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# Phytochemical and antibacterial profile of five aqueous plant extracts

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## الملف الكيميائي النباتي والمضاد للبكتيريا لخلاصات مائية لخمس نباتات

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**Abstract:**

The increasing global interest in plant-derived therapeutics has prompted renewed investigation into medicinal herbs as alternative treatments for infections caused by pathogenic bacteria. The present study investigated the antibacterial effects of five medicinal plants "*Nigella sativa*", "*Allium sativum*", "*Eucalyptus rostrata*", "*Hibiscus sabdariffa*" and "*Syzygium aromaticum*" against five clinical bacterial isolates from human patients in Babylon Governorate, Iraq. We used pus, urine, ascitic fluid, stool and blood samples for the isolation of bacterial strains, which comprised *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. The antibacterial activity was evaluated by the agar well diffusion method and a qualitative phytochemical screening of each aqueous extract was done. All extracts from the tested plants showed inhibitory activity against the bacterial isolates, with the highest antibacterial effect being obtained from the extract of *S. aromaticum* against all the tested strains. Activity was observed against all bacteria at 5 mg/mL, except *E. rostrata*, which had no activity against *S. typhimurium*. The phytochemical analysis confirmed the presence of most of the extracts, flavonoids, tannins, steroids, alkaloids, saponins, and phenols in most extracts with the exception of flavonoids in *E. rostrata*, cardiac glycosides in *S. aromaticum* and terpenoids in *H. sabdariffa*. These findings support the traditional use of these medicinal plants in the treatment of bacterial infections and highlight their potential as sources of novel antimicrobial agents.

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**Keywords:** *Salmonella typhimurium*; antibacterial activity; aqueous plant extracts; phytochemical screening.

**الملخص**

أدى الاهتمام العالمي المتزايد بالعلاجات مستخلصات النباتات إلى تحفيز إجراء تحقيقات متجددة حول الأعشاب الطبية كعلاجات بديلة للالتهابات التي تسببها البكتيريا الممرضة .بحثت الدراسة الحالية التأثيرات المضادة للبكتيريا لخمسة نباتات طبية، وهي: الحبة السوداء(*Nigella sativa*) ، والثوم (*Allium sativum*)، واليوكالبتوس أو الكافور(*Eucalyptus rostrata*) ، والكركديه (*Hibiscus sabdariffa*)، والقرنفل(*Syzygium aromaticum*) ، ضد خمس عزلات بكتيرية سريرية مأخوذة من مرضى بشر في محافظة بابل، العراق .لقد استخدمنا عينات من القيح، والبول، والسائل الاستسقائي، والبراز، والدم لعزل السلالات البكتيرية، والتي شملت السالمونيلا التيفيمورية (*Salmonella typhimurium*)، والكلبيلا الرئوية(*Klebsiella pneumoniae*) ، والإشريكية القولونية (*Escherichia coli*)، والمكورات العنقودية الذهبية(*Staphylococcus aureus*) ، والمكورات العنقودية البشرية .(*Staphylococcus epidermidis*) جرى تقييم الفعالية المضادة للبكتيريا باستخدام طريقة الانتشار في حفر الأغار، كما أُجري الكشف الكيميائي النباتي النوعي لكل مستخلص مائي .أظهرت جميع المستخلصات النباتية المختبرة نشاطاً تثبيطياً ضد العزلات البكتيرية، حيث تم الحصول على أعلى تأثير مضاد للبكتيريا من مستخلص القرنفل (*S. aromaticum*) ضد جميع السلالات المختبرة .وقد لوحظ نشاط مضاد ضد جميع أنواع البكتيريا عند تركيز 5 مغم/مل، باستثناء نبات الكافور (*E. rostrata*) الذي لم يُظهر أي نشاط ضد السالمونيلا التيفيمورية .(*S. typhimurium*) وأكد التحليل الكيميائي النباتي وجود معظم المستخلصات، والفلافونويد، والتانينات، والستيرويدات، والقلويدات، والصابونين، والفينولات في غالبية المستخلصات، باستثناء الفلافونويد في نبات الكافور(*E. rostrata*) ، والجليكوسيدات القلبية في القرنفل(*S. aromaticum*) ، والتيربينويدات في الكركديه .(*H. sabdariffa*) تدعم هذه النتائج الاستخدام التقليدي لهذه النباتات الطبية في علاج الالتهابات البكتيرية وتبرز إمكاناتها كمصادر لعوامل مضادة للميكروبات جديدة .

