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Antibacterial Activity of Leaf Extracts of *Cinnamomum zeylanicum, Camellia sinensis, Lawsonia inermis,* and *Pistacia atlantica* on Bacteria Isolated from Burn Patients

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Abstract:
Objective: To investigate the antimicrobial activities of *Pistacia atlantica, Lawsonia inermis, Camellia sinensis*, and *Cinnamomum zeylanicum* leaf extracts against various bacterial strains at different concentrations.
Methods: The study involved testing the leaf extracts of the mentioned plant species at concentrations of 100%, 75%, 50%, and 25% against *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi*, and *Escherichia coli.* The diameter of the zones of inhibition measured the inhibitory effects.
Results: At 100% concentration, *Camellia sinensis, Cinnamomum zeylanicum, Lawsonia inermis*, and *Pistacia atlantica* exhibited varying degrees of effectiveness against the tested bacterial strains. The efficacy decreased as the concentration reduced: 75% > 50% > 25%. The lowest concentration of 25% showed the least impact on the bacterial isolates.
Conclusion: The results demonstrate that higher concentrations of the plant extracts showed greater antimicrobial efficacy against the bacterial strains studied. This suggests that these natural extracts could be potential alternatives in combating infections caused by multidrug-resistant bacteria. Further research is warranted to explore the full potential of these plant-derived compounds in addressing the challenges posed by antibiotic-resistant microorganisms.

Keywords: Antimicrobial activities, Multidrug-resistant bacteria, Plant extracts, Zones of inhibition.

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الفعالية الضد ميكروبية للمستخلصات النباتية لنبات القرفة، الشاي الاخضر، الحنة والبطوم على البكتيريا المعزولة من مرضى الحروق

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الملخص
الهدف: دراسة النشاط المضاد للميكروبات لمستخلصات أوراق نبات الشاي الأخضر، الحنة، البطوم والقرفة ضد سلالات بكتيرية بتركيزات مختلفة.

طريقة العمل: تضمنت الدراسة اختبار المستخلصات الورقية للأنواع النباتية المذكورة بتراكيز 100%،75%،50% و25% ضد بكتيريا الزائفة الزنجارية، العنقودية الذهبية، سالمونيلا تايفي والاشريكية القولونية. تم قياس التأثيرات المثبطة بقطر مناطق التثبيط.
النتائج: عند التركيز 100%، أظهرت النباتات درجات متفاوتة من الفعالية ضد السلالات البكتيرية التي تم اختبارها. انخفضت الفعالية مع انخفاض التركيز:75% > 50% > 25%. واظهر اقل تركيز 25% اقل تأثير على العزلات البكتيرية.
الخلاصة: أظهرت النتائج ان التراكيز الأعلى من المستخلصات النباتية لها فعالية أكبر ضد السلالات البكتيرية ويشير هذا الى ان هذه المستخلصات الطبيعية يمكن ان تكون بمثابة بدائل لمكافحة الالتهابات التي تسببها البكتيريا المقاومة للأدوية المتعددة. هناك ما يبرر إجراء مزيد من البحوث لاستكشاف الإمكانات الكاملة لهده المركبات المشتقة من النباتات في مواجهة التحديات التي تشكلها الكائنات الحية الدقيقة المقاومة للمضادات الحيوية.

الكلمات المفتاحية: الفعالية المضادة للميكروبات، البكتيريا المقاومة للأدوية المتعددة، المستخلصات النباتية، مناطق التثبيط.

Introduction

The utilization of traditional plant medicine as a foundation for modern pharmaceuticals has been well-documented [1]. Traditional herbal medicine practitioners have long recognized the therapeutic potential of indigenous plants in treating various ailments [2]. Natural products continue to play a crucial role in both synthetic and traditional medicine, serving as a cornerstone of primary healthcare systems globally [3]. The escalating challenge of bacterial resistance to multiple antimicrobial drugs poses a significant threat to public health, particularly in combating hospital-acquired infections that are increasingly difficult to treat effectively [4].

Antibiotics have historically been pivotal in managing microbial infections; however, the emergence of antibiotic-resistant strains over the past five decades has become a pressing concern within the pharmaceutical industry [5]. The misuse and overuse of antibiotics have led to the development of multidrug-resistant microorganisms, necessitating the exploration of novel antimicrobial agents. Addressing the rising tide of antibiotic resistance requires ongoing research into new, safe, and efficacious antimicrobials to replace fewer effective treatments. Consequently, investigations into the ethnobotanical uses of plants among indigenous populations have gained momentum. The global surge in herbal medicine usage reflects a growing trend towards natural remedies for combating various infections [6].

