

Exploring the antibacterial efficacy of select plants against diverse pathogenic bacteria

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ABSTRACT

This research delves into the antimicrobial potential of aqueous extracts from six distinct plant species against clinically relevant bacterial pathogens. *Hibiscus sabdariffa* exhibits efficacy against *Streptococcus faecalis*, *Staphylococcus aureus*, and *Klebsiella sp.*, positioning it as a promising natural antimicrobial agent. *Trigonella filipes* and *Trigonella strangulata* display significant zones of inhibition against *Citrobacter sp.*, *Escherichia coli*, and *Pseudomonas aeruginosa*, underscoring their potential as sources of potent antimicrobial compounds. The study reveals variations in Minimal Inhibitory Concentration (MIC) values among the plant extracts, emphasizing their diverse antimicrobial profiles. *Allium sativum* (garlic) and *Medicago sativa* exhibit the lowest MIC of 4 μ l against *Staphylococcus aureus*, highlighting their efficacy, while *Trigonella strangulata* requires the highest MIC of 50 μ l. The research methodology, including the extraction process, bacterial cultures, and evaluation techniques such as the cup diffusion and MIC determination methods, is detailed. These findings provide valuable insights into the antimicrobial potential of six plant extracts, encouraging further exploration of their mechanisms and therapeutic applications in addressing bacterial infections. Keywords: Plant extracts, Antimicrobial activity, Minimal Inhibitory Concentration (MIC), Clinical pathogens, Natural remedies.

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Introduction

The natural world has emerged as a source of enormous promise in the persistent hunt of alternatives to battle bacterial illnesses. Throughout this investigation, particular plants have taken center stage, exhibiting an enthralling capacity to repel dangerous microorganisms. This article takes you on a trip through the vast tapestry of nature's defenses in search of latent antibacterial potential in certain plant species (1). The fight against bacterial diseases is an ongoing one, made more difficult by the frightening growth of antibiotic resistance. Scientists and researchers are increasingly looking to nature's medicine for novel remedies as we navigate these turbulent seas (2). Plants have long been regarded as the unsung heroes of the Earth's ecosystem due to their broad array of bioactive chemicals. Among these substances is a possible breakthrough in our continuing battle against harmful germs (3).

Our investigation into the antibacterial capabilities of specific plants is more than just a scientific investigation; it is an attempt to bridge the gap between old herbal wisdom and current

medicinal demands (4). Plant-based medicine has a long history, with societies all over the world using the therapeutic abilities of plants for ages. With the urgent issues posed by antibiotic-resistant forms of bacteria today, the necessity to reexamine and scientifically evaluate these ancient therapies has taken on tremendous importance (5). Our investigation into this botanical world takes us beyond the laboratory and into the heart of various ecosystems. The plants under consideration constitute a cross-section of botanical variety, with each containing distinct chemicals with intriguing antibacterial capabilities. These plants have adapted and developed, building natural defenses against microbial dangers from lush rainforests to parched deserts (6). Pathogenic bacteria are microscopic organisms that pose a significant threat to human, animal, and plant health by causing various infectious diseases. These bacteria have evolved sophisticated mechanisms to exploit host environments, often resulting in a range of illnesses with diverse clinical manifestations. The ability of pathogenic bacteria to evade the host's immune defenses, adhere to host tissues, and produce toxins contributes to their virulence. Understanding the biology,

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epidemiology, and pathogenesis of these bacteria is crucial for developing effective strategies for disease prevention, diagnosis, and treatment. As researchers delve into the intricacies of host-pathogen interactions, the importance of combating antibiotic resistance and exploring innovative therapeutic approaches becomes increasingly apparent. This article explores the world of pathogenic bacteria, shedding light on their impact on global health and the ongoing efforts to address the challenges they present (7).

The importance of this research rests not only in the potential discovery of novel antibacterial drugs, but also in the long-term and environmentally beneficial nature of plant-based remedies. Plant-derived chemicals are frequently more harmonious than manufactured antibiotics, which can lead to environmental deterioration and antibiotic resistance (8). "Antibacterial Potential of Some Plants Against Various Pathogenic Bacteria" becomes more than a scientific study; it becomes a story of hope and promise. It's an investigation into the delicate dance of flora

and fauna, a search for answers woven into the very fabric of the natural world.