**الكلمات المفتاحية:** السالمونيلا التيفيمورية؛ النشاط المضاد للبكتيريا؛ المستخلصات النباتية المائية؛ الكشف الكيميائي النباتي.

## 1. Introduction

The problem of human bacterial infections continues to be a significant global health issue, especially due to the growing rates of antimicrobial resistance. *Staphylococcus aureus* is a pathogen that is one of the most prevalent causes of human infections and has acquired a high level of resistance to β-lactam antibiotics, such as penicillins. There is therefore an imminent need to find new antimicrobial agents or alternative measures to improve the effectiveness of the current antibiotics. Natural products, especially those of medicinal plants, are a promising source of new antimicrobial compounds [1]. Explain The Extraintestinal pathogenic *Escherichia coli* (ExPEC) causes a broad spectrum of human infections, such as urinary tract infections, bacteraemia and meningitis [2]. On the same note, *Salmonella* species, particularly *S. typhimurium*, are the leading causes of foodborne diseases in the global scene and are a significant burden to the population. The widespread use of antibiotics in animal farming has helped to create multidrug-resistant strains of *Salmonella* and *E. coli* that can be passed to humans via contaminated food [3]. Moreover, an opportunistic pathogen, *Staphylococcus epidermidis*, is a major cause of nosocomial infections related to indwelling medical equipment and bloodstream infections [4].

Medicinal plants are becoming more and more popular in developing countries, in part due to the inaccessibility of modern drugs [5]. Plant extracts are generally safe, biodegradable and can

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be used in food [6]. It has been shown in numerous studies that plants with antibacterial activity do have bioactive secondary metabolites including tannins, alkaloids, flavonoids, phenols, saponins and glycosides [7, 8]. Due to the high rate of antimicrobial resistance transmission around the world, the need to find new antibacterial agents in natural sources has gained more and more significance [9, 10].

*Nigella sativa* (black cumin) seeds are famous due to their medicinal qualities and a number of bioactive compounds, such as alkaloids, tannins, phenols and saponins [11]. Eucalyptus species have long been used in traditional medicine for the treatment of respiratory and urinary tract infections [12], and their leaves are rich in secondary metabolites such as alkaloids, saponins, tannins, phenols, steroids and terpenoids [12, 13]. *Hibiscus sabdariffa* (roselle) contains organic acids, phytosterols, polyphenols and anthocyanins, which contribute to its antimicrobial activity [3]. Anthocyanins are phytochemicals belonging to the flavonoid family; two main anthocyanins were identified in the calyces of *H. sabdariffa* in a previous study [10, 14]. *Syzygium aromaticum* (clove) is widely recognised for its antimicrobial, antifungal and pesticidal properties [15, 16], while *Allium sativum* (garlic) exhibits broad-spectrum antibacterial activity largely attributed to allicin and other organosulfur compounds [6, 17, 18]. Garlic contains various bioactive chemical components, including alliin, allicin, alliinase and S-allylcysteine, which are responsible for its pharmacological and antibacterial effects [1]. Garlic has been shown to have an antibacterial effect in vitro and in animal studies against numerous human pathogenic micro-organisms [19].

The present study aimed to evaluate the antibacterial activity of aqueous extracts from *N. sativa*, *A. sativum*, *E. rostrata*, *H. sabdariffa* and *S. aromaticum* against multidrug-resistant Gram-positive and Gram-negative clinical bacterial isolates. In addition, phytochemical screening of these extracts was conducted to identify the bioactive compounds potentially responsible for the observed antimicrobial effects.