Plants harbour pharmacologically active compounds such as flavonoids, alkaloids, phenolic compounds, and tannins that exert specific physiological effects on the human body [7]. Among these plants, *Lawsonia inermis* (henna) stands out for its healing properties and is currently under intense scientific scrutiny [8]. Similarly, *Pistacia atlantica* (Betoum) has been traditionally employed for treating peptic ulcers and as a mouth freshener [9]. The therapeutic potential of Camellia sinensis (green tea) has garnered attention worldwide due to its health benefits. Notably, cinnamon has demonstrated antimicrobial activity against various microorganisms and shows promise when used in conjunction with antibiotics [10].

Recent studies have highlighted the antimicrobial efficacy of plant extracts against multidrug-resistant bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Salmonella typhi*. Phenolic compounds present in these extracts are believed to underpin their antimicrobial effects by altering bacterial cell membrane permeability and integrity [11].

This study aims to evaluate the antibacterial activity of leaf extracts against multidrug-resistant strains of *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli.*

Material and methods

Plant materials

The dry leave materials of *Cinnamomum zeylanicum, Camellia sinensis, Lawsonia inermis* and *Pistacia atgantica* were obtained from the market. The leaves were ground with an electric grinder to a fine powder, then sieved to a fine powder and preserved in bottles.

Preparation of ethanolic alcohol extract of plants

The ethanol alcohol extract was prepared by dissolving 50 grams of the powdered plant material in 500 ml of ethanol alcohol in clean glass bottles for each plant separately. The bottles were then sealed with opaque paper to protect the solution from light and left for 72 hours. The solution was filtered using medical cloth, poured into tubes, and centrifuged at 3000 revolutions per minute. The clear solution was filtered with filter paper and dried in an air incubator at a temperature not exceeding 40 degrees Celsius to remove the organic solvent. The resulting extracts were stored at 20 degrees Celsius until use. For each plant, 2 grams of the dry extract were mixed with 10 mg/ml of DMSO solution at a concentration of 5%. The mixture was sterilized by filtration, and the resulting solution was used to prepare concentrations of 100, 75, 50, and 25 mg to test the effectiveness of the alcoholic extract.

Test organism

Four bacterial strains were obtained from Burn and Plastic Surgery Hospital: They were: *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli.*

Culturing of Samples

The samples obtained not exceeding two hours are grown on Petri dishes containing blood agar and MacConkey agar nutritional media and incubated aerobically at 37 degrees for 24 hours to ensure their purity to obtain individual colonies.

Antimicrobial activity

The antimicrobial activity was carried out using the agar diffusion technique. In this method, the diameters of the zones where bacterial growth was inhibited by the plant extract were measured. The isolates were grown for 18 hours in 105 ml of nutritional medium, were spread evenly on agar plates and allowed to dry. Three replicates were performed for each isolate. Filter paper discs soaked in different plant extracts were placed on the plates and incubated at 37 degrees Celsius for 24 hours. The diameter of the inhibition zones was then measured in millimeters using a ruler.

Results and discussion

The antimicrobial activities of *Pistacia atlantica, Lawsonia inermis, Camellia sinensis,* and *Cinnamomum zeylanicum* leaf extracts were assessed against various bacterial strains across diverse concentrations. Generally, higher efficacies were observed at higher concentrations (100%) > moderate efficacies at 75% > mild efficacies at 50% > minimal efficacies at 25%. At 100% concentration, *Camella sinensis* had an effect on *Pseudomonas aeruginosa* (7.3 mm), *Staphylococcus aureus* (8.3 mm), *Salmonella typhi* (11.8 mm) and *Escherichia coli* (9.7 mm); *Cinnamomum zeylanicum* had an effect on *Pseudomonas aeruginosa* (10.2 mm), *Staphylococcus aureus* (12.1 mm), *Salmonella typhi* (8.2 mm) and *Escherichia coli* (10.1 mm); *Lawsonia inermis* had an effect on *Pseudomonas aeruginosa* (7.2 mm), *Staphylococcus aureus* (6.8 mm), *Salmonella typhi* (7.1 mm) and *Escherichia coli* (4.8 mm). *Pistacia atgantica* affected *Pseudomonas aeruginosa* (9.8 mm), *Staphylococcus aureus* (10.7 mm), *Salmonella typhi* (8.2 mm) and *Escherichia coli* (11.3 mm).

