Al-Farouk journal of sciences
Volume (2), Issue (5) (Nov, 2026)
ISSN: 3135-2359

مجلة الفاروق للعلوم
المجلد (2) العدد 5 نوفمبر 2026
عدد خاص بالمؤتمر العلمي الرابع للعلوم والتقنية
28-29 نوفمبر 2026 مصراتة – ليبيا
إدارة المعاهد العليا – وزارة التعليم التقني

Antibacterial Activity of Thyme, Sidr and Black Seed Preparations against Selected Pathogenic Bacteria

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Received: 29/7/2026 Revised: 20/8/2026 Accepted: 28/8/2026 Publication: 28/11/2026

أ- **الملخص**

أصبحت مقاومة مضادات الميكروبات تحديًا صحيًا عالميًا رئيسيًا مما زاد من الحاجة إلى البحث عن مصادر بديلة للعوامل المضادة للبكتيريا. تحتوي النباتات الطبية على مجموعة متنوعة من المركبات النشطة حيويًا التي قد تثبط نمو الكائنات الحية الدقيقة الممرضة وقد تمثل مصادر واعدة لتطوير عوامل طبيعية مضادة للميكروبات.هدفت هذه الدراسة إلى **تقييم النشاط المضاد للبكتيريا لمستحضرات الزعتر والسدر وحبة البركة بصورة منفردة ومجتمعة ضد مجموعة مختارة من البكتيريا الممرضة** باستخدام طريقة الانتشار في الآبار على وسط الآجار. تم اختبار ستة مستحضرات نباتية وهي: الزعتر (T)، والسدر(S) ، ومزيج الزعتر والسدر(T+S) ، وحبة البركة الكبيرة(NL) ، وحبة البركة الصغيرة(NS) ، ومزيج حبة البركة الكبيرة والصغيرة.(NL+NS) تم تقييم النشاط المضاد للبكتيريا ضد كل من **Staphylococcus aureus** : والعقديات الحالّة للدم ألفا(Alpha-hemolytic *Streptococcus*) ، **Escherichia coli**، **Klebsiella pneumoniae**، **Pseudomonas aeruginosa**. وتم تحديد النشاط المضاد للبكتيريا من خلال قياس قطر مناطق التثبيط بالمليمتر.أظهرت النتائج وجود اختلافات ملحوظة في مدى حساسية البكتيريا للمستحضرات النباتية المختبرة. وكانت **Staphylococcus aureus**أكثر الكائنات البكتيرية حساسية للمستحضرات النباتية. وسجل **مزيج الزعتر والسدر (T+S)** أكبر منطقة تثبيط بلغت **21مم**، يليه الزعتر (18 مم)، وحبة البركة الكبيرة (17 مم) والسدر (13 مم)، وحبة البركة الصغيرة (8 مم)، ومزيج حبة البركة الكبيرة والصغيرة (8 مم.(ولم يُلاحظ تثبيط قابل للقياس يتجاوز قطر البئر البالغ **6 مم** ضد العقديات الحالّة للدم ألفا، **E. coli**، **P. aeruginosa**. كما لوحظ نشاط محدود ضد **K. pneumoniae**، حيث سجل مزيج الزعتر والسدر (T+S) منطقة تثبيط بلغت **8مم**.تشير النتائج إلى أن مستحضرات **الزعتر والسدر وحبة البركة الكبيرة** تمتلك نشاطًا واعدًا مضادًا للبكتيريا، وخاصة ضد **S. aureus**، مع إظهار مزيج الزعتر والسدر أعلى نشاط بين المستحضرات النباتية المختبرة. ومع ذلك، هناك حاجة إلى إجراء دراسات إضافية تشمل تحديد **التركيز المثبط الأدنى(MIC)** ، **التركيز القاتل الأدنى(MBC)** والتحليل الكيميائي النباتي للمستحضرات واختبارات التآزر المعيارية من أجل تأكيد هذه النتائج وتحديد طبيعة التفاعل بين المستحضرات المختلفة.

1- الكلمات المفتاحية:الزعتر؛ السدر؛ *Ziziphus spina-christi*؛ *Nigella sativa*؛ حبة البركة؛ النشاط المضاد للبكتيريا؛ *Staphylococcus aureus*؛ طريقة الانتشار في آبار الآجار.

