Comparative Evaluation of the Antimicrobial Properties of Selected Plant Extracts from Solanaceae and Grossulariaceae Families Against Clinical Isolates of Gram-Positive and Gram-Negative Bacteria

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Abstract

The Solanaceae family of vegetables significantly contributes to human health and well-being. This study investigates the antioxidant and antibacterial activity of four alcoholic plant extracts belonging to the Solanaceae family. The aim of the current study is to evaluate the inhibitory effect of Physalis angulata, Solanum niger, Lycium shawii, and Ribes uva-crispa against bacterial isolates obtained from various clinical specimens. A total of 200 clinical samples were collected from patients in Anbar City between September 2024 and January 2025, including specimens from burns, wounds, ear, nasal, and throat swabs, urine, blood, fluids, and abscesses. The experimental work was conducted at the Department of Biology, College of Education for Women, University of Anbar, during the academic year 2024–2025. The results of this study contribute to the growing interest in the medicinal potential of Solanaceae-derived compounds, particularly their antibacterial properties against clinical pathogens. Antibacterial activity of extracts were evaluated by a well diffusion method against two species of Gram-negative (Escherichia coli, and Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus, and Staphylococcus epidermis) and compared to standard antibiotic Clindamycin 10 ug (DA 10)/ Doxycycline 10 ug (DO 10 The study results showed variations in the inhibitory efficacy of the four plant extracts against clinically isolated bacterial strains. S. nigrum extract recorded the highest efficacy against S. aureus, with an average inhibition diameter of 15.33 ± 1.5 mm, followed by P. angulata (12 ± 0 mm), compared to antibiotic (DA 10), which recorded 30 ± 1 mm (P = 0.0001). For S. epidermidis, P. angulata and R. crispa outperformed with approximately the same efficacy (14.33 ± 2.1 and 14.33 ± 4.1 mm, respectively), compared to 18 ± 2 mm for the antibiotic (DO 10) (P = 0.002). Against E. coli, the effectiveness was limited, with the extracts recording between 3 ± 2.6 and 7.33 ± 2.1 mm, compared to 20 ± 2 mm for the antibiotic (C30) (P = 0.001). Efficiency against P. aeruginosa was also low, with L. shawii recording the least effectiveness, with an average of 1.33 mm, while P. angulata recorded the highest among plants, with 9.66 mm, compared to 20 ± 2 mm for the antibiotic (MRP) (P = 0.001). These results demonstrate that the plant extracts were more effective against Gram-positive bacteria than Gram-negative bacteria, reflecting the influence of cell wall composition on bacterial sensitivity to active plant compounds.

Keywords: Solanaceae family, antioxidant activity, antibacterial activity, against Gram-positive bacteria, Gram-negative bacteria .

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Introduction

The Solanaceae family is one of the largest and most economically significant families of flowering plants (angiosperms), encompassing approximately 83 to 90 genera and over 3,000 to 4,000 species. This family includes essential food crops such as potatoes, tomatoes, eggplants, chili peppers, as well as medicinally important species like Physalis angulata and Solanum nigrum (1). These plants are not only vital to global nutrition but also hold considerable economic value due to their diverse agricultural, nutritional, and pharmacological uses (2). The genus Solanum alone accounts for more than half of the known Solanaceae species and is characterized by extensive morphological variation and widespread global distribution, with the highest diversity observed in Latin America and Australia (3.(

In addition to their nutritional role, many Solanaceae species are well-documented for their therapeutic properties. They are rich in bioactive compounds such as flavonoids, alkaloids, phenolic acids, carotenoids, and saponins, which contribute to their antioxidant, antimicrobial, antiviral, anti-inflammatory, antihypertensive, antiplatelet, and cardioprotective effects. Among the species of interest, P. angulata (commonly known as wild gooseberry) is native to tropical regions of the Americas. It contains high levels of carotenoids (e.g., beta-carotene), flavonoids (e.g., quercetin, rutin), vitamin C (ascorbic acid), and several alkaloids, all of which contribute to its potent antioxidant capacity (4.(

S. nigrum, known regionally as black nightshade or black eggplant, is an annual herbaceous plant widely distributed across Asia, Europe, and Africa. It can grow up to one meter in height and bears small white flowers in clusters, producing dark, juicy berries rich in active phytochemicals such as solanine, asparagine, lutein, tannins, linoleic acid, and palmitic acid (5). These constituents are believed to underlie the plant's strong antioxidant and medicinal effects. This species typically grows in fields, orchards, gardens, canal banks, and arid zones, highlighting its ecological adaptability (6.(

Lycium shawii, a thorny perennial shrub, thrives on sandy and rocky slopes. It naturally blooms between March and April but can flower year-round under irrigation. Recent studies have demonstrated the antioxidant and antibacterial potential of its thorn extracts, further underscoring its therapeutic relevance (7.(

It is worth noting that Ribes uva-crispa (commonly referred to as gooseberry or Indian gooseberry) does not belong to the Solanaceae family but rather to the Grossulariaceae family (8,9). Despite this taxonomic distinction, it remains of interest due to its strong antioxidant activity. Native to temperate and cold climates—especially in Europe and parts of Asia—Ribes species are cultivated for their high content of polyphenols, ascorbic acid, vitamin E, and essential minerals. These compounds contribute to its health-promoting effects and adaptability to diverse environmental conditions, including loamy soils, partial shade, and freezing temperatures (10,11.(

Taken together, the selected plants exhibit a wide range of bioactive profiles and ecological adaptations, making them suitable candidates for comparative phytochemical and pharmacological evaluation. Therefore, the current study aims to assess and compare the levels of phenolic compounds, flavonoids, alkaloids, and terpenoids among these species, in an effort to establish a form of chemical taxonomy and explore their potential therapeutic applications, particularly in the

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context of antioxidant and antimicrobial activity.

Materials And Methods

Plant Sample Collection:

Fresh plants (Physalis angulata, Solanum niger, Lycium shawii, and Ribes uva-crispa) were collected over several field visits in the summer and fall .

The intact parts of the four plants mentioned above were taken to the herbarium of the College of Education for Women, University of Anbar, for identification and classification under the supervision of the supervising professor (Professor Dr. Ashwaq Talib Hameed). They were air-dried with continuous stirring, ensuring they were not exposed to direct sunlight to preserve the active compounds. They were then ground using an electric grinder to obtain a fine plant powder and stored in dry, clean, tightly sealed glass containers, each labeled with the name, place of collection, and surrounding environmental conditions.

Extract Preparation:

Five g of dried plant leaf powder was placed in 100 ml of methanol-water (80%) in an ultrasonic water bath for 30 minutes at 30°C. The sample was then filtered using Whatman No. 0.1 filter paper and extracted using a rotary evaporator. The extract was then dried using an electric oven at 40°C and stored in opaque glass bottles in the refrigerator until use (12.(

Isolation and identification of bacterial isolates

A total of 200 clinical specimens were collected from various sources, including (burn, wound, ear, nasal, throat swabs, urine, blood, fluid and abscesses) from patients in Anbar City between September 2024 and January 2025. After obtaining a single colony of isolated bacteria, the isolates were identified depending on phenotypic colony characteristics, including size, shape, mannitol fermentation, and Novobiocin disc diffusion.

Culture specimens

Immediately after collection, the samples were cultivated on MacConkey agar and Blood agar using the planning method, and the plates were incubated for 24 hours at a temperature of 37°C (13.(

Diagnosis of Bacteria (Morphological Diagnosis(

Colonies were initially identified through the phenotypic characteristics of isolated bacterial colonies grown on MacConkey medium and blood agar media, which included the shape, color, texture, odor, and size of the colonies. S. aureus bacteria were grown on Mannitol agar, while P. aeruginosa were grown on Cetrimide medium (Cetrimide agar). The diagnosis was confirmed through biochemical tests (indole test, methyl red test, urease test, oxidase test, and catalase test.(

Antibacterial activity test of plant extracts

The antibacterial activity of Physalis angulata, Solanum niger, Lycium shawii, and Ribes uva-crispa extracts against two species of Gram-negative (Escherichia coli, and Pseudomonas aeruginosa) and Gram-positive (Staphylococcus aureus, and Staphylococcus epidermis) and compared to standard antibiotic Clindamycin 10 ug (DA 10)/ Doxycycline 10 ug (DO 10), using agar well diffusion method. Muller Hinton agar (MHA) was performed and left until it solidifies, then circular wall holes with a diameter of (6) mm were made for each dish, and then the study fungi were planted and distributed homogeneously on the culture medium, then (50) microliters of extract were added at concentrations (25, 50, 75 and 100) mg/ µL in the holes prepared for them, and then the dishes were placed in the incubator at a temperature of 37°C. After (48) hours, the activation diameter (inhibition zone) was read and the positive control factor was prepared using distilled water(14, 15 .(

Ethics approval

This study was conducted in accordance with the ethical guidelines outlined in the Declaration of Helsinki. Prior to obtaining the sample, the patient's consent was obtained through written and verbal communication, after the review and acceptance of the study protocol and subject's information by the local ethics committee (3055 in 15/3/2024.(

Statistical Analysis

The results of the investigation were analyzed using SPSS version 22.0, and each experiment was carried out three times to ensure accuracy. All data were presented as Mean ± Standard Deviation (16.(

Results and Discussion

Morphological Characteristics

Diagnosis of E. coli and P. aeruginosa was based on the morphological characteristics of bacterial colonies on both rich and selective culture media. On MacConkey agar, E. coli bacteria were fermenting lactose sugar, so they gave pink, smooth, shiny colonies with sharp edges on the differential MacConkey agar medium containing bile salts and crystal violet dye, which allows the growth of Gram-negative bacteria, including the Enterobacteriaceae family, and inhibits the growth of Gram-positive bacteria, as in Figure (2-A). while P. aeruginosa colonies in MacConkey agar appeared pale because they did not ferment lactose as show in Figure (2-B). This is consistent with the findings of the researcher (17.(

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(A)

(B)

Figure 2: Show **(A**) *E. coli* **,** and **(B)** *P. aeruginosa* colonies on MacConkey agar medium

Bacterial isolates (Staphylococcus aureus and Staphylococcus epidermidis) were identified based on morphological characteristics after being grown on Blood Agar under aerobic conditions. The results showed that S. aureus formed large, smooth, convex, slightly elevated colonies with an opaque appearance and a distinctive creamy yellow color. These colonies were also surrounded by areas of beta-hemolysis, indicating its ability to lyse red blood cells (17), as shown in Figure (3-A). Staphylococcus epidermidis was identified based on the same morphological characteristics, forming large, white to gray, convex colonies but not non-hemolytic when grown on Blood Agar(18), as shown in Figure (3-B. (

(A)

(B)

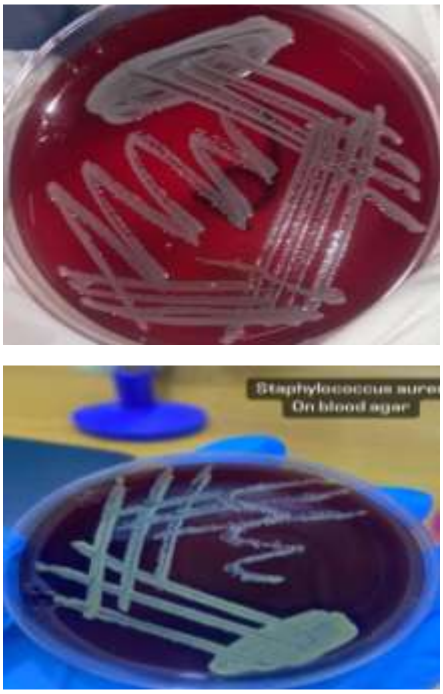

Figure 3: Identification of *Staphylococcus* spp. **A-** *S. aureus* on blood agar, and **B-** S. *epidermidis* on blood agar

Microscopic Examination

The bacterial isolates isolated in this study were stained using Gram stain. E. coli, and P. aeruginosa appeared pink upon microscopic examination, indicating that they are Gram-negative. Their rod-shaped shapes were observed, and their cells were arranged singly, in pairs, or in short chains (18), as shown in Figure 1.

(A)

(B)

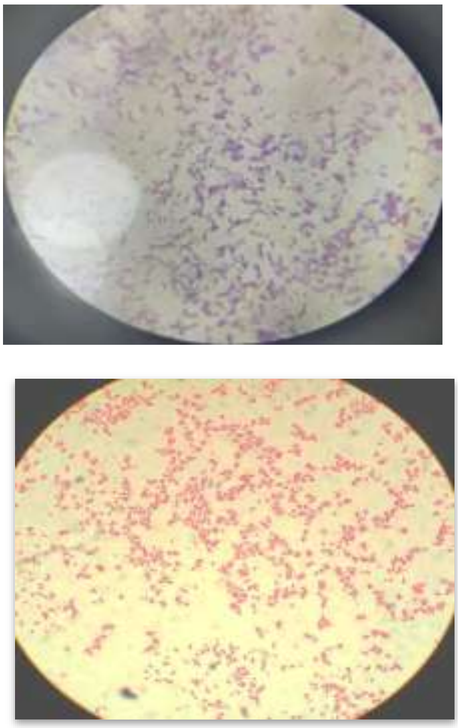

Figure 4:(A) *E. coli* and (B) P. *aeruginosa* bacteria under a light microscope (at a power of 40X and 100X

The results of microscopic examination of the Gram-positive bacterial isolates showed spherical cells clustered in the shape of grape clusters as shown in Figure (5 A and B), which agreed with the results of (19,20.(

Biochemical Tests

Biochemical tests were performed to identify both Gram-negative (P. aeruginosa, E. coli) and Gram-positive (S. aureus, S. epidermidis) bacterial isolates, as shown in Table 1. E. coli showed positive results for catalase, methyl red, indole, gas production, and motility tests, but were negative for urease, Voges-Proskauer, citrate consumption, oxidase, and H_2 S production.

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(A)

(B)

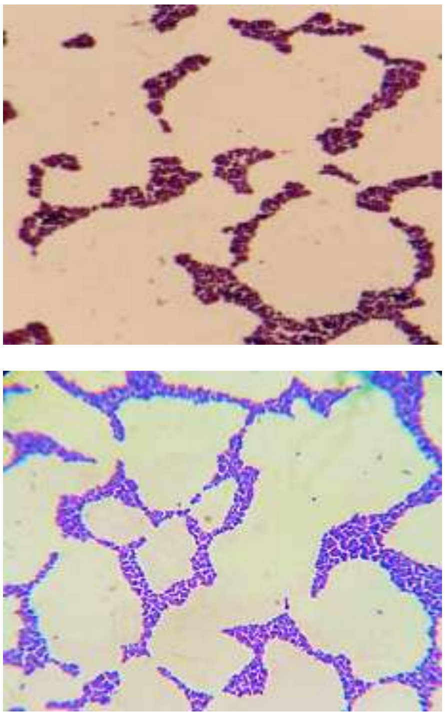

Figure 5. (A) *S. aureus* and (B) *S. epidermidis* under the microscope with Gram stain (lens power 1000X)

It grew on EMB medium and produced metallic green colonies indicative of lactose fermentation .

Table 1. Biochemical Test

EMB Result	Isolates	Catalase	Oxidase	Urease	MR	VP	Indole	Citrate	Motility	H_2 S	Gas	Gelatin	Mannitol
Green metallic	*E. coli*	+	–	–	+	–	+	–	+	–	+	–	+
Colorless colonies	*P. aeruginosa*	+	+	–	–	–	–	+	+	–	–	–	+
Not applicable	*S. aureus*	+	–	+	+	+	–	–	–	–	–	+	+
Not applicable	*S. epidermidis*	+	–	–	–	+	–	–	–	+	–	–	–

+ Positive - Negative

It also grew on TSI (A/A) and mannitol medium. P. aeruginosa isolates showed positive results for catalase, oxidase, citrate consumption, and motility tests, but were negative for urease, methyl red, indole, Voges-Proskauer, gas production, and H_2 S. When cultured on EMB medium, they produced clear, non-lactose-fermenting colonies and did not grow on iron (K/K) medium. Variations in growth were also observed on mannitol medium.

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As for Gram-positive bacteria, S. aureus showed positive results for catalase, urease, methyl red, Voges-Proskauer, coagulase, nitrate reduction, and gelatin liquefaction tests. It was also capable of growing and fermenting mannitol, but was negative for motility, indole, oxidase, citrate consumption, and H_2S production. S. epidermidis showed positive results in catalase, Voges-Proskauer, susceptibility to Novobiocin, and H_2S production tests, but was negative in oxidase, urease, indole, methyl red, motility, and gelatin liquefaction tests (19,20.(

Biological study

In this study, the inhibitory activity of four plant extracts (Physalis angulata, Solanum niger, Lycium shawii, Ribes uva-crispa) against four clinically isolated bacterial species was evaluated and compared with standard antibiotics as shown in Table 2, and Figure 6 (A,B, and C). The results showed that the standard antibiotic (DA 10) was the most effective against S. aureus with an average inhibition diameter of 30 ± 1 mm, indicating its strong conventional effect .

As for the plant extracts, Solanum nigrum came in first with an average inhibition diameter of 15.33 ± 1.5 mm, followed by Physalis angulata with 12 ± 0 mm. The lowest values were for Ribes uva-crispa (11 ± 3.6 mm) and Lycium shawii (11.33 ± 1.2 mm). Statistical analyses showed a significant difference between the extracts and the control group (P = 0.0001), as well as between the plant extracts themselves (P = 0.02), indicating the presence of significant differences.

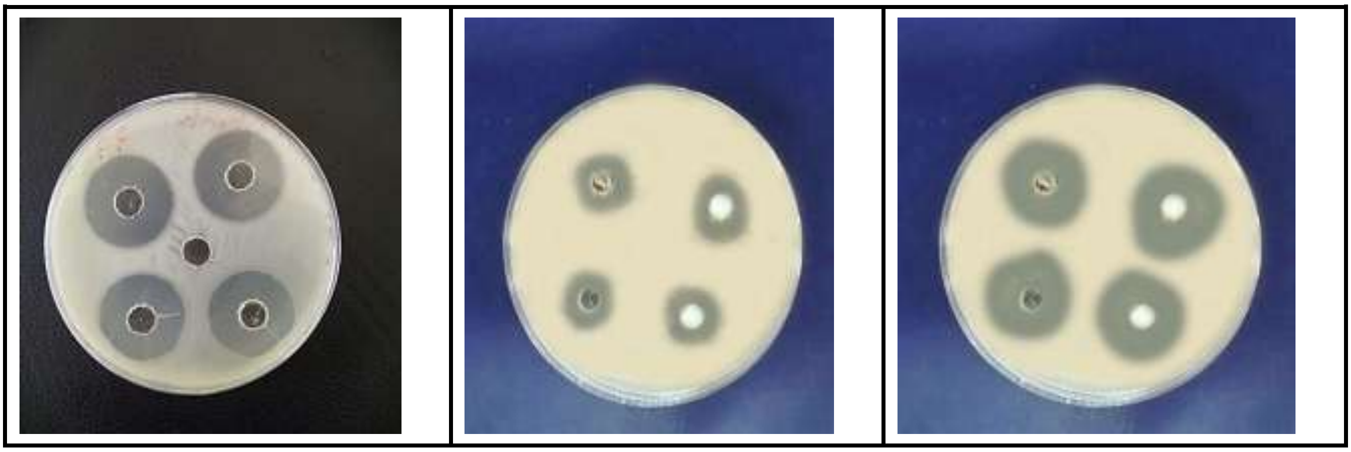

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Table 2. Zones of inhibition in (mm) for the four extracts and comparison with standard antibiotics

Type of organisim	*Physalis angulata*	*Solanum niger*	*Lycium shawii*	*Ribes uva-crispa*	Antibiotic	P value Between tested groups and control	P value Between tested groups only
Staphylococcus aureus	C 12±0	B 15.33±1.5	C 11.33±1.2	C 11±3.6	A 30±1 (DA 10)	0.0001	0.02
Staphylococcus epidermis	B 14.33±2.1	C 11±1	D 9.33±1.2	B 14.33±4.1	A 18±2 (DO 10)	0.002	0.05
Escherichia coli	6.33±2.6	7.33±2.1	3±2.6	7±4.4	20±2 **(C30)**	0.001	0.09 N.SIG
Pseudomonas aurous	9.66	4	1.33	3.333	20±2 **(MRP)**	0.001	0.07 N.SIG

*LSD test was used to calculate the significant differences between tested mean, the letters (A, B, C and D) LSD for represented the levels of significant, highly significant start from the letter (A) and decreasing with the last one.. Similar letters mean there are no significant differences between tested mean p≤0.05 were considered significantly different. Clindamycin 10 ug (DA 10)/ Doxycycline 10 ug (DO 10).

While the antibiotic (DO 10) recorded an activity of 18 ± 2 mm against S. epidermidis, it was partially superior to Physalis angulata and Ribes uva-crispa with almost the same diameter (14.33 ± 2.1 mm and 14.33 ± 4.1 mm, respectively). Solanum nigrum performed weaker (11 ± 1 mm) and so did Lycium shawii (9.33 ± 1.2 mm). Statistical analysis revealed significant differences between groups compared to the antibiotic (P = 0.002), while the differences between the extracts themselves were at the significance threshold (P = 0.05). For E. coli, the antibiotic C30 showed good efficacy with an inhibition diameter of 20 ± 2 mm, while all plant extracts showed poor efficacy, with diameters ranging from 3 ± 2.6 mm for Lycium shawii to 7.33 ± 2.1 mm for Solanum nigrum. There was no significant difference between the effects of the different plant extracts (P = 0.09), while a significant difference was found when compared to the antibiotic (P = 0.001), indicating the weak efficacy of the extracts against this Gram-negative strain.

The plant extracts (Physalis angulata, Solanum niger, Lycium shawii, Ribes uva-crispa) showed good results against P. aeruginosa, as the diameters of Physalis angulata (9.66 mm), Solanum nigrum (4 mm), Ribes uva-crispa (3.333 mm), and Lycium shawii (1.33) were recorded, while the antibiotic (MRP) showed the strongest effectiveness with a diameter of 20 ± 2 mm against P. aeruginosa.

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Gram-positive bacteria generally exhibit higher sensitivity to plant extracts than Gram-negative bacteria, primarily due to structural differences in their cell walls. Gram-positive bacteria have a thick cell wall composed of multiple layers of peptidoglycan, which lacks an outer membrane. This allows active plant compounds—such as flavonoids, tannins, and volatile oils—to easily penetrate and interact with internal cell components (21). In contrast, Gram-negative bacteria have a lipopolysaccharide-rich outer membrane that acts as a barrier against most hydrophobic compounds and increases their natural resistance to many antibiotics (21). Furthermore, some Gram-negative bacterial strains exhibit increased activity of multidrug-resistant (MDR) efflux pumps, which reduce the intracellular concentration of active compounds, limiting their efficacy(22). These properties explain the experimental results that showed a remarkable efficacy of plant extracts against Staphylococcus aureus and Staphylococcus epidermidis, versus weak efficacy against Escherichia coli and Pseudomonas aeruginosa.

Conclusion

The research concluded that the tested plant extracts, particularly from the Solanaceae family, are promising sources of antimicrobials. Their remarkable effectiveness against Gram-positive bacteria, compared to Gram-negative bacteria, is primarily attributed to the fundamental differences in the composition of their cell walls. This suggests that the active compounds in these plants are able to easily penetrate the less complex cell walls of Gram-positive bacteria, while the outer membrane of Gram-negative bacteria acts as a strong barrier.

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