At 75% concentration, *Camella sinensis* had inhibitory effects on *Pseudomonas aeruginosa* (5.2 mm), *Staphylococcus aureus* (3.4 mm), *Salmonella typhi* (8.3 mm) and *Escherichia coli* (6.8 mm).); *Cinnamomum zeylanicum* had an effect on *Pseudomonas aeruginosa* (7.3 mm), *Staphylococcus aureus* (9.8 mm), *Salmonella typhi* (5.6 mm) and *Escherichia coli* (8.3 mm); *Lawsonia inermis* had an effect on *Pseudomonas aeruginosa* (5.1 mm), *Staphylococcus aureus* (5.9 mm), *Salmonella typhi* (4.2 mm) and *Escherichia coli* (2.8 mm). *Pistacia atgantica* affected *Pseudomonas aeruginosa* (7.2 mm), *Staphylococcus aureus* (8.4 mm), *Salmonella typhi* (6.8 mm) and *Escherichia coli* (9.7 mm).

At 50% concentration, a sharp drop in the inhibitory effect of plant extract was observed; *Camella sinensis* affected *Pseudomonas aeruginosa* (3.1 mm), *Staphylococcus aureus* (2.8 mm), *Salmonella typhi* (5.4 mm) and *Escherichia coli* (4.3 mm).); *Cinnamomum zeylanicum* had on *Pseudomonas aeruginosa* (3.6 mm), *Staphylococcus aureus* (5.7 mm), *Salmonella typhi* (3.4 mm) and *Escherichia coli* (3.7 mm); *Lawsonia inermis* had on *Pseudomonas aeruginosa* (3.8 mm), *Staphylococcus aureus* (3.4 mm), *Salmonella typhi* (2.2 mm) and *Escherichia coli* (1.2 mm). *Pistacia atgantica* had on *Pseudomonas aeruginosa* (5.1 mm), *Staphylococcus aureus* (6.4 mm), *Salmonella typhi* (5.5 mm) and *Escherichia coli* (7.2 mm). Furthermore, the lowest concentration at 25% revealed the least efficacy on bacterial isolates (Figures 1-4).