Abstract

Antimicrobial resistance has become a major global health challenge and has increased the need to investigate alternative sources of antibacterial agents. Medicinal plants contain diverse bioactive compounds that may inhibit the growth of pathogenic microorganisms and may provide promising candidates for the development of natural antimicrobial agents. This study aimed to evaluate the antibacterial activity of thyme, Sidr, and black seed preparations, individually and in combination, against selected pathogenic bacteria using the agar well diffusion method. Six plant preparations were investigated: thyme (T), Sidr (S), thyme plus Sidr (T+S), large black seed (NL), small black seed (NS), and a combination of large and small black seed (NL+NS). The antibacterial activity was evaluated against *Staphylococcus aureus*, alpha-hemolytic *Streptococcus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Antibacterial activity was determined by measuring the diameter of inhibition zones in millimeters. The results demonstrated considerable differences in bacterial susceptibility. *Staphylococcus aureus* was the most susceptible organism. The largest

inhibition zone was produced by the thyme plus Sidr combination (T+S), measuring 21 mm, followed by thyme (18 mm), large black seed (17 mm), Sidr (13 mm), small black seed (8 mm), and the large plus small black seed combination (8 mm). No measurable inhibition beyond the 6-mm well diameter was observed against alpha-hemolytic *Streptococcus*, *E. coli*, or *P. aeruginosa*. Limited activity was observed against *K. pneumoniae*, with an inhibition zone of 8 mm produced by T+S.The findings suggest that thyme, Sidr, and large black seed preparations possess promising antibacterial activity against *S. aureus*, with the thyme-Sidr combination showing the greatest activity among the tested preparations. However, further studies involving minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), phytochemical analysis, and standardized synergy testing are required to confirm these findings.

Keywords: Thyme; Sidr; *Ziziphus spina-christi*; *Nigella sativa*; Black seed; antibacterial activity; *Staphylococcus aureus*; agar well diffusion.

1. Introduction

Antimicrobial resistance (AMR) is an increasing public health problem that threatens the effectiveness of conventional antimicrobial therapy. The emergence of resistant bacterial pathogens has encouraged researchers to investigate alternative antimicrobial sources, particularly medicinal plants and their bioactive constituents. Plant-derived preparations contain a wide variety of secondary metabolites, including phenolic compounds, flavonoids, tannins, terpenoids, alkaloids, and essential oils, which may interfere with bacterial growth through several mechanisms. Among medicinal plants, thyme (*Thymus vulgaris*) has attracted considerable attention because of its antimicrobial properties. Recent experimental research has demonstrated that extracts of *T. vulgaris* can inhibit clinically relevant bacterial pathogens, including *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Wirtu *et al*. (2024) reported antibacterial activity of different *T. vulgaris* extracts against these organisms and highlighted the importance of extract composition in determining antibacterial efficacy. The antimicrobial properties of thyme have also been associated with its chemically active constituents. Recent research on thyme essential oil has demonstrated concentration-dependent inhibition of bacterial growth, including activity against *S. aureus* and *E. coli*. These findings support the continued investigation of thyme as a potential source of natural antibacterial compounds. Sidr (*Ziziphus spina-christi*) is another medicinal plant widely used in traditional medicine. Recent research has confirmed that extracts of *Z. spina-christi* possess antibacterial properties. Alamsjah *et al*. (2023) investigated fresh extract, infusion, and ethanol extract of Sidr leaves against Gram-positive bacteria, including *S. aureus*, and demonstrated measurable antibacterial activity. More recent research has further supported the antimicrobial potential of *Z. spina-christi*. Gebrehiwot *et al*. (2024) isolated and evaluated phytochemicals from the roots of *Z. spina-christi* and reported antibacterial activity against several pathogenic bacteria. Their results also showed that antibacterial activity varied according to extract concentration and bacterial species. Recent studies have also investigated Sidr-derived materials against resistant microorganisms. For example, El-Sawaf *et al*. (2024) demonstrated significant antimicrobial activity of a green-synthesized nanocomposite produced using *Z. spina-christi* extract, including strong effects against *S. aureus* and *E. coli*. Although such nanocomposite results cannot be directly compared with crude plant preparations, they further demonstrate the antimicrobial potential associated with *Z. spina-christi*. Black seed (*Nigella sativa*) has likewise received increasing scientific attention because of its biologically active constituents, particularly thymoquinone. A 2022 study by Shafodino *et al*. demonstrated that different *N. sativa* seed extracts exhibited antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, and *Bacillus subtilis*, although the magnitude of activity varied according to the extraction solvent and preparation. Sutrisna *et al*. (2022) also reported that ethanolic seed extracts of *N. sativa* inhibited *S. aureus* and *Streptococcus epidermidis* in vitro. Another study published in 2023 investigated *N. sativa* against bacteria isolated from wound infections and found that *S. aureus* showed measurable susceptibility to the methanolic seed extract, while many Gram-negative isolates showed little or no inhibition. The same study reported antibacterial activity of thymoquinone against *S. aureus*. More recently, Rahman *et al*. (2024) investigated the phytochemical and antimicrobial properties of *N. sativa* ethanolic extract and identified several compounds, including thymoquinone and thymol, that may contribute to its antimicrobial properties. The extract demonstrated activity against a broad range of bacteria, including *S. aureus*, *E. coli*, and *P.*

