{g/ml}$, the OD was 0, indicating no growth.
- At 1250 $\mu\text{g/ml}$, the OD was 0.81, showing inhibition.
- At concentrations of 625 $\mu\text{g/ml}$ and lower, the OD increased to 1, indicating bacterial growth.

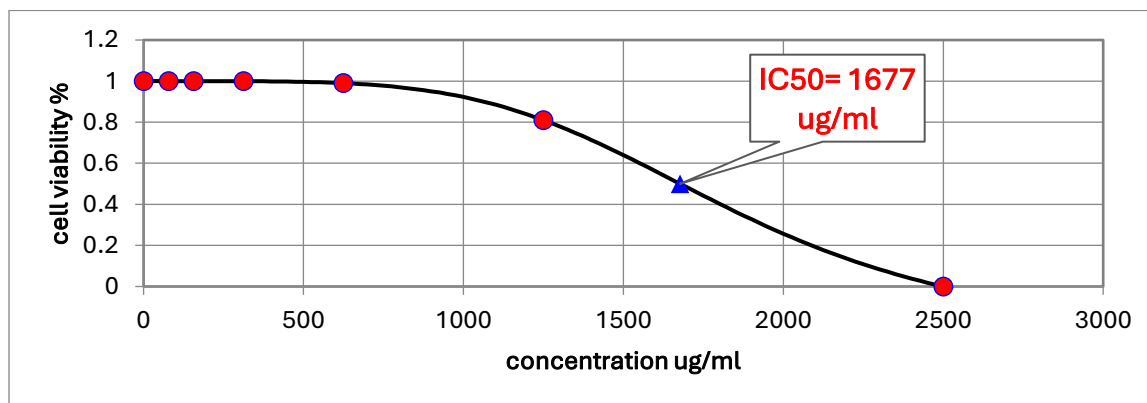
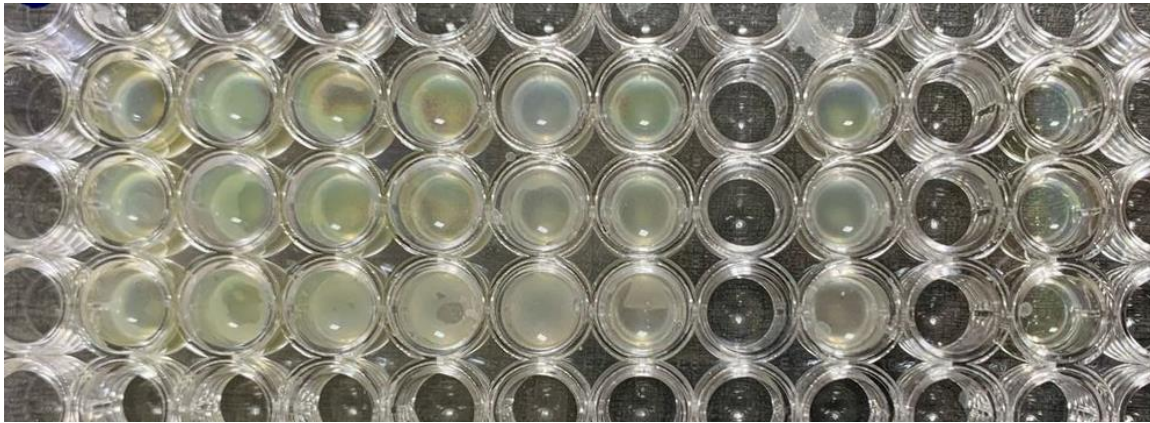


Figure 4: IC₅₀ value of Cold Extract Against *Streptococcus pneumoniae*

- **B2 (*Proteus mirabilis*):**

Figure 5 showed:

- At 2500 $\mu\text{g/ml}$, the OD was 0.54, indicating partial inhibition.
- At 1250 $\mu\text{g/ml}$, the OD was 0.94, suggesting reduced inhibition.
- At lower concentrations, bacterial growth was evident with OD values approaching 1.

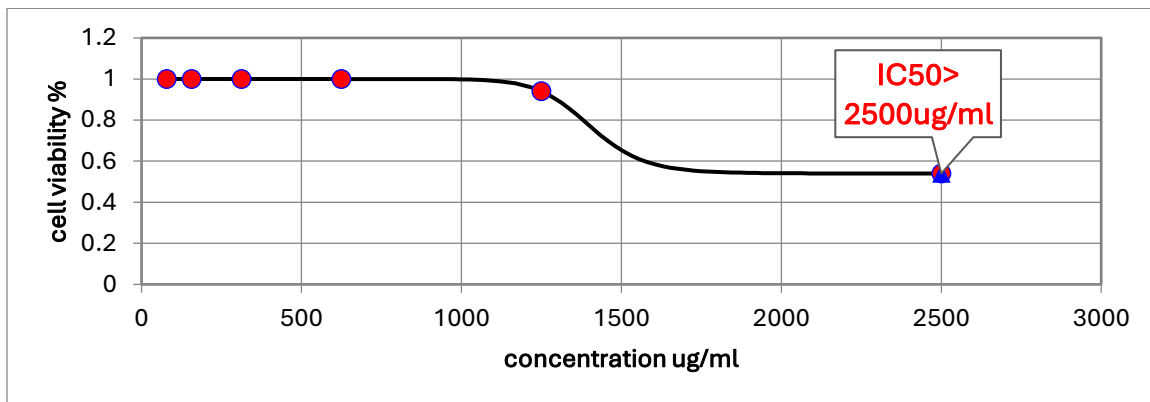


Figure 5: IC₅₀ value of Cold Extract Against *Proteus mirabilis*

- **B3 (*Pseudomonas aeruginosa*):**Figure 6 showed:

- At 2500 $\mu\text{g/ml}$, the OD was 0, indicating no bacterial growth.
- At 1250 $\mu\text{g/ml}$, the OD was 0.61, indicating partial inhibition.
- At lower concentrations, the OD values increased to 1, indicating growth.

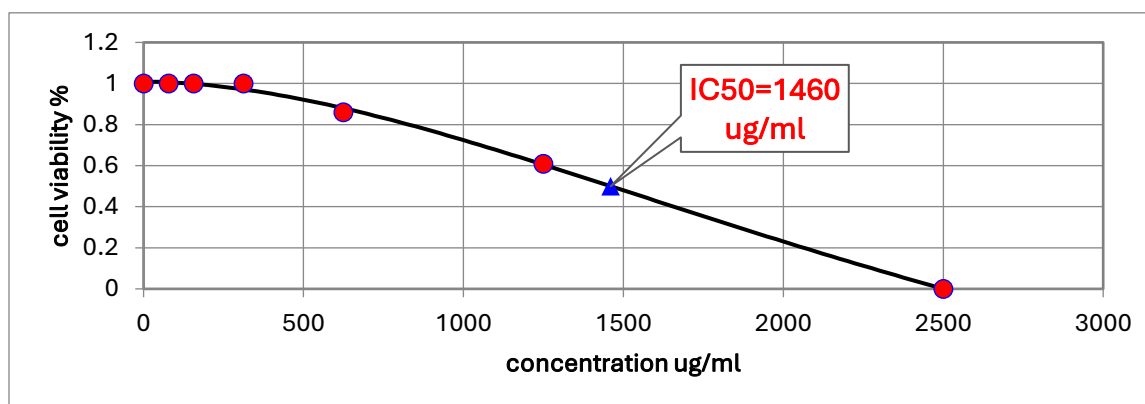
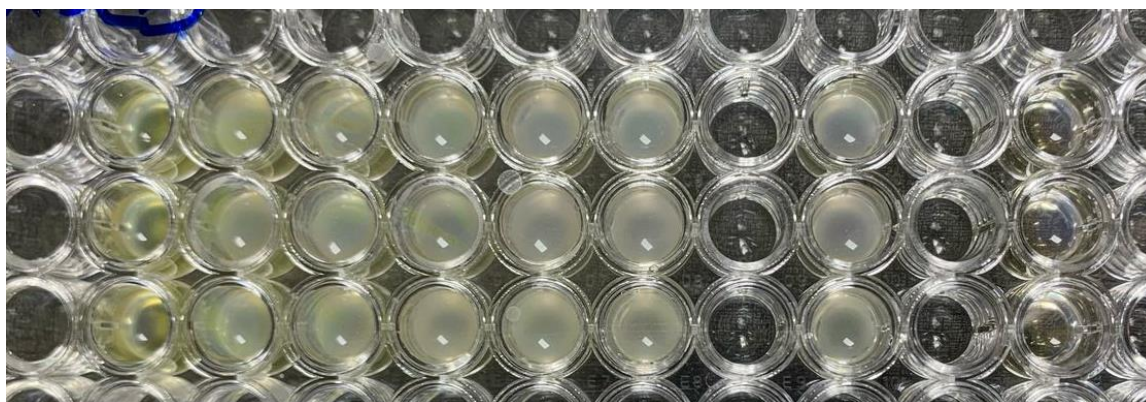