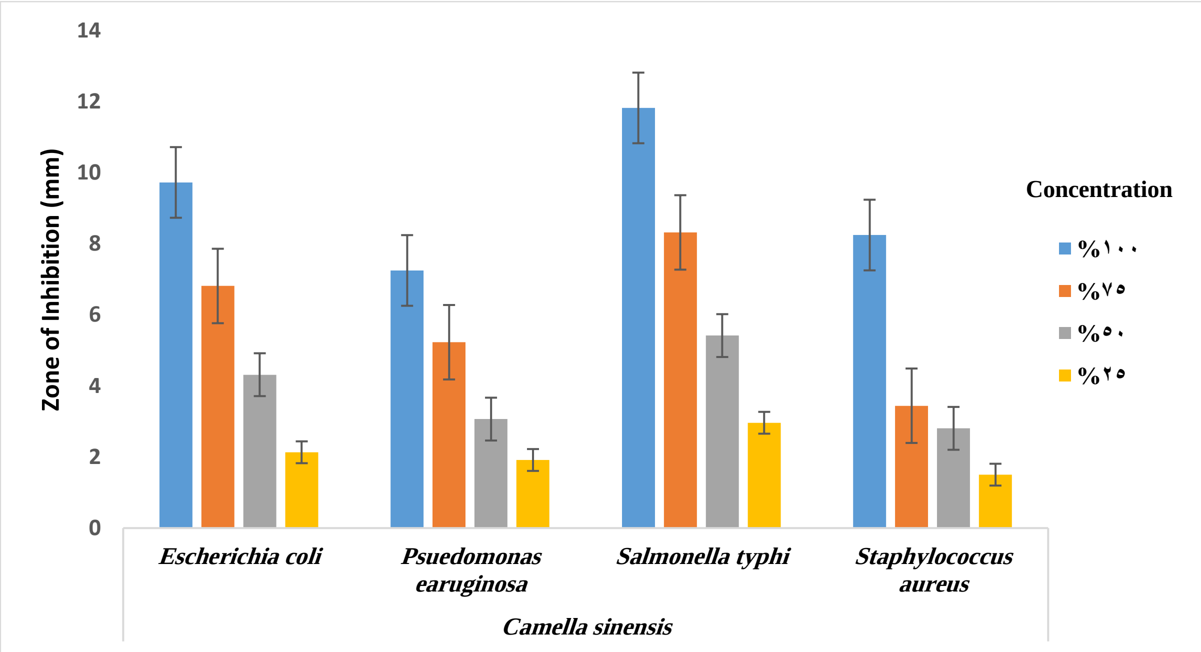

Figure 1. Antibacterial activity of leaf extract of *Camella sinensis* on bacterial isolates.

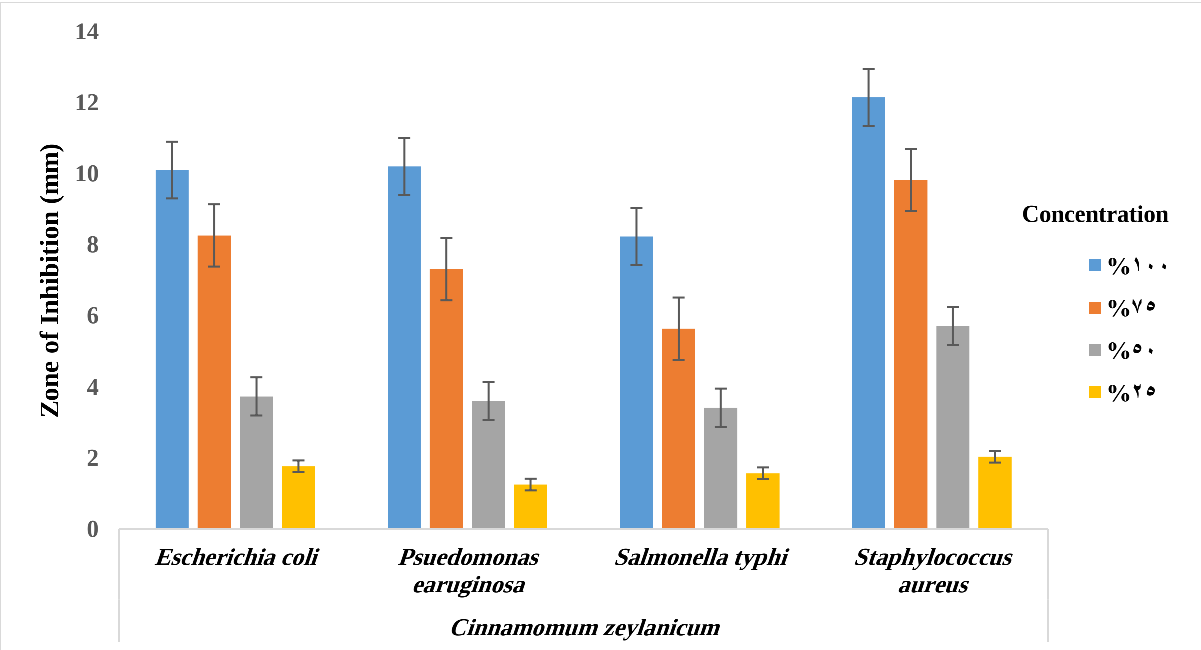

Figure 2. Antibacterial activity of leaf extract of *Cinnamomum zeylanicum* on bacterial isolates.