aeruginosa. A recent review by Abbas *et al*. (2024) further summarized the antimicrobial properties of *N. sativa* and emphasized the potential contribution of its bioactive constituents to antibacterial activity. Although numerous studies have investigated thyme, Sidr, or black seed individually, comparative evaluation of these three medicinal plant preparations under the same experimental conditions, particularly the combination of thyme and Sidr, remains limited. Therefore, the present study was designed to compare the antibacterial effects of these preparations against selected Gram-positive and Gram-negative pathogenic bacteria. The aim of this study was to evaluate and compare the antibacterial activity of thyme, Sidr, and black seed preparations, individually and in combination, against selected pathogenic bacteria.

2. Materials and Methods

2.1 Study Design and Study Locations

An experimental laboratory study was conducted to evaluate the antibacterial activity of thyme, Sidr, and black seed preparations against selected pathogenic bacterial isolates. **The extraction process was carried out at the Olive Research Center, Tarhuna, Libya.** The preparation and extraction of the investigated plant materials were performed under the appropriate laboratory conditions at the center. **The microbiological analysis of the prepared samples was conducted at the Microbiology Laboratory, Department of Medical Laboratories, Higher Institute of Science and Technology, Soq Al-Khamis Emsehel, Libya.** The antibacterial activity of the prepared plant extracts was evaluated against the selected bacterial isolates using the agar well diffusion method. This division of the experimental procedures ensured that the plant extraction and microbiological evaluation were conducted under appropriate laboratory conditions.

2.2 Preparation of Plant Materials

The extraction of the investigated plant materials was carried out at the **Olive Research Center, Tarhuna, Libya.** The plant materials included thyme, Sidr, and black seed preparations. The plant materials were cleaned, dried, and processed before extraction. The prepared materials were subjected to the extraction procedure under controlled laboratory conditions. Following extraction, the obtained preparations were appropriately filtered and concentrated before being transferred for microbiological evaluation.

2.3. Extraction Procedures

Alcoholic extracts of the plant materials were prepared using ultrasonic-assisted extraction (UAE), with methanol as the extraction solvent. Briefly, 10 g of powdered plant material was mixed with 100 mL of 70% methanol to obtain a solid-to-solvent ratio of 1:10 (w/v). The mixtures were sonicated in an ultrasonic water bath for 60 min at a controlled temperature below 35–40 °C to minimize the potential degradation of heat-sensitive compounds. Following sonication, the mixtures were filtered through Whatman No. 1 filter paper. The resulting filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C until the final volume reached approximately 10 mL.

2.4. Extract Preparation and Dilution

Following concentration, the crude methanol 100% (v/v) extracts were used as working solutions for antibacterial testing without diluted. The prepared solutions were handled under consistent conditions before antibacterial evaluation.

2.5 Microbiological Analysis −2

The microbiological analysis was performed at the **Microbiology Laboratory, Department of Medical Laboratories, Higher Institute of Science and Technology, Soq Al-Khamis Emsehel, Libya.**

The antibacterial activity of the prepared plant extracts was evaluated against five bacterial isolates: *Staphylococcus aureus,* alpha-hemolytic *Streptococcus, Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

The agar well diffusion method was used to determine the antibacterial activity. The bacterial suspensions were standardized to approximately 0.5 McFarland turbidity before inoculation onto Mueller-Hinton agar plates. After applying the plant preparations to the wells, the plates were incubated at 37 °C for 24 hours. The inhibition zones were subsequently measured in millimeters.