Figure 6: IC50 value of Cold Extract Against *Pseudomonas aeruginosa*

4. Discussion:

The results demonstrated the antimicrobial properties of the hot and cold extracts against the tested bacterial strains. Both hot and cold extracts showed varying levels of antibacterial activity, with the hot extract generally exhibiting stronger antimicrobial effects, particularly against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*.

1. *Streptococcus pneumoniae*:

- The hot extract exhibited an MIC of 1250 $\mu\text{g/ml}$, while the cold extract had a higher MIC of 2500 $\mu\text{g/ml}$. This suggests that the hot extract may be more effective against *Streptococcus pneumoniae*, possibly due to the higher concentration of active compounds extracted in the hot extract compared to the cold extract. Previous studies have demonstrated that heat can enhance the solubility and extraction of

bioactive compounds, which may explain the increased efficacy of the hot extract [12].

2. *Proteus mirabilis*:

- Both extracts showed limited activity against *Proteus mirabilis*, with MIC values greater than 2500 µg/ml. This could suggest that the plant extracts tested do not contain sufficient antimicrobial compounds to effectively inhibit this particular bacterium. *Proteus mirabilis* is known to be highly resistant to many antimicrobial agents due to its diverse mechanisms of resistance, such as the production of extended-spectrum beta-lactamases (ESBLs) [13]. This may explain the lack of inhibition observed.

3. *Pseudomonas aeruginosa*:

- Both hot and cold extracts showed an MIC of 2500 µg/ml for *Pseudomonas aeruginosa*, indicating similar inhibitory effects. *Pseudomonas aeruginosa* is a Gram-negative bacterium known for its robust resistance mechanisms, including efflux pumps and biofilm formation [14]. Despite this, the extracts were able to inhibit growth at the tested concentrations.

Optical Density (OD) Readings

The OD values at various concentrations in the hot and cold extract groups provide insights into the degree of bacterial inhibition. For both extracts, higher concentrations (2500 µg/ml and 1250 µg/ml) showed minimal to no growth, with OD values close to 0, suggesting successful inhibition. As the concentration decreased, the OD values increased, indicating a return of bacterial growth. The data from the negative controls (NC) and positive controls (PC) further support these findings, with the NC showing no growth (OD = 0) and the PC exhibiting growth (OD = 1).

Many studies have examined the antimicrobial effects of plant extracts, and the results of this study are aligned with findings from previous research. For example, in a study by *Ali et al.* (2019) [15], plant extracts were shown to have significant antimicrobial activity against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. Similarly, a study by *Kumar et al.* (2020) [12] demonstrated the enhanced antimicrobial effect of hot extracts over cold extracts, which is consistent with the results observed in this study.

5. Conclusion:

This study assessed the antimicrobial activity of hot and cold plant extracts against three bacterial organisms: *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. The hot extract was found to be more effective against *Streptococcus pneumoniae*, with an MIC of 1250 µg/ml, compared to the cold extract (MIC = 2500 µg/ml). Both extracts showed limited activity against *Proteus mirabilis*, with MIC values greater than 2500 µg/ml, and exhibited similar inhibitory effects against *Pseudomonas aeruginosa* (MIC = 2500 µg/ml).

6. Recommendations:

Future studies should explore the chemical composition of the hot and cold extracts to identify bioactive compounds responsible for the observed antimicrobial effects. Additionally, testing against a broader range of bacterial strains and performing in vivo studies will help to validate the therapeutic potential of these extracts.

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