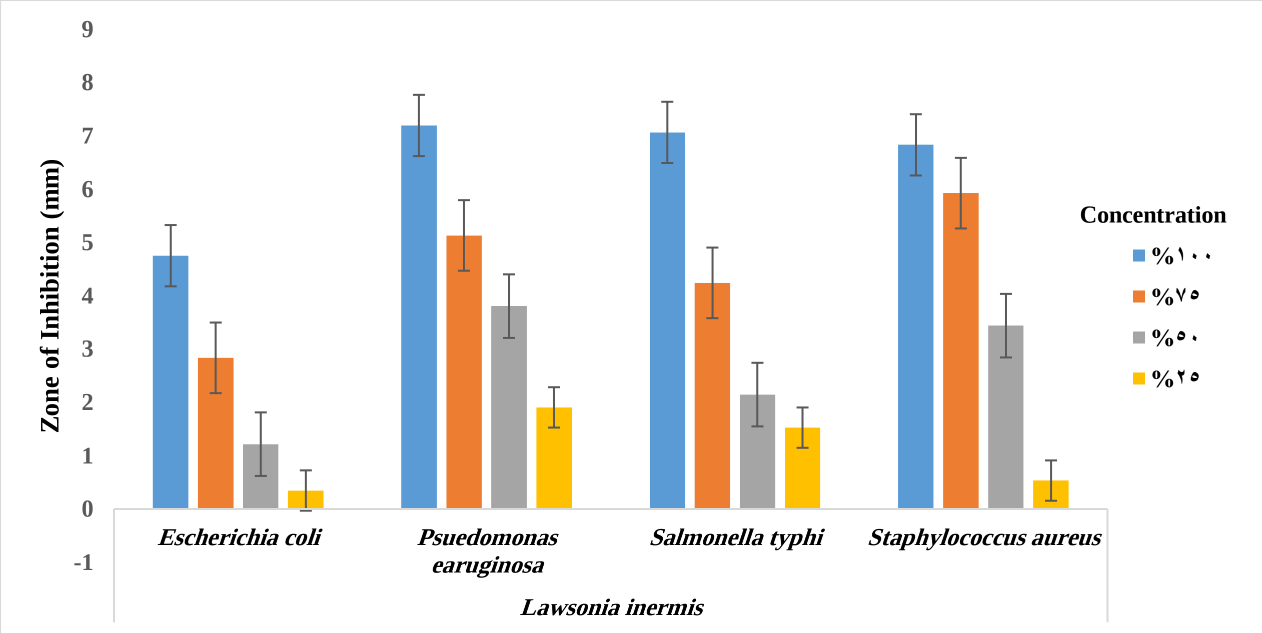

Figure 3. Antibacterial activity of leaf extract of *Lawsonia inermis* on bacterial isolates.