## 2. Materials and methods

### 2.1 Plant material

Plant materials of *N. sativa*, *A. sativum*, *E. rostrata*, *H. sabdariffa* and *S. aromaticum* were obtained from a local market in Babylon Governorate, Iraq, in June 2021. The plant species were authenticated by a qualified botanist. The collected materials were thoroughly cleaned to remove impurities, washed with tap water followed by distilled water, and air-dried in the shade. A fine powder was obtained by mortar and pestle grinding of the dried parts of the plants and storing in airtight containers at room temperature until extraction (Table 1).

**Table 1.** Data on the plant species used and the percentage extract yield.

| Plant species | Family | Common name | Plant part used | Extract yield (%) |
|---|---|---|---|---|
| *Nigella sativa* | Ranunculaceae | Black cumin | Seeds | 10.34 |
| *Eucalyptus rostrata* | Myrtaceae | Eucalyptus | Leaves | 25.15 |
| *Hibiscus sabdariffa* | Malvaceae | Roselle | Flowers | 16.38 |
| *Syzygium aromaticum* | Myrtaceae | Clove | Flowers | 15.17 |
| *Allium sativum* | Amaryllidaceae | Garlic | Bulbs | 13.49 |

### 2.2 Isolation and identification of bacterial isolates

Five clinical bacterial strains were isolated from patients attending Hilla Teaching Hospital and Marjan Medical City Hospital in Babylon, Iraq, between June and September 2021. The

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isolates included three Gram-negative bacteria (*E. coli*, *S. typhimurium* and K. pneumoniae) and two Gram-positive bacteria (S. aureus and S. epidermidis) [20]. Standard microbiological and biochemical tests were used to culture and identify clinical samples (urine, blood, pus, stool and ascitic fluid). The isolates were maintained in brain heart infusion broth which was supplemented with 15% glycerol, and kept at -20 C until use [2]. The sources and clinical details of the isolates are presented in Table 2.

**Table 2.** Clinical isolates from humans and their sources.

| Organism | Disease | Age/gender | Source |
|---|---|---|---|
| *Escherichia coli* | Cystitis | 17 yr/M | Urine |
| *Staphylococcus aureus* | Stomach ulcer/infection | 26 yr/M | Ascitic fluid |
| *Klebsiella pneumoniae* | Wound at surgical site | 50 yr/F | Pus |
| *Staphylococcus epidermidis* | Bacteraemia | 10 yr/F | Blood |
| *Salmonella typhimurium* | Intestinal infection (diarrhoea) | 51 yr/M | Stool |

### 2.3 Preparation of bacterial inocula

Each bacterial isolate was subcultured on Mueller–Hinton agar and incubated overnight at 37 °C. Bacterial suspensions were prepared in sterile saline and adjusted spectrophotometrically at 580 nm to achieve a final concentration of approximately $1 \times 10^7$ CFU/mL [6].

### 2.4 Preparation of plant extracts

Two hundred grams of each powdered plant material were mixed with 1000 mL of distilled water and extracted at room temperature for 24 h. The mixtures were homogenised using a blender for 3 min and subsequently filtered through a plastic sieve, sterile gauze and a 0.22 µm nitrocellulose membrane. The filtrates were lyophilised, and the resulting dried extracts were weighed and stored at 4 °C until further use [21].

### 2.5 Phytochemical screening of aqueous extracts

Qualitative phytochemical analyses of the aqueous extracts were conducted to detect the presence of alkaloids, flavonoids, steroids, terpenoids, tannins, phenols, saponins and cardiac glycosides using standard published protocols [15, 22].

### 2.6 Antibacterial activity of plant extracts

The antibacterial activity of the plant extracts was evaluated using the agar well diffusion method as described by Javed et al. [5]. A stock solution was prepared by dissolving the powdered concentrates in distilled water at 50 mg/mL, and working solutions were obtained by diluting the stock solution in distilled water to final concentrations of 5, 10 and 25 mg/mL. Mueller–Hinton agar plates were inoculated with 60 µL of bacterial suspension, and wells (8 mm diameter) were aseptically created using a cork borer. Each well was filled with 70 µL of the respective plant extract, and the plates were incubated at 37 °C for 24 h. Distilled water was used as a negative control. Antibacterial activity was expressed as the mean diameter of the inhibition zones (mm) [11].

### 2.7 Statistical analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Minitab software was used to determine statistical significance, with $p < 0.05$ being statistically significant.