Materials and Methods

Plant Collection: The plant collection for this study was conducted in the Erbil city. The plant species were chosen based on a combination of traditional knowledge and scientific literature highlighting their potential antibacterial properties (Table-1). Local communities were consulted to gather insights into traditional uses of plants in the region, ensuring the inclusion of species with historical significance. Upon collection, plant specimens were carefully processed for preservation. Techniques such as drying or freezing were employed to maintain the integrity of bioactive compounds. Proper labeling and documentation ensured traceability and facilitated subsequent analyses (9, 10).

Table 1. List of plant species tested for antibacterial activity

Sl.No.	Plant name	Family
1	<i>Allium sativum</i>	Amaryllidaceae
2	<i>Hibiscus sabdariffa</i>	Malvaceae
3	<i>Medicago sativa</i>	Fabaceae
4	<i>Teucrium polium</i>	Lamiaceae
5	<i>Trigonella filipes</i>	Fabaceae
6	<i>Trigonella strangulate</i>	Fabaceae

Extraction of Plant Compounds: Plant samples (100g) from the plants underwent a meticulous process: they were thoroughly cleansed, gently dried with blotting, and then macerated using a waring blender with 100ml of sterile distilled water for duration of 10 minutes. The resulting macerate underwent a two-step filtration process, first through a double-layered muslin cloth and subsequently via centrifugation at 4000g for 30 minutes. The supernatant, obtained post-centrifugation, was further filtered through Whatman No. 1 filter paper and subjected to heat sterilization at 120°C for 30 minutes, establishing it as the mother extract. This extract was aseptically preserved in sterile brown bottles at 5°C until it was ready for subsequent use (9, 11).

Bacterial Cultures: Clinical strains of *Citrobacter sp.*, *Escherichia coli*, *Klebsiella sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus faecalis* were sourced from the Department of Biology at Education College, Salahaddin University, Erbil. These bacteria were subcultured every two weeks and frequently maintained on nutrient agar slopes. In the antibacterial activity experiments, the aforementioned microorganisms were used as test pathogens (12).

Assessment of Antibacterial Activity: The antibacterial efficacy of the aqueous extracts was evaluated using the cup diffusion method on nutrient agar medium (13). The cup diffusion method involved creating wells in nutrient agar plates using a cork borer (5 mm). Subsequently, an inoculum containing 10 CFU/ml of bacteria was evenly spread on six solid

plates using a sterile swab moistened with the bacterial suspension. Following this, 50 µl of the aqueous extract was introduced into the wells on the inoculated plates. As a control, 50 µl of sterilized distilled water was also included. The plates were then incubated at 37°C for 24 hours, and any observed zones of inhibition around the wells were measured in millimeters (mm). Each treatment was replicated 12 times for statistical robustness. As a reference, antibiotics (10mcg) Bacitracin and Ciprofloxacin were employed to determine the sensitivity of each bacterial species under examination.

Determining Minimal Inhibitory Concentration (MIC): MIC was assessed using both agar and broth dilution methods (14). In the broth dilution tests, 0.1ml of a standardized bacterial suspension (10 CFU/ml) was added to each tube, containing varying concentrations of the aqueous extracts (02-50 µl/ml), and then incubated for 24 hours at 37°C. Alternatively, in the agar plating method, dilutions ranging from 02-50 µl of the aqueous extracts were placed in wells on inoculated plates and incubated as described earlier. The lowest concentration in either the tube or the plate that exhibited no visible growth upon microscopic evaluation was identified as the MIC.

The obtained data underwent statistical analysis using SPSS for Windows.

Results and Discussion

Table 2. outlines the antibacterial activity of aqueous extracts from various plant species, measured in terms of the zone of inhibition (mm), against different pathogenic microorganisms. Notably, *Allium sativum* (Garlic) displays robust antibacterial efficacy across the board, exhibiting significant zones of inhibition against all tested bacteria, with the largest inhibition observed against *S. aureus* (17.87±0.98 mm). *Hibiscus sabdariffa* also demonstrates notable activity, particularly against *Citrobacter sp.* and *Klebsiella sp.*

Medicago sativa exhibits moderate inhibition against several bacteria. *Teucrium polium*, on the other hand, shows no inhibition against *Citrobacter sp.* and *P. aeruginosa*, but displays a moderate inhibitory effect against *E. coli*, *S. faecalis*, and *S. aureus*. *Trigonella filipes* and *Trigonella strangulata* exhibit varying degrees of antibacterial activity, with the former being particularly effective against *S. aureus*. These findings suggest that the examined plant extracts possess varying degrees of potency against different pathogenic microorganisms, emphasizing the need for tailored approaches in harnessing their antibacterial properties for potential therapeutic applications. Moreover, the results underscore the diverse and nuanced nature of plant-based

antibacterial agents, supporting further exploration and research into their specific mechanisms and potential clinical applications. The growth of the tested bacteria was impeded by both *Allium sativum* (garlic) and *Medicago sativa* (alfalfa), highlighting the potential antimicrobial attributes of these natural substances. Garlic, historically recognized for its medicinal qualities, contains bioactive compounds such as allicin, known for its antibacterial effects against a wide range of pathogens (15). Likewise, *Medicago sativa*, or alfalfa, is abundant in phytochemicals that may contribute to its antimicrobial characteristics (16). The observed inhibition of the tested bacteria implies that these plant-derived substances could be valuable resources for developing innovative antimicrobial agents. Further research is needed to comprehend the specific mechanisms of action and explore the potential applications of *Allium sativum* and *Medicago sativa* in the realm of antimicrobial therapy. These findings contribute to the expanding knowledge about natural compounds with antibacterial potential, offering promising paths for developing alternative and supplementary approaches to counter bacterial infections.

Table 2. Antibacterial activity of aqueous extracts of plant species on pathogenic microorganisms assessed as a zone of inhibition (mm).

Sl.No. Plants	<i>Citrobacter sp.</i>	<i>E. coli</i>	<i>Klebsiella sp</i>	<i>P. aeruginosa</i>	<i>S. faecalis</i>	<i>S. aureus</i>
<i>Allium sativum</i>	14.89±0.11	15.14±0.71	15.23±0.64	13.15±0.31	16.64±0.79	17.87±0.98
<i>Hibiscus sabdariffa</i>	10.57±0.37	9.97±0.39	14.19±0.42	10.02±0.51	14.65±0.68	13.51±0.34
<i>Medicago sativa</i>	11.84±0.63	09.73±0.16	10.89±0.17	09.69±0.20	10.05±0.18	9.98±0.19
<i>Teucrium polium</i>	0.00±0.00	8.01±0.12	7.67±0.11	0.00±0.00	8.63±0.21	9.21±0.16
<i>Trigonella filipes</i>	13.76±0.49	9.83±0.34	8.89±0.42	9.17±0.36	14.76±0.62	15.07±0.68
<i>Trigonella strangulata</i>	14.85±0.60	11.81±0.63	9.93±0.20	8.12±0.13	12.71±0.45	13.06±0.59

Table 3. Minimum inhibitory concentration of plant extracts on pathogenic bacteria.

Sl.No. Plants	<i>Citrobacter sp.</i>	<i>E. coli</i>	<i>Klebsiella sp</i>	<i>P. aeruginosa</i>	<i>S. faecalis</i>	<i>S. aureus</i>
<i>Allium sativum</i>	10	20	10	08	08	04
<i>Hibiscus sabdariffa</i>	20	10	20	20	10	20
<i>Medicago sativa</i>	08	10	20	10	20	04
<i>Teucrium polium</i>	nd	30	40	10	nd	40
<i>Trigonella filipes</i>	20	10	nd	20	10	20
<i>Trigonella strangulata</i>	20	40	50	50	nd	20

Hibiscus sabdariffa was effective against *Streptococcus faecalis*, *Staphylococcus aureus* and *Klebsiella sp.*, indicating its potential as a powerful agent with antimicrobial properties. This finding suggests that compounds present in *H. sabdariffa* may possess antibacterial activity, making it a promising candidate for further exploration in the development of natural antimicrobial agents (17). The ability of *H. sabdariffa* to inhibit the growth of these bacterial strains highlights its potential role in addressing infections caused by these pathogens. Further research is warranted to elucidate the specific mechanisms underlying the

antibacterial effects of *Hibiscus sabdariffa* and to explore its applications in the context of antimicrobial therapy. These results contribute to the growing body of evidence supporting the medicinal properties of *Hibiscus sabdariffa* and its potential as a natural remedy against specific bacterial infections (18).

Trigonella filipes and *Trigonella strangulata* exhibited the most significant zone of inhibition against *Citrobacter sp.*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This notable outcome suggests a strong antimicrobial effect of these plant species against the mentioned bacterial strains (19). The observed high degree of

zone of inhibition indicates the potential of *Trigonella filipes* and *Trigonella strangulata* as potent sources of antimicrobial compounds (20). Further investigation into the specific bioactive constituents responsible for this inhibitory activity would contribute to a deeper understanding of their antimicrobial mechanisms. These findings underscore the potential utility of *Trigonella filipes* and *Trigonella strangulata* in the development of natural antimicrobial agents, particularly in combating infections associated with *Citrobacter sp.*, *Escherichia coli*, and *Pseudomonas aeruginosa* (21).

- ***Allium sativum* (Garlic):** It exhibits relatively low MIC values against most bacteria, suggesting strong inhibitory effects. Particularly effective against *S. aureus* with an MIC of 04.
- ***Hibiscus sabdariffa*:** Shows varying effectiveness. Effective against *E. coli*, *P. aeruginosa*, and *S. aureus* but less so against *Klebsiella sp.*
- ***Medicago sativa* (Alfalfa):** Demonstrates good inhibitory activity, especially against *S. aureus* and *E. coli*.
- ***Teucrium polium*:** The MIC values vary widely, and it is not determined (nd) for *Citrobacter sp.* and *S. faecalis*. It is particularly effective against *P. aeruginosa*.
- ***Trigonella filipes* and *Trigonella strangulata*:** These plants show mixed results. *Trigonella strangulata* has a higher MIC against most bacteria compared to *Trigonella filipes*.

Comparative Analysis: Different bacteria respond differently to the same plant extract, highlighting the specificity of the antibacterial properties. The choice of plant extract for treatment may need to be tailored to the specific pathogen (Table 3).

The Minimal Inhibitory Concentration (MIC) of the all different plant extracts exhibited variations against distinct test pathogens. Notably, some plant extracts demonstrated no discernible activity, even at a concentration of 50 µl. The MIC values, representing the minimum concentration of the plant extract necessary to inhibit the growth of the test pathogens, are detailed in (Table 3). These findings underscore the diverse and selective antimicrobial potential of the studied plant extracts, highlighting the importance of understanding their specific efficacy against different pathogens. The variability in MIC values emphasizes the need for a comprehensive assessment of each plant extract's antimicrobial properties, paving the way for targeted applications in the development of therapeutic interventions against specific microbial infections.

Lowest MIC of 4 µl against *S. aureus* was observed *Allium sativum* (garlic) and *Medicago sativa*. Whereas highest MIC of 50 µl was needed to inhibit *Trigonella strangulata* against *Klebsiella sp.* and *P. aeruginosa*. *Allium sativum* (garlic) and *Medicago sativa* exhibited the lowest Minimal Inhibitory Concentration (MIC) of 4 µl against *S. aureus*, indicating a potent inhibitory effect on the growth of this pathogen (22). In contrast, *Trigonella strangulata* necessitated the highest MIC of 50 µl for inhibition. These findings

underscore the notable antimicrobial efficacy of *Allium sativum* and *Medicago sativa*, particularly against *S. aureus*, while highlighting the comparatively lower potency of *Trigonella strangulata* at the tested concentrations (23). Understanding these MIC variations provides valuable insights into the differential antimicrobial activities of these plant extracts, contributing to their potential application as natural agents for controlling bacterial infections. Further investigation into the specific bioactive compounds responsible for these effects could enhance our understanding of the mechanisms underlying their antimicrobial properties (24).

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