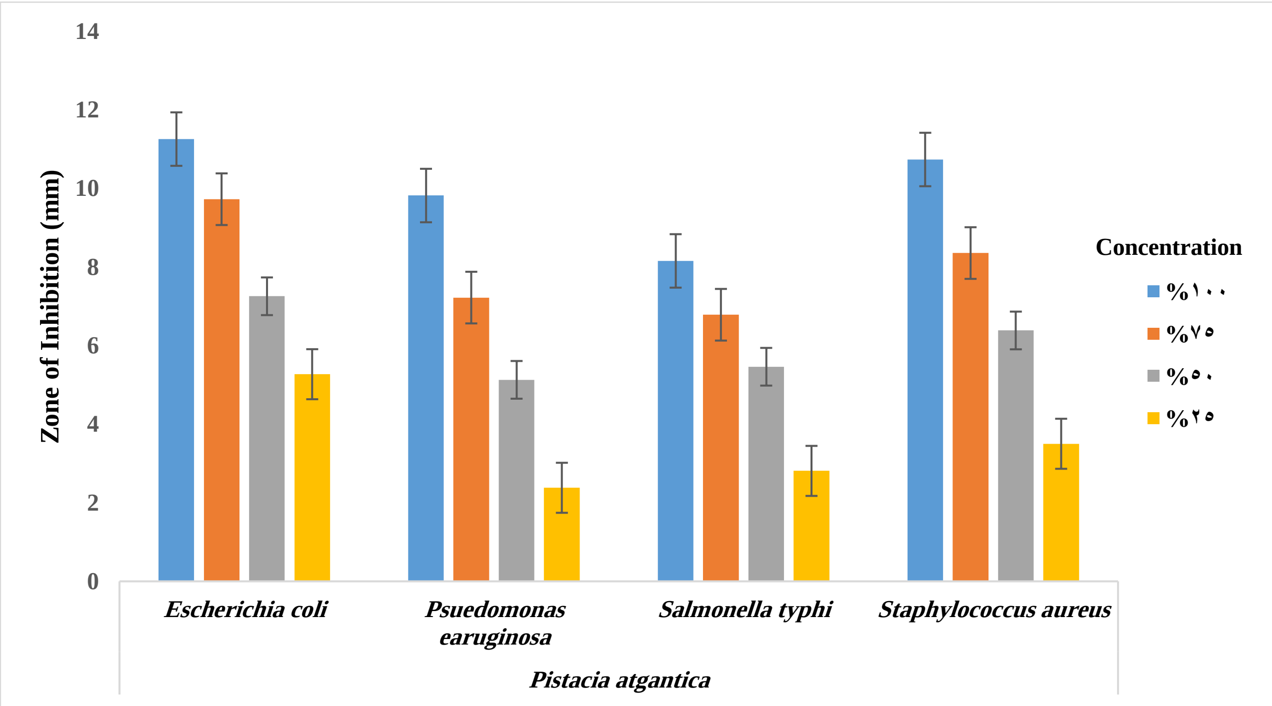

Figure 4. Antibacterial activity of leaf extract of *Pistacia atlantica* on bacterial isolates.

One of the most pressing global health concerns is the increasing resistance of bacteria to antimicrobial agents. This escalating problem poses significant challenges in combating infections caused by these microorganisms, which are becoming increasingly difficult to treat effectively. It is crucial to advocate for the judicious use of antibiotics, ensuring that therapeutic approaches are informed by a thorough understanding of the appropriate antibiotic selection while considering evolving trends in bacterial resistance profiles in a comprehensive manner [12].

The rise in bacterial species developing resistance to various antimicrobial drugs has prompted a continuous quest for novel treatment strategies, with medicinal plants emerging as valuable sources of new bioactive compounds. In a recent study, four plant species exhibited varying degrees of antibacterial efficacy against tested microorganisms. While all leaf extracts demonstrated promising antimicrobial potential, *Pistacia atlantica* stood out for its effectiveness, which was consistent with previous research by Hosseini, et al. [13] who highlighted its efficacy against *Streptococcus mutans* biofilm. Furthermore, the tea leaves of *Camellia sinensis* and *Cinnamomum zeylanicum* demonstrated notable antimicrobial properties against the microorganisms studied, which was in line with previous findings of Senthamil, et al. [14]; Ahuokpeme, et al. [15], Malongane, et al. [16], Mayyas, et al. [17], Antolak, et al. [18]. Notably, the ethanol alcohol extract of green tea exhibited stronger inhibitory effects compared to black tea, supported by Mukhtar and Ghori [19], Shaaban [20], attributing this difference to the solubility of *C. zeylanicum* bark components in ethanol over water. Salma, et al. [21] investigation revealed ethanolic extract proved more potent against *Staphylococcus aureus* than *Escherichia coli*, showing zone of inhibition measurements of 17 mm starting from a 60% concentration versus 18 mm beginning at 80% concentration for *E. coli*. Green tea's polyphenols-epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) are primarily accountable for impeding bacterial proliferation according to Radji, et al. [22]. An earlier study conducted by Kumar, et al. [23] reported comparable zone of inhibition diameter values ranging between 8-12 mm when using ethanolic extract derived from green tea leaves against *S. aureus*. Additionally, henna (*Lawsonia inermis*) leaf extracts displayed inhibitory effects on microorganisms implicated in burn wound infections, corroborating Goris, et al. [24] findings and advocating for henna's potential role in managing such infections effectively. Furthermore, this study revealed a substantial decrease in the effectiveness of plant extracts at 50% concentration relative to the 100% concentration. This is in line with the findings of Yang and Lee [25], Roozegar, et al. [26], Gerchman [27]. The emergence of multidrug-resistant strains of pathogenic bacteria like *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi,* and *Escherichia coli* presents a formidable challenge for clinicians worldwide in treating burn wound infections due to their resistance to multiple antibiotics. To address this growing concern, exploring novel antimicrobial compounds from herbal sources is imperative as an alternative therapeutic approach to combat the rise of multidrug-resistant bacteria and provide clinicians with additional treatment options in the near future [28].

Conclusion

The plant leaf extracts investigated in this study have demonstrated promising potential as alternatives in combating infestations of multidrug-resistant bacteria. The findings suggest that these natural extracts, with their antimicrobial properties and inhibitory effects on various bacterial strains, could serve as valuable resources in the ongoing battle against multidrug-resistant pathogens. Further research and exploration of these plant-derived compounds may offer new avenues for developing effective strategies to address the challenges posed by multidrug-resistant bacterial infections.

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