## 3. Results and discussion

### 3.1 Phytochemical screening

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Qualitative phytochemical screening revealed that the aqueous extracts of the investigated medicinal plants contained a diverse range of bioactive secondary metabolites, including alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids and cardiac glycosides (Table 3). Phenols, steroids, tannins and saponins were detected in all five extracts, whereas the remaining classes of compounds showed species-specific distribution: alkaloids were not detected in *A. sativum*, flavonoids were not detected in *E. rostrata*, cardiac glycosides were not detected in *S. aromaticum*, and terpenoids were not detected in *H. sabdariffa*.

**Table 3.** Qualitative phytochemical screening of the aqueous plant extracts.

| Compound | *A. sativum* | *N. sativa* | *H. sabdariffa* | *E. rostrata* | *S. aromaticum* |
|---|---|---|---|---|---|
| Alkaloids | − | + | + | + | + |
| Phenols | + | + | + | + | + |
| Flavonoids | + | + | + | − | + |
| Steroids | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| Saponins | + | + | + | + | + |
| Cardiac glycosides | + | + | + | + | − |
| Terpenoids | + | + | − | + | + |

*Note: (+) present; (−) not present.*

### 3.2 Antibacterial activity of plant extracts

The antibacterial activity of aqueous extracts from the five plants, each tested at three concentrations (5, 10 and 25 mg/mL), was assessed by measuring the inhibition zone (mm) on agar using the well diffusion method (Table 4). All of the aqueous extracts possessed antimicrobial activity, and the diameter of the inhibition zones increased with extract concentration. The aqueous extract of *S. aromaticum* (clove) exhibited the strongest bactericidal action (57 mm) against *S. typhimurium* and, at 25 mg/mL, was the most effective extract in inhibiting the growth of all pathogenic micro-organisms tested, whereas the aqueous extract of *E. rostrata* was the least effective, showing no activity against *S. typhimurium* at 5 mg/mL. Antimicrobial activity was variable for the other plant extracts against the bacterial strains.

**Table 4**. Antibacterial activity (inhibition zone, mm) of aqueous extracts from five plants, each tested at 5, 10 and 25 mg/mL, against pathogenic bacteria, determined using agar well diffusion assays**.**

| Bacterial species | *N. sativa* 25 | *N. sativa* 10 | *N. sativa* 5 | *A. sativum* 25 | *A. sativum* 10 | *A. sativum* 5 | *S. aromaticum* 25 | *S. aromaticum* 10 | *S. aromaticum* 5 | *E. rostrata* 25 | *E. rostrata* 10 | *E. rostrata* 5 | *H. sabdariffa* 25 | *H. sabdariffa* 10 | *H. sabdariffa* 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. typhimurium* | 33 | 24 | 18 | 27 | 23 | 19 | 57 | 49 | 43 | 17 | 12 | − | 22 | 17 | 15 |
| S. epid | 20 | 15 | 9 | 24 | 19 | 15 | 33 | 25 | 20 | 20 | 12 | 9 | 24 | 12 | 14 |

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| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ermidis | | | | | | | | | | | | | | | |
| *E. coli* | 22 | 16 | 11 | 19 | 17 | 14 | 41 | 37 | 13 | 20 | 14 | 8 | 25 | 23 | 19 |
| K. pneumoniae | 21 | 18 | 12 | 24 | 20 | 17 | 23 | 17 | 11 | 17 | 11 | 7 | 24 | 20 | 17 |
| S. aureus | 20 | 14 | 12 | 25 | 22 | 16 | 20 | 13 | 9 | 23 | 19 | 12 | 26 | 19 | 17 |

### 3.3 Discussion

Qualitative phytochemical profiles of the five extracts provide a reason, at least partially, why the antibacterial activity of the extracts varied and points to species-specific metabolic pathways that can modulate biological activity. Phenols and flavonoids are known to disrupt the cell membrane of bacteria, prevent the synthesis of nucleic acids and disrupt some vital enzymatic activities. Tannins especially have the ability to precipitate membrane proteins and prevent microbial adhesion and saponins have antibacterial effects by permeabilising membranes. The fact that most of the extracts contain these compounds gives a mechanistic explanation for the antimicrobial activity in this study. Phytochemical reports on *Nigella sativa* describe seeds rich in alkaloids, saponins, phenolic compounds and fixed and volatile oils, which is consistent with the alkaloids, phenols, tannins and saponins detected in the present extract [5]. In Eucalyptus, alkaloids, flavonoids, tannins, polyphenols, triterpenes and saponins are among the phytochemicals that characterise the genus, according to previous studies [23, 24], and phytochemical analyses of Eucalyptus aqueous extracts have revealed the presence of alkaloids, terpenoids, flavonoids, steroids, saponins and tannins [22, 25]; notably, terpenoids were detected in the present *E. rostrata* extract, whereas flavonoids were not, possibly reflecting the limited water solubility of lipophilic flavonoid aglycones. Previous reports on *Hibiscus sabdariffa* indicate the presence of flavonoids, alkaloids, saponins, terpenes, carbohydrates, tannins and sterols [26], and phytochemical analysis of an aqueous methanolic extract of *H. sabdariffa* identified cardiac glycosides, flavonoids, saponins and alkaloids among the constituents [10]; in the present study, terpenoids were the only screened class not detected in *H. sabdariffa*. Several secondary metabolites, including alkaloids, saponins, flavonoids and phenols, have been found in the phytochemical analysis of *Syzygium aromaticum* [15], in agreement with the present results, in which cardiac glycosides were the only class not detected in this species.

In medicinal plants, diverse secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins and sterols have documented healing properties. Preliminary evaluation of bioactive compounds can aid in the discovery of bioactive properties that may lead to drug development. Plants contain a wide range of secondary metabolites, some of which have demonstrated antibacterial effects in vitro. These are powerful bioactive compounds that can be utilised as a therapeutic agent or as a starting material to make any useful pharmaceutical [11]. One of the most common classes of secondary metabolites exhibiting antibacterial activity is phenols and polyphenols. The structure and composition of flavonoids, specifically the number and location of methoxy and phenolic groups, are known to affect the diversity of their antibacterial action, and tannins have been demonstrated to have antibacterial activity [22]. Disruption of the bacterial cell wall and membrane by essential oils and fatty acids may render organisms more susceptible to other phytochemicals, such as flavonoids, saponins, tannins and alkaloids, which have been reported as antibacterial agents. Oluwasina et al. [15] posit that the

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antibacterial effect of saponins is an action of membrane-lytic capabilities but not just a modification of the surface tension of the extracellular medium; the antibacterial effect of saponins can thus be elicited through this action of membrane disruption.

Mostafa et al. [6] demonstrated that *S. aromaticum* yields 4.38% extract. *H. sabdariffa* extracts have been reported to inhibit *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* and *Klebsiella pneumoniae* in previous studies [10, 14]. Abdallah [27] demonstrated the antibacterial activity of a methanolic extract of *H. sabdariffa* calyces against hospital isolates of multidrug-resistant Acinetobacter baumannii, and Bokaeian et al. [28] investigated the antibacterial activity of roselle (*H. sabdariffa*) ethanol extract against antibiotic-resistant *E. coli* and S. aureus isolated from urinary tract infections. The present findings are consistent with earlier reports on the antimicrobial effects of *H. sabdariffa* extracts against multidrug-resistant *Salmonella* and *E. coli* [3]. Antimicrobial screening of *H. sabdariffa* extracts likewise indicated activity against *E. coli* and S. aureus, with inhibition observed at a concentration of 2000 μg/mL [26]. The flavonoids in *H. sabdariffa* have been suggested to contribute to this antibacterial activity [14].

Oluwasina et al. [15] reported that *Syzygium aromaticum* exhibits antibacterial activity against *Escherichia coli* (6.67 mm), *Staphylococcus aureus* (14 mm) and *Staphylococcus epidermidis* (10.3 mm). Similarly, Mostafa et al. [6] found that an ethanolic extract of *S. aromaticum* had antibacterial activity against S. aureus. The antimicrobial properties of aqueous garlic extracts observed here are consistent with prior findings that garlic exhibits inhibitory effects on bacterial strains (*S. aureus*, 19.1 mm; *E. coli*, 16.9 mm) [18]. Garlic has been shown to possess antibacterial activity against S. aureus, *E. coli* and *Salmonella* in several studies [6, 7, 11].

Venâncio et al. [1] noted that *Allium sativum* did not show any antibacterial activity against methicillin-resistant strains of *S. aureus*, although it showed activity against penicillin-susceptible strains. In the current work, all the clinical isolates tested were found to have antibacterial activity, such as *Klebsiella pneumoniae*, *S. aureus* and *E. coli* [17]. Overall, the tested strains were clinical isolates exhibiting high resistance to many antibiotics, rendering these results particularly encouraging.

The antibacterial effects of *Nigella sativa* align with previous findings in which aqueous extracts of *N. sativa* seeds exhibited significant bactericidal activity against Staphylococcus sp. and *E. coli* [5]. Islam et al. [11] likewise found that *N. sativa* methanol extracts strongly inhibit both Gram-positive and Gram-negative clinical bacterial strains. Another study reported that a 50 mg/mL aqueous extract of *N. sativa* seeds was effective against *Klebsiella pneumoniae* (zone of inhibition 11 mm).

The findings indicate that the aqueous extract of *E. rostrata* exhibited bactericidal activity against the bacterial strains, which is consistent with numerous recent investigations that tested aqueous extracts of *E. rostrata* against diverse strains of *E. coli* and S. aureus. Ali and Hussein [8] demonstrated bactericidal activity of *E. rostrata* aqueous extract against S. aureus and *E. coli*. Al-Manhel and Niamah [29] also demonstrated an effect of *E. rostrata* aqueous extract on a variety of infectious organisms. Salman et al. [30] found that Eucalyptus aqueous extract showed varying antibacterial activity (inhibition zones ranging from 7 to 14 mm) against eight strains of multidrug-resistant S. aureus. Pandey and Singh [31] demonstrated in vitro antibacterial activity of aqueous extracts of Eucalyptus leaves against S. epidermidis, S. aureus and *E. coli*.

The mechanisms underlying the antibacterial effects of secondary metabolites are not fully understood, and multiple mechanisms may operate in concert rather than any single compound acting alone. Flavonoids could exert their effects by disrupting the cytoplasmic membranes, inhibiting the DNA gyrase and the hydroxyacyl-acyl carrier protein dehydratase activities [5]. Roselle extract flavonoids have been implicated in antibacterial activity in vitro, possibly via interactions with bacterial cell walls that enhance extract permeability. Other metabolic

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consequences, including electron transport chain component inhibition and phosphorylation steps, can also play a role in the observed activity, leading to higher membrane permeability and cellular contents leakage [10].

This is the first report of the antimicrobial effects of these five medicinal plants on the significant pathogenic bacteria identified in Babylon Governorate, Iraq, in clinical sources. The antimicrobial effects witnessed justify the traditional applications of these plants as a treatment of various diseases. Further research is warranted to identify and characterise the specific bioactive compounds responsible for the observed antibacterial activity.

## 4. Conclusions

The findings of the present research indicate that aqueous extracts of *N. sativa*, A. sativum, *E. rostrata*, *H. sabdariffa* and *S. aromaticum* have a strong antibacterial activity against the clinically relevant pathogenic bacteria. These results confirm the traditional application of these medicinal plants and indicate their possible use as safe, cost-effective and natural antimicrobial agents. To identify the particular bioactive compounds that mediate the observed antibacterial effects, further research is suggested to isolate and characterise them.

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**Author contributions**

I.J.K. and I.A.A.H.A. collected the plant material, prepared the extracts and performed the experimental work; I.H.Y. designed and supervised the study, analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

**Conflict of interest**

The authors declare no conflict of interest.

**Ethics approval and consent to participate**

The use of clinical bacterial isolates obtained from patients was approved by the ethics committee of the University of Al-Hamdaniya (Approval No.: UH.2026.016). All procedures were performed in accordance with the applicable institutional guidelines, and the confidentiality of the participants' identities was maintained throughout the study.

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