2.6 Bacterial Isolates

Five bacterial isolates were tested:

2.6.1 Gram-positive bacteria:

- *Staphylococcus aureus*
- Alpha-hemolytic *Streptococcus*

2.6.2 Gram-negative bacteria:

- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Pseudomonas aeruginosa*

The bacterial inocula were standardized to approximately 0.5 McFarland, corresponding to approximately 1.5×10^8 colony-forming units (CFU)/mL.

2.7 Antibacterial Assay

The antibacterial activity was evaluated using the agar well diffusion method. Mueller-Hinton agar plates were inoculated uniformly with the standardized bacterial suspensions. Wells were prepared in the agar, and the plant preparations were introduced into the wells.

The plates were incubated at 37 °C for 24 hours. Following incubation, the inhibition zones were measured in millimeters (mm).

2.8 Antibiotic Control

The antibiotic control was included for comparison. The inhibition zones recorded for the antibiotic control were 30 mm for *S. aureus*, 14 mm for alpha-hemolytic *Streptococcus*, and 30 mm for each of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Ciprofloxacin and vancomycin were used among the positive controls.

2.9 Statistical Analysis

The inhibition-zone measurements were summarized descriptively for each plant preparation and bacterial isolate. Because a single value was recorded for each preparation–isolate combination and no replicate measurements were available, no inferential statistical analysis was performed.

3. Results and Discussion

3.1 Results

Table 1. Antibacterial activity of the tested plant preparations against selected bacterial isolates (inhibition-zone diameter, mm)

Bacterial isolate	T	S	T+S	NL	NS	NL+NS	C
Staphylococcus aureus	18	13	21	17	8	8	30
Alpha-hemolytic Streptococcus	6	6	6	6	6	6	14
E. coli	6	6	6	6	6	6	30

Bacterial isolate	T	S	T+S	NL	NS	NL+NS	C
Klebsiella pneumoniae	6	6	8	6	6	6	30
P. aeruginosa	6	6	6	6	6	6	30

Abbreviations: T = Thyme; S = Sidr; T+S = Thyme + Sidr; NL = Large black seed; NS = Small black seed; NL+NS = Large + small black seed; C = Antibiotic control.

Note: Values are expressed as inhibition-zone diameter (mm). A value of approximately 6 mm corresponds to the well diameter and indicates no measurable inhibition beyond the well.

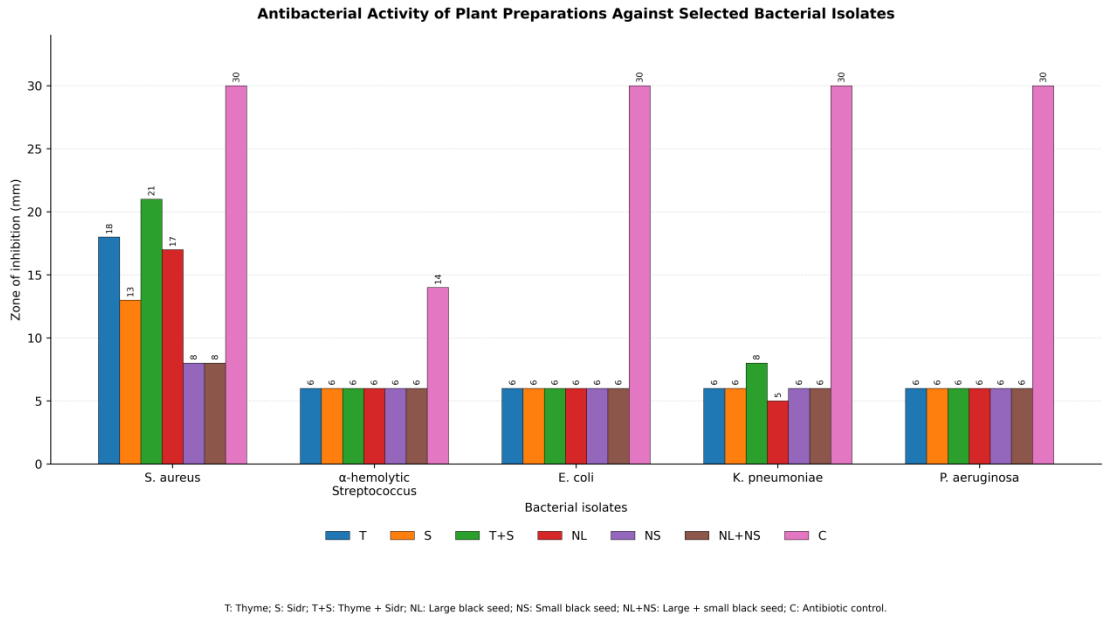


Figure 1. Comparison of the Antibacterial Activity of Thyme, Sidr, and Black Seed Preparations −3

Against Selected Bacterial Isolates

3.1.1 *Staphylococcus aureus*

Staphylococcus aureus was clearly the most susceptible organism. The highest inhibition zone was observed with the thyme and Sidr combination (T+S) at 21 mm, followed by thyme alone (18 mm), large black seed (17 mm), Sidr alone (13 mm), small black seed (8 mm), and the large plus small black seed combination (8 mm). The antibiotic control produced a 30-mm inhibition zone. Representative inhibition plates are shown in Figures 2–7.

3.1.2 *Alpha-hemolytic Streptococcus, E. coli,* and *P. aeruginosa*

All six plant preparations produced 6-mm measurements against alpha-hemolytic *Streptococcus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, indicating no measurable antibacterial inhibition beyond the well diameter under the experimental conditions.

3.1.3 *Klebsiella pneumoniae*

Limited activity was observed against *Klebsiella pneumoniae*, with an inhibition zone of 8 mm produced by the T+S combination, indicating weak antibacterial activity, while the other preparations produced 6-mm zones, equal to the well diameter (no measurable inhibition).

3.2 Discussion

The present study demonstrated a clear difference in the susceptibility of the tested bacteria to the investigated plant preparations. The most important finding was the pronounced susceptibility of *Staphylococcus aureus*, particularly to the thyme-Sidr combination. The T+S combination produced the largest inhibition zone (21 mm). This was greater than the inhibition zones produced by thyme alone (18 mm) and Sidr alone (13 mm). This observation suggests that combining the two preparations may increase antibacterial activity against *S. aureus*. However, the present experiment does not by itself establish a synergistic interaction. A true synergistic effect should be confirmed using quantitative methods such as checkerboard analysis and calculation of the fractional inhibitory concentration index. The strong activity of thyme against *S. aureus* is consistent with recent literature. Wirtu *et al*. (2024) demonstrated antibacterial activity of *Thymus vulgaris* extracts against several pathogenic bacteria, including *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. The present study similarly found that thyme produced a substantial inhibition zone against *S. aureus* (18 mm), whereas no measurable activity was detected against *E. coli* or *P. aeruginosa*. This difference emphasizes that antibacterial activity depends not only on the plant preparation but also on the bacterial species and experimental conditions. The observed activity of Sidr is also supported by recent research. Alamsjah *et al*. (2023) reported antibacterial activity of *Ziziphus spina-christi* leaf preparations against Gram-positive bacteria, including *S. aureus*. In the present study, Sidr produced a 13-mm inhibition zone against *S. aureus*. Thus, although the magnitude of inhibition cannot be directly compared because extraction procedures and concentrations differ, both studies support the antibacterial potential of Sidr against Gram-positive bacteria. Further support comes from Gebrehiwot *et al*. (2024), who demonstrated antibacterial activity of phytochemicals isolated from *Z. spina-christi* roots and reported that activity varied according to concentration and bacterial species. This variability may help explain why Sidr showed measurable activity against *S. aureus* in the current experiment but not against the other organisms. The results obtained with black seed are also biologically plausible. Previous research has demonstrated that *Nigella sativa* extracts can inhibit *S. aureus*, although activity varies according to extraction solvent and preparation. Shafodino *et al*. (2022) found antimicrobial activity of different *N. sativa* preparations against both Gram-positive and Gram-negative bacteria, with substantial differences among extraction methods. Similarly, a study of *N. sativa* against wound-associated bacterial isolates reported that *S. aureus* showed susceptibility to the methanolic seed extract, while many Gram-negative isolates showed no inhibition. This pattern is particularly relevant to the current findings, in which large black seed produced a 17-mm inhibition zone against *S. aureus*, while no measurable activity was observed against *E. coli* and *P. aeruginosa*. The difference between large and small black seed preparations is noteworthy. In the present experiment, NL produced a 17-mm inhibition zone against *S. aureus*, whereas NS produced only 8 mm. This may reflect differences in chemical composition, active-compound concentration, maturity, storage conditions, or preparation characteristics. However, because the chemical composition of the two seed groups was not measured in the present study, the reason for this difference cannot be established conclusively. Recent research has identified thymoquinone and other compounds among the constituents potentially responsible for the antimicrobial effects of *N. sativa*. Rahman *et al*. (2024) reported thymoquinone and thymol among compounds identified in *N. sativa* extract and demonstrated activity against several bacterial species. A 2024 review also emphasized the importance of the bioactive constituents of *N. sativa* in its antimicrobial potential. An important observation in the present study is the low susceptibility of Gram-negative bacteria. *E. coli* and *P. aeruginosa* showed no measurable inhibition, while *K. pneumoniae* showed only weak activity. This contrasts with some recent studies in which plant extracts demonstrated broader activity against Gram-negative bacteria. For example, recent work on *N. sativa* has reported activity against *E. coli* and *P. aeruginosa* under particular extraction conditions. Such differences are expected because antibacterial activity is strongly affected by extraction solvent, concentration, plant origin, preparation method, bacterial strain, and assay conditions. Therefore, absence of activity in the present experiment should not be interpreted as evidence that these plants are universally ineffective against Gram-negative bacteria. The relatively low susceptibility of the Gram-negative organisms may also be related to their outer membrane, which can restrict penetration of some plant-derived antimicrobial compounds. The additional outer membrane and associated resistance mechanisms can contribute to reduced susceptibility compared with many Gram-positive

organisms. The *K. pneumoniae* result is particularly interesting. The T+S combination produced an 8-mm zone, compared with 6 mm for most other preparations. Although this represents only a small inhibition beyond the 6-mm well, it suggests that the combination may have some activity against this organism. Similar recent studies of *Z. spina-christi* have demonstrated variable activity against *Klebsiella* and other Gram-negative bacteria depending on the preparation and extraction conditions. The current finding of 21 mm against *S. aureus* for T+S is therefore the major result of the study. It provides preliminary evidence that combining thyme and Sidr may produce stronger antibacterial activity than either preparation alone. Nevertheless, because the current experiment measured inhibition zones only, the finding should be regarded as a preliminary indication of enhanced activity rather than proof of synergism.

4. Conclusion

The present study demonstrated that the antibacterial activity of thyme, Sidr, and black seed preparations varied considerably according to the bacterial species and plant preparation. The most susceptible organism was Staphylococcus aureus, against which all six plant preparations demonstrated measurable inhibition. The thyme + Sidr combination (T+S) produced the highest inhibition zone (21 mm), followed by thyme (18 mm) and large black seed (17 mm). Sidr produced moderate activity (13 mm), while small black seed and the large + small black seed combination produced lower activity (8 mm each). No measurable inhibition was observed against alpha-hemolytic *Streptococcus*, *E. coli*, or *P. aeruginosa*. *K. pneumoniae* showed only weak activity, with the highest value being 8 mm for T+S.The results support previous studies demonstrating antibacterial properties of thyme, *Ziziphus spina-christi*, and *Nigella sativa*, while also highlighting the importance of extraction method, plant preparation, bacterial species, and experimental conditions. The particularly strong activity of T+S against *S. aureus* suggests that this combination deserves further investigation. However, additional quantitative experiments are required before concluding that the combination has synergistic antibacterial activity.

5. Recommendations

1. Conduct further studies using a larger number of independent replicates to improve the reliability and statistical power of the findings.

2. Determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the most active preparations, particularly thyme, Sidr, large black seed, and the thyme–Sidr combination.

3. Investigate the phytochemical composition of the plant preparations, including major bioactive compounds, to help explain differences in antibacterial activity between preparations.

4. Standardize extraction conditions, extract concentrations, plant origin, and storage conditions in future experiments to improve reproducibility and allow meaningful comparisons between studies.

5. Expand the investigation to additional bacterial strains and clinically relevant resistant isolates to determine whether the observed activity is consistent across different bacterial populations.

6. Consider evaluating different extract concentrations and extraction solvents, since antibacterial activity may vary substantially according to concentration and preparation method.

7. The promising activity of the thyme–Sidr combination against *Staphylococcus aureus* (21 mm) warrants further laboratory investigation before any therapeutic application is considered.

Acknowledgments −4

The authors would like to express their sincere gratitude to the Olive Research Center, Tarhuna, Libya, for providing the facilities and technical support required for the extraction of the investigated plant materials. The authors also extend their appreciation to the Microbiology Laboratory, Department of Medical Laboratories, Higher Institute of Science and Technology, Soq Al-Khamis Emsehel, Libya, for providing the laboratory facilities and technical assistance required to conduct the microbiological analyses and antibacterial assays.

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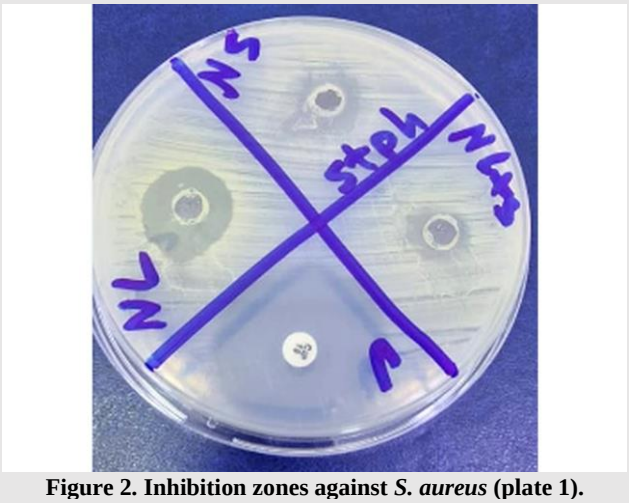


Figure 2. Inhibition zones against *S. aureus* (plate 1).

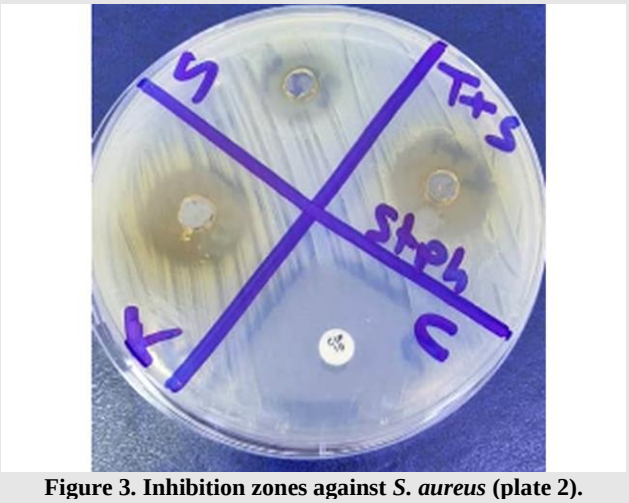


Figure 3. Inhibition zones against *S. aureus* (plate 2).

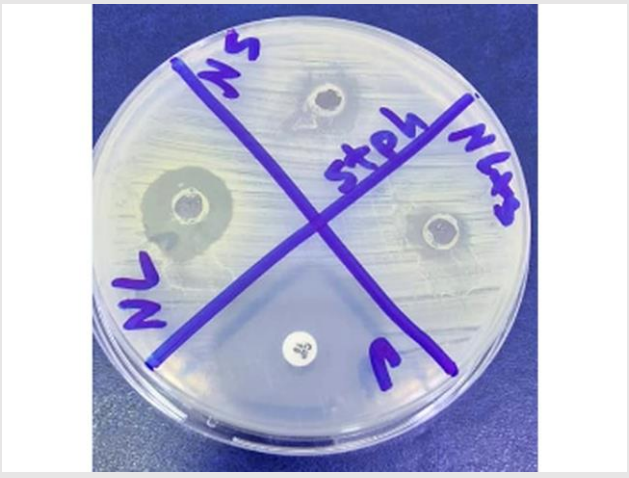


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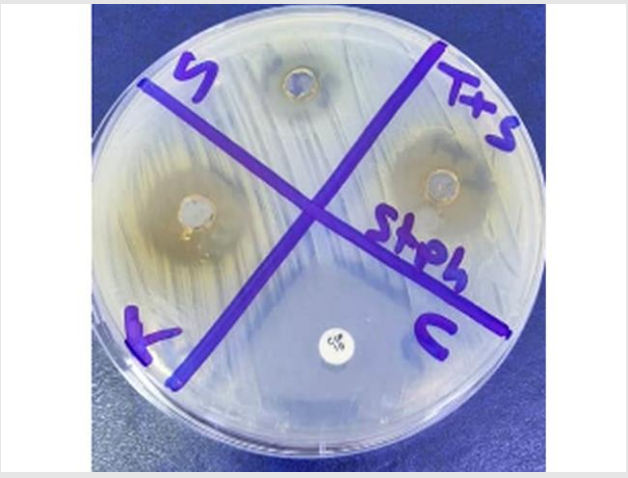


Figure 3. Inhibition zones against *S. aureus* (plate 2).



Figure 4. Inhibition zones against alpha-hemolytic *Streptococcus*.

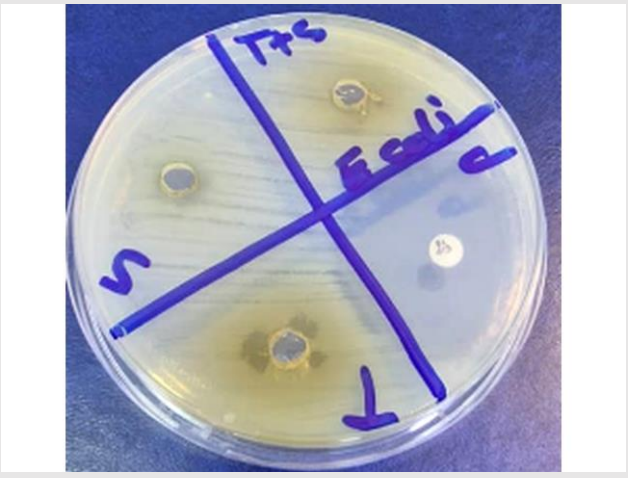


Figure 5. Inhibition zones against *E. coli*.

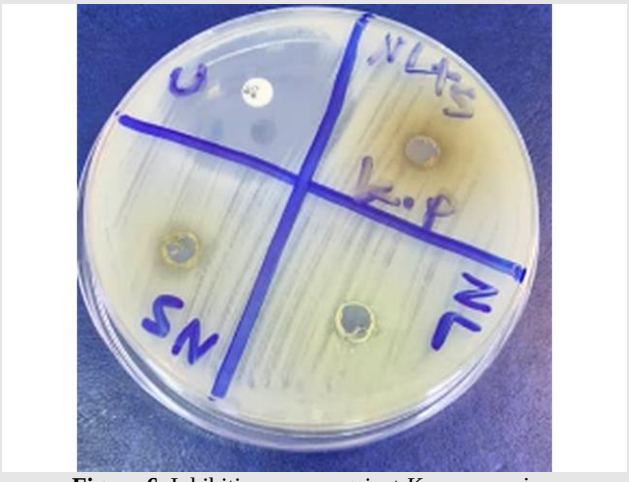


Figure 6. Inhibition zones against *K. pneumoniae*.

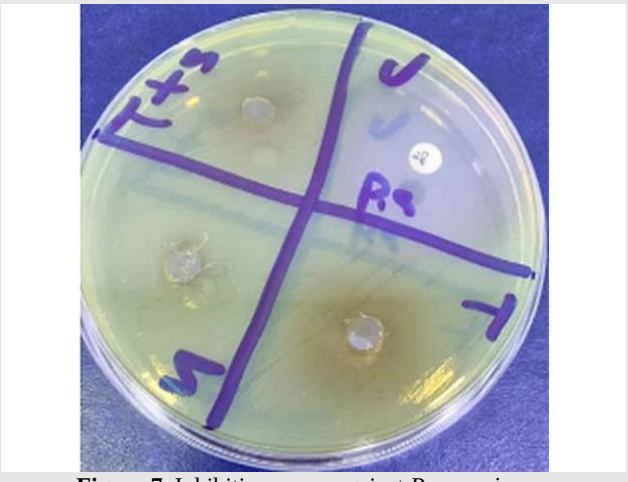


Figure 7. Inhibition zones against *P. aeruginosa*.