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Research Article

Evaluation of Aqueous Extract of (Ziziphus spina-christi) leaves as a Natural Antiseptic

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Abstract

Background and Objective: Excessive use of chemical antiseptics like ethanol causes adverse skin conditions, while rising antimicrobial resistance necessitates sustainable alternatives. This study evaluated the antibacterial efficacy of aqueous leaf extracts of *Ziziphus spina-christi* (Sidr) from Karbala Governorate, Iraq, as an eco-friendly disinfectant against pathogenic bacteria. **Materials and Methods:** Dried leaves were prepared using two aqueous extraction methods: infusion (40°C for 24 hours) and decoction (boiled to 100°C). Phytochemical screening tested for phenols, alkaloids, flavonoids, and saponins. Antibacterial profiles were evaluated against five Gram-positive and four Gram-negative bacterial isolates using the agar well diffusion method and compared with Gentamycin (0.01 mg/ml). **Results:** Phytochemical screening confirmed an abundance of bioactive secondary metabolites including, alkaloids, flavonoids, phenols and saponin. Both aqueous preparations demonstrated significant broad-spectrum antibacterial activity. The aqueous infusion consistently exhibited superior inhibition over the boiled decoction across all strains. Infusion zones of inhibition ranged from 10±0.1 mm to 15±0.3 mm for Gram-positive bacteria, and 12±0.1 mm to 21±0.2 mm for Gram-negative bacteria. *Escherichia coli* was the most susceptible strain (21±0.2 mm), while *Streptococcus pneumoniae* was the least. Crucially, most tested clinical strains exhibited resistance to Gentamycin but remained highly vulnerable to the Sidr infusion extract. **Conclusion:** The aqueous infusion of *Ziziphus spina-christi* leaves possesses potent antimicrobial properties capable of overcoming conventional antibiotic resistance. Due to its energy-efficient preparation, lack of side effects, and robust antagonistic performance, it serves as a promising natural alternative for antiseptic and disinfectant formulations.

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1. Introduction

Modern daily lifestyle practices have significantly contributed to the overuse of antiseptic agents. The overuse is driven by increased awareness of hygiene, fear of infections and the widespread of antimicrobial products (Lompo and Jacobs,2023). The most important agent that is currently used, particularly since the post outbreak of the COVID-19 pandemic, ethanol, is a clear organic liquid used in the manufacturing of many products and medical preparations. Medically, alcohol is used as a sterilising, disinfecting, and antiseptic agent. It's applied to the skin to clean it before medical procedures like blood draws or surgical operations. It is also used to disinfect the hands of doctors, nurses, and other healthcare workers (López-Gigosos *et al*., 2017). Ethanol kills bacteria by destroying the bacterial membrane and causing the bacterial cell to die. Since single-celled microorganisms like bacteria and viruses are made of molecules that alcohol molecules can bind to, this effectively helps to eliminate these microorganisms very quickly. Ethanol also affects other targets, including the synthesis of DNA, RNA, and peptidoglycan proteins, and these are considered secondary effects after the destruction of the cell membranes (Aka, 2015; Liu *et al*., 2020). Despite the potent antimicrobial efficacy of ethanol, the prolonged or excessive application of alcohol can result in adverse effects on cellular integrity and skin barrier function. The side effects of medical ethanol are that it is a fast-evaporating substance, which leads to the skin losing water and heat. This loss causes skin dryness and can lead to cracks. Through these cracks, the skin becomes more susceptible to absorbing any infection, which may cause inflammation. Studies have shown that excessive use of medical alcohol can lead to dry eczema, which is accompanied by severe itching that causes the appearance of wounds, ulcers, and skin spots (Hübner *et al*., 2011).

The scarcity of current antimicrobial drugs and the growing need for new ones for therapeutic purposes are the main reasons that prompt specialists to search for new sources for such medications (Muteeb *et al*.,2023; Sharma *et al*.,2026). Among them is the search for an alternative antimicrobial agent to be used instead of conventional chemical ones that lead to the emergence of resistant microbial strains (Murugaiyan *et al*., 2022). One of these plants is the Sidr plant extract, where numerous laboratory studies, particularly the aqueous extract, possess antibacterial, antifungal, and antiviral properties (Abdulqahar, *et al*., 2023). Inspired by the botanical wealth of Iraq and the diverse medicinal species found in the Iraqi plant encyclopedia and considering the widespread use of the Sidr plant in traditional medicine (Ali *et al*., 2017).

Plants are rich in renewable, effective natural compounds that have the ability to eradicate the growth of a wide range of microorganisms. Plant extracts are traditionally used to treat infectious diseases and organ disorders (Asgarpanah at *et al*.,2012; El-Shahir *et al*.,2022).

Ziziphus spina-christi belongs to the family Rhamnaceae and is locally called Sidr. The Sidr is widely distributed in various regions of the world, especially in tropical, subtropical, and arid regions (Gibreel, 2019; Rizwan *et al*., 2016). It's a perennial that grows naturally in the Arabian Peninsula, adapting to desert conditions and tropical regions (Ali *et al*., 2017). The fact behind this plant is the active compound that can be found in all parts, including the leaves, branches, roots, fruits, and flowers. Thus, it has been linked to medicinal, therapeutic, and spiritual benefits since ancient times (Taghipour *et al*., 2020).

The biological activity of the Sidr is attributed to its content of compounds with an inhibitory effect on the growth of microorganisms. The most notably active compound are tannins, saponins, glycosides, alkaloids, phenols, steroids, and resins (Elghaffar *et al*.,2022). In addition to the organic acids, vitamins, amino acids, fats, waxes, and sugars (Ashraf *et al*., 2015; Bai *et al*., 2016). Collectively, makes it as a promising candidate as a natural antimicrobial agent against pathogenic microorganisms (Elghaffar *et al*.,2022) The aim of this study is to evaluate Sidr leaf extract (using both boiled and infused aqueous extraction methods) as an alternative environmentally friendly disinfectant against pathogenic bacterial species.

2. Materials and Methods

2.1 Collection of Plant Samples

To conduct laboratory studies, Sidr plant leaves were collected from Karbala Governorate. The samples were transported to the laboratory, where they were washed with distilled water. Afterwards, the leaves were placed in an electric oven to dry at a temperature of 55°C for approximately 10-12 hours. Finally, the leaves were ground to obtain a powder, then stored at room temperature for later use (A Elanany & Salem., 2024).

2.2 Extraction of Plant Samples

The aqueous extract, both infusion and a decoction were prepared according to the method described by (Fotakis *et al*.,2016). This was done by mixing 20 grams of the dried leaves with 400 ml of distilled water in a 1000 ml volumetric flask. The mixture was stirred in a shaking water bath for 24 hours at a temperature of 40°C. Afterwards, the extracts were filtered using several layers of sterile medical gauze. As for the decoction, it was prepared in the same way, but the mixture was boiled until it reached a temperature of 100°C. Then allowed to cool and filtered with sterile medical gauze. Finally, both extracts are sterilised by filtration using a Millipore filter with a pore size 0.22 µm.

2.3 Detect the active compound of the aqueous extract

As described by **(**Dahanayake *et al*.,2019), the following active compounds were detected: **Phenols** were detected using the lead acetate test, where three drops of Pb(OAc)2 were added to 5 mL of the extract and mixed well; the formation of a yellow precipitate indicated the presence of phenols. **Alkaloids** were detected using the Wagner's reagent test. Two drops of Wagner's reagent (1 g of iodine and 3 g of potassium iodide dissolved in 50 mL of distilled water) were added to 2 mL of the extract and mixed well; the appearance of a reddish-brown color indicated the presence of alkaloids. **Flavonoids** were tested by adding 5 mL of the extract to 5 mL of an ammonia solution, followed by the addition of a concentrated solution of H2SO4. The formation of a yellow color indicated the presence of flavonoids. **Saponins** were detected by adding 5 mL of the extract and 2.5 mL of water to a test tube, shaking vigorously, and allowing it to stand for 10 minutes. The froth was then mixed with 3 drops of olive oil and shaken vigorously to observe the formation of an emulsion. The presence of a stable froth indicated that saponins were present in the extract.

2.4 Antibiotics Used

To test the sensitivity of bacteria to antibiotics and compare it with the plant extracts and their active compounds. The Gentamycin 0.01 mg/ml was used as a positive control, since it considers as a broad-spectrum antibiotic.

2.5. Bacterial isolates

The tested bacteria, including *Streptococcus pneumonia* , *Staphylococcus aureus*, *Staphylococcus epidermidis, Enterococcus* and *Bacillus subtilis* as a gram-positive bacterium, in addition to *Proteus mirabilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as gram-negative bacteria, were kindly provided by Razaza and Western Euphrates research unit, College of Science, Kerbala University. The bacterial suspension was prepared by culturing in a test tube containing 5 ml of nutrient broth. The inoculated tubes were incubated for 18 hours at 37°C.

2.6. Testing Bacterial Susceptibility to Antibiotics as a positive control

The gel well diffusion method was used to test the susceptibility of bacterial isolates to antibiotics, following the method by (Egorove, 1985). This method involves pouring 20 ml of Muller-Hinton agar into each plate. Then, 50 microliters of each bacterial culture were spread onto the plates using a glass spreader. The plates were left to dry for half an hour before forming the well. Under a septic condition, two wells per plate using a sterile Cork-Borer with a diameter of 6mm were made. Afterwards, 25 microliters of the prepared antibiotic Gentamycin: 0.01mg/ml was loaded into the well. As for the control plate, sterile water was added. After that, the plates were left in a refrigerator for 1 hour to let the antibiotic diffuse in the agar. Then, the plates were incubated in an incubator for 18-24 hours at 37°C. Finally, the diameter of the inhibition zone was measured using a ruler to determine the effectiveness of the antibiotic.

2.7. Testing the antagonistic effectiveness of the aqueous extract

The agar well diffusion method (El Kahlout *et al*.,2020) was used to test the sensitivity of bacterial isolates to the aqueous extract of Sidr in an infusion and a decoction form. The same steps were repeated as in a previous section, except that 50 microliters of the plant extract were added to each well. Sterile distilled water without the plant extract was applied as a negative control. The diameter of the inhibition zone was measured using a ruler to determine the effectiveness of the plant extract.

2.8. statistical analysis

All experiments were performed as a triplicate. The average of inhibition zone for each test was calculated. One-way ANOVA was used to identify significant differences between and among samples. Additionally, a descriptive analysis was performed using IBM SPSS statistical program version 20. A p-value of ≤ 0.05 was deemed significant.

3. Results

The antibacterial activity of the aqueous infusion and aqueous decoction was evaluated against several Gram-positive and Gram-negative bacterial strains by measuring the average diameter of the zone of inhibition (mm). The results are summarized in Table 3. Both the aqueous infusion and aqueous decoction exhibited noticeable antibacterial activity against all tested bacterial strains compared to water.

The aqueous infusion extract demonstrated higher antibacterial activity compared to the aqueous decoction across all tested strains:

Gram-Positive Bacteria: the aqueous infusion, the zone of inhibition ranged from 10±0.1 mm (for *Staphylococcus aureus*) to 15±0.3 mm (for *Staphylococcus epidermidis*). For the aqueous decoction, the zones were notably smaller, ranging from a minimum of 3±0.2 mm (*Streptococcus pneumonia*) to a maximum of 7 mm (*Bacillus subtilis*, *Staphylococcus epidermidis*, and *Enterococcus*). For Gram-Negative Bacteria: A similar trend was observed. The aqueous infusion produced large inhibition

zones ranging from 12±0.1 mm (*Klebsiella pneumoniae*) to 21±0.2 mm (*E. coli*), whereas the decoction yielded significantly lower values ranging from 3 mm (*Proteus mirabilis* and *Pseudomonas aeruginosa*) to 8±0.2 mm (*E. coli*).

Table 1: Diameter of the inhibition zone for the aqueous infusion and aqueous decoction extract of dried Sidr leaves against some pathogenic microorganisms.

The studied bacteria		**Average Diameter of the Inhibition Zone (mm)± SD**		
		Aqueous infusion	Aqueous decoction	Water negative control
Gram positive bacteria	*Streptococcus pneumonia*	10±**0.3**	3±**0.2**	0±**0.0**
	Bacillus subtilis	13±**0.3**	7±**0.1**	0±**0.0**
	Staphylococcus aureus	14±**0.1**	4±**0.1**	0±**0.0**
	Staphylococcus epidermidis	15±**0.3**	7±**0.3**	0±**0.0**
	Enterococcus	11±**0.1**	7±**0.2**	0±**0.0**
Gram negative bacteria	*Proteus mirabilis*	14±**0.1**	3±**0.1**	0±**0.0**
	Pseudomonas aeruginosa	17±**0.3**	3±**0.1**	0±**0.0**
	Klebsiella pneumoniae	14±**0.1**	5±**0.1**	0±**0.0**
	E. coli	21±**0.2**	8±**0.2**	0±**0.0**

Among all the studied bacteria, *Escherichia coli* was the most susceptible strain to both preparations, yielding the highest inhibition zones of 21±0.2 mm and 8±0.2 mm for the infusion and decoction, respectively. Conversely, *Streptococcus pneumonia* exhibited the lowest susceptibility overall, with an inhibition zone of only 3±0.2 mm in the decoction extract, although it remained moderately susceptible to the infusion (10±0.3 mm).

As a positive control, Gentamycin (0.01 mg/ml) was evaluated against the tested bacteria using the inhibition zone diameter. The typical average inhibition zone diameters (mm) and the standard interpretive guidelines from the Clinical and Laboratory Standards Institute (CLSI) for a standard Gentamicin (10 µg) disk against the studied strains demonstrated in Table 2. All tested bacteria showed a resistance to gentamycin antibiotic except *Bacillus subtilis* that showed a sensitivity towards this antibiotic

Table 2: Diameter of the inhibition zone for the antibiotic Gentamycin (0.01 mg/ml) against testing bacteria.

The studied bacteria		**Average Diameter of the Inhibition Zone (mm)± SD**	
		Gentamycin 0.01 mg/ml	
Gram positive bacteria	*Streptococcus pneumonia*	8±**0.2**	**R**
	Bacillus subtilis	21 ±**0.2**	S
	Staphylococcus aureