

Article

The Effectiveness of Sidr Leaf Extract (*Ziziphus Spina-Christi*) Against Some Types of Microorganisms Isolated from UTI Patients

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Abstract: *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the leading causes of urinary tract infections. These bacteria have also become resistant to antibiotics, and accordingly, attention has been directed towards medicinal plants as alternative treatments. Objective: To study the inhibitory effect of alcoholic and aqueous extract of *Ziziphus spina-christi* against pathogenic bacteria. In this study, **100 samples** were collected from urine from patients attending and lying in a hospital (**Mosul Teaching Hospital - Public Health Laboratory**) of various ages and genders for the period from December 1, 2023 to March 10, 2024, Preparation different concentrations (25, 50, 75 and 100) mg/ml of an alcoholic and aqueous extract of the Sidr plant. The results indicated that the number of isolates that showed positive growth was 57 out of **100 isolates** distributed between *S. aureus* and *P. aeruginosa*. In contrast, the rest of the isolates showed negative growth. Laboratory tests were conducted on 26 of 50 samples positive for *P. aeruginosa*. The Gram-positive isolates included 31 isolates, with a percentage of (50%), *S. aureus*, out of a total of 50 isolates, and the Gram-negative isolates, with a rate of (50%), *P.aeruginosia*, out of a total of 37 isolates. At (25, 50, 75 and 100) mg/ml, the alcoholic extract recorded inhibition zone was (5.5, 10.12, 12.7, 15.55)mm and (6.77, 12.90, 17.77, 20.1) mm against *P.aeruginosa* and *S.aureus* respectively, while aqueous extract recorded inhibition zone (1.01, 2.22, 3.05, and 3.75) mm, against *S.aureus*. It was also observed that the cold aqueous extract had no inhibitory effect on *P.aeruginosa* isolates. Alcoholic extracts were more effective against both bacteria types than aqueous extracts for all concentrations.

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1. Introduction

Medicinal plants have been extensively investigated due to their nutritional [1] and pharmaceutical value [2]. The presence of carbohydrates, proteins, and lipids in medicinal plants allows them to meet the human body's dietary needs. The components above also significantly impact various morphological, physiological, and metabolic processes [3]. Studies have indicated that consuming 100 grams of a nourishing plant can provide more than 10 to 12% of the daily required dietary allowance (RDA). Plants also serve as highly significant reservoirs of medicinal molecules. Several novel biologically active therapeutic compounds and their phytochemical components, such as hypolipidemic, hypoglycemic, and antioxidant, can be extracted from plants. Many medicines derived from plants, directly or indirectly, are now being extensively used [4]. Humanity has been fascinated with the

therapeutic use of plant-derived remedies for managing illnesses since ancient times [5].

Plants serve as ecological repositories of antimicrobial substances with therapeutic properties. It is often believed that these agents are nearly devoid of the typical adverse effects linked with antimicrobials of synthetic origin [6, 7]. Many contemporary pharmaceuticals have been derived from natural sources, and their isolations were primarily based on the knowledge of the properties of the substances in traditional medicine. *Ziziphus* is a genus consisting of circa 40 species of prickly shrubs and small trees belonging to the buckthorn family, Rhamnaceae. These plants are found in warm-temperate and subtropical climates worldwide [8]. Within traditional medicine, Sidr is employed for its antibacterial properties, demulcent effects, toothache astringency, and mouthwash [9]. Furthermore, it treats several medical conditions, including liver disorders, urinary problems, digestive disorders, weakness, obesity, diabetes, skin infection, loss of appetite, fever, bronchitis, pharyngitis, anaemia, sleeplessness, and diarrhoea [10]. Historically, the fruits and leaves of the Sidr plant were considered the most significant components for medicinal purposes [11]. Ferulic acid, rutin, hydroxybenzoic acid, and chlorogenic acid are phenolic compounds found in Sidr fruits and leaves with antioxidant properties. The leaves are abundant in ceanothus, betulinic acids, saponins, flavonoids, triterpenes, tannins, and flavonoids [12].

This work aimed to study the inhibitory effect of alcoholic and aqueous extracts of *Ziziphus spina-christi* against pathogenic bacteria.



Figure 1: Leaves of *Ziziphus mauritiana* [13]

2. Materials and Methods

Sample Collection

In the current study, the leaves of the Sidr plant (*Zizyphusspin-csit*), which grows in Iraq, were used. Plant leaves are collected from the garden of the College of Agriculture / Tikrit University. The plant leaves were washed with tap water to remove dirt and dust, then with distilled water. After that, the leaves were placed in the shade to dry completely for seven days. The dried leaves were pounded until they became homogeneous powder using an electric blender. The plant powder was stored for further use [14].

Preparation of Extract

In the present study, two solvents, **distilled water and ethanol**, were employed for the extraction process. 50 gm of dried plant materials (purified powder) were. They were incubated in 500 ml of distilled water and methanol at ambient temperature for 24 hours. The extracts were filtered using three layers of muslin cloth filter paper No.1 and subjected to centrifugal force of 3000 (g) cycles per minute for

15 minutes. The resulting liquid was collected and dried in an oven at 40°C. The dry powder was then stored in the refrigerator for future use in testing the efficacy against the bacteria used in the experiment. The plant sample weighed 15 g and was dissolved in 15 ml of distilled water to obtain a 100% viable stock solution.

The concentrations employed in this investigation were 10%, 20%, 30%, 40%, and 50% of the plant's aqueous and methyl alcohol extracts. To prepare the extracts for the inhibition tests, they were sterilised using a 0.45 µm diameter membrane filter [15].

Patient specimen sampling

The present investigation involved the collection of 100 urine samples from patients with urinary tract infections attending the Mosul Teaching Hospital - Public Health Laboratory, various age groups and genders from December 1, 2023, to March 10, 2024.

Culture specimens

Immediately after collection, the samples were cultivated on MacConkey agar and mannitol saline medium using the planning method, and the plates were incubated for 24 hours at 37°C [16].

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, including the colonies' shape, colour, texture, odour, and size. *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, methyl red, urease, oxidase, and catalase tests**).

Antibacterial activity test of plant extracts

The antibacterial activity of *Ziziphus spina-christi* extracts against *Streptococcus mutans* using agar well diffusion method. Muller Hinton agar (MHA) was performed and left until it solidified. Circular wall holes with a diameter of (6) mm were made for each dish. Then, the study fungi were planted and distributed homogeneously on the culture medium, and (50) microliters of extract were added at concentrations (25, 50, 75 and 100) mg/ µL in the holes prepared for them. 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[7, 17].

Ethics approval

This study was conducted according to the ethical guidelines outlined in the Declaration of Helsinki. Before obtaining the sample, the patient's consent was obtained through written and verbal communication after the local ethics committee reviewed and accepted the study protocol and the subject's information (3055 on 15/3/2023).

Statistical Analysis

The investigation's results were analysed using SPSS version 22.0. Each experiment was carried out three times to ensure accuracy. All data were presented as the Mean ± Standard Deviation [18].

3. Results

This study, 100 urine samples were collected from patients of various ages and genders attending and lying in hospitals (Azadi General—Kirkuk General—Public

Health Laboratory) from 1/12/2023 to 10/3/2024. The number of positive urine samples reached 57, which contained bacterial growth, while the remaining 43 were negative for bacterial growth, as shown in Figure (2).

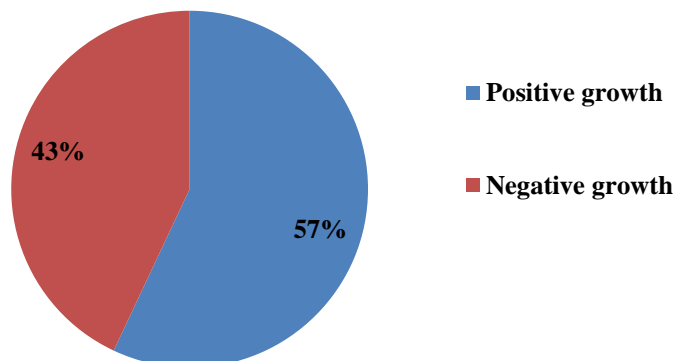


Figure 2: Rate of distribution of bacterial isolates according to growth

Cultural Characteristics

In the current study, **26 positive growths for *Pseudomonas spp* and 31 for *S. aureus* isolates** were obtained; it was grown for the first time on MacConkey medium and blood agar, in addition to Cetrimide agar. Its colonies were pale white on MacConkey medium due to its inability to ferment the sugar lactose, in addition to its ability to cause lysis of blood cells on blood agar to give β . Haemolysis pattern [19] and its ability to grow on selective medium Cetrimide agar, as shown in Figure (3).

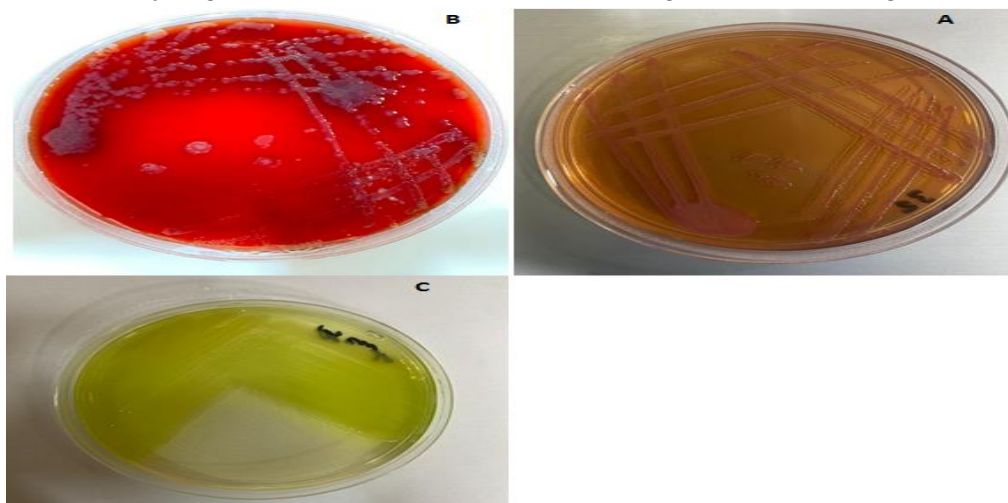
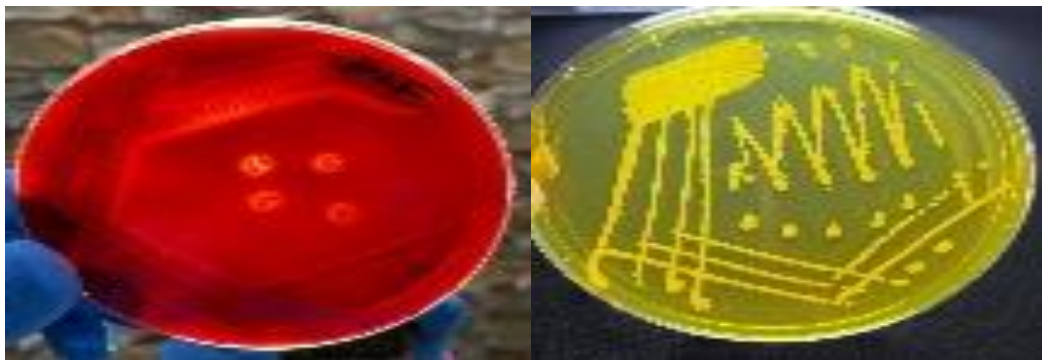


Figure 3: *P.aeruginosa* colonies (A) on blood agar (B) on MacConkey agar and (C) on Cetrimide agar

Staphylococcus aureus was diagnosed to grow on blood medium and does not grow on McConkey medium. By relying on its phenotypic characteristics, a preliminary diagnosis was made of it, as 31 of them were confirmed to be *S. aureus* bacteria, which formed small, convex colonies tending to a yellowish-white colour, surrounded by a halo of completely decomposed blood (haemolysis- β). In addition to its ability to grow in the mannitol salt agar medium and turn it from pink to yellow as a result of its

fermentation of the mannitol sugar contained in the culture medium, its colonies appear in the form of golden yellow, spherical and small, as shown in Figure (4).



Biochemical test

The results of biochemical tests on 26 isolates of *Pseudomonas spp* bacteria showed that all of these isolates were positive for both **oxidase and catalase tests** due to the appearance of purple when the oxidase reagent was added. This is an indication that the bacteria contain cytochrome oxidase as a receptor for hydrogen and catalase through the appearance of air bubbles containing hydrogen peroxide gas, which can reduce citrate and does not produce H₂S gas [20], **negative for the indole test** because the red ring does not appear on the surface, as well as **the methyl red test** because the colour of the medium does not turn red, and **negative for the urease test**. After all, the colour of the medium does not turn pink, as shown in **Table 1**.

Table 1: Biochemical tests for identification of *P.aeruginosa*

Tests	Results
Catalase test	+
Oxidase test	+
Indole test	-
Methyl-red	-
Urease test	-

The results of the biochemical tests Showed that all *S. aureus* isolates gave positive results for **the catalase, urease, and Methyl red tests** and **negative results for the oxidase and indol tests**.

Table 2: Biochemical tests for identification of *S. aureus*

Tests	Results
Catalase test	+
Oxidase test	-
Indol test	-
Methyl-red	+
Urease test	+

Testing the inhibitory effectiveness of extracts

A study showed the effect of cold aqueous and alcoholic (ethanol) plant extracts of the *Ziziphus spina-christi* plant on the growth of *P.aeruginosa* and *S.aureus* bacteria isolated from the urinary tract.

The results of Figure 6 show the bacterial isolates and the effectiveness of the extract of the *Ziziphus spina-christi* plant. The alcoholic extract recorded the highest rate of inhibition on both isolated *P.aeruginosa* and *S.aureus* bacteria, which reached an average diameter of (15.55 mm and 20.1mm) respectively at a concentration of 100 mg/ml, as it was better than low concentrations (25, 50, and 75) mg/ml, which recorded (5.5, 10.12, 12.75) mm against *P.aeruginosa*, and recorded (6.77, 12.90, 17.77)mm against *S.aureus* respectively.

The effect of the extracted aqueous extract on *S.aureus* isolates isolated from the urinary tract gave the concentrations mentioned above an inhibitory effect, which amounted to 1,01, 2.22, 3.05, and 3.75 mm, respectively. It was also observed that there was no inhibitory effect of the cold aqueous extract of the *Ziziphus spina-christi* plant on the *P.aeruginosa* isolates. From the results, we find that the higher the concentration of the alcoholic extract, the greater the diameter of inhibition against the two types of bacteria used in the study.

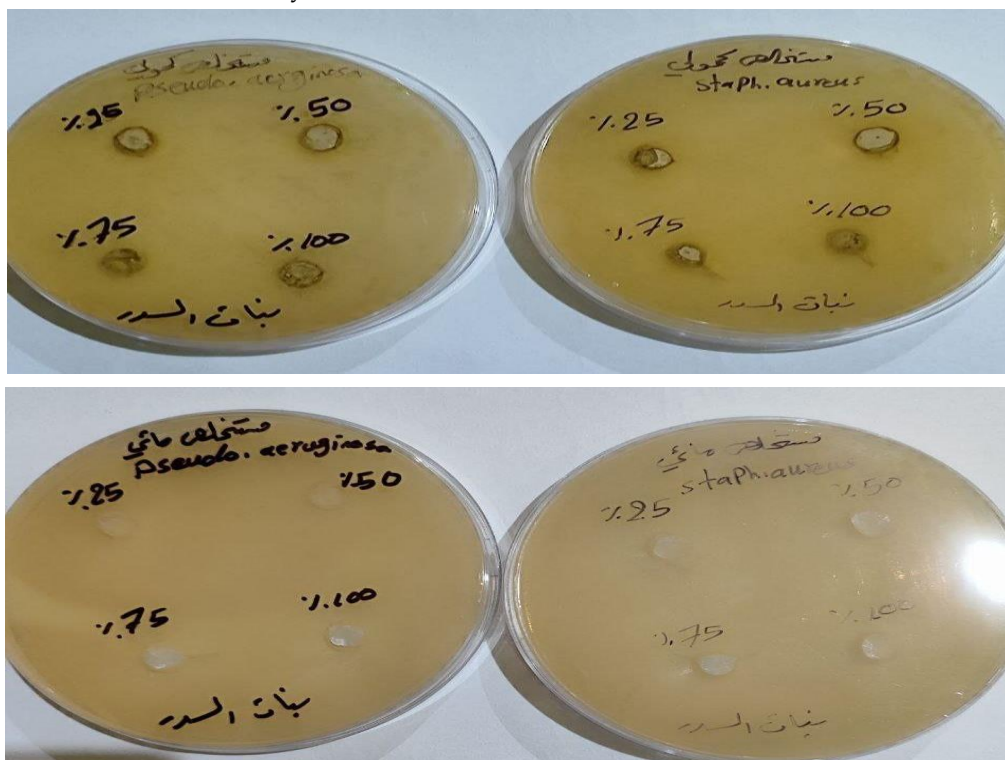
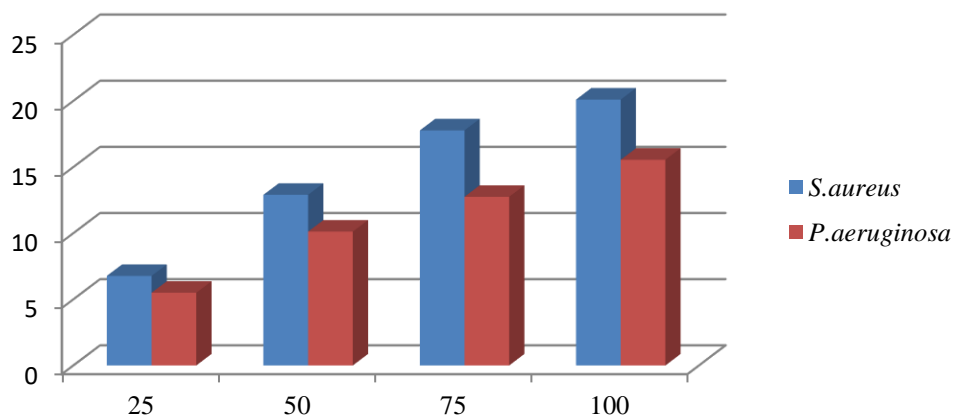


Figure 5: Inhibitory zone effect of (a) alcoholic (b) aqueous extract against *P.aeruginosa* and *S.aureus* isolates, respectively.

a



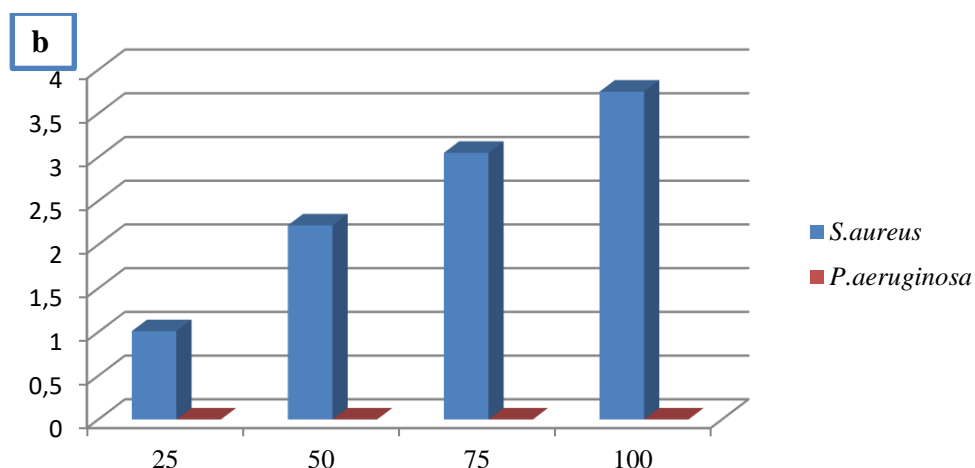


Figure 6: Inhibitory effect of (a) alcoholic (b) aqueous extract against *P.aeruginosa* and *S.aureus* isolates

In recent years, medicinal plants have taken a significant role in research. Plants have been used to treat many types of microorganisms. The *Ziziphus Christi* plant has been used as a disinfectant for wounds and many bacterial and fungal germs. Infusing its leaves and branches could also treat diabetes and high blood pressure.

The *Ziziphus spina-christi* plant has inhibitory activity because it contains glycosides, saponins, tannins, flavonoides, and alkaloids [21]. The results were similar to the research presented by El-Hashash *et al.*, where they concluded that the *Ziziphus spina-christi* plant extracts have high effectiveness against some organisms[12]. Microscopic organisms, including *P.aeruginosa* and *S.aureus*, agreed with[22]. Our results also agreed with the findings of the researcher, who showed the effect of the alcoholic extract of the garlic plant with a higher inhibitory diameter on *S. aureus* than the aqueous extract because the alcoholic extract contains many active compounds that have an inhibitory effect on pathogenic bacteria, and the reason for the ineffectiveness of the aqueous extract is the lack of effectiveness of the compounds against bacterial isolates[23].

4. Conclusion

Medicinal plants are considered an important alternative to medicines for treating many diseases, including urinary tract infections, abdominal pain, minor wounds, and burns. Alcoholic extracts of the Sidr plant are more effective than aqueous extracts. The higher the concentration of the extract, the greater its inhibitory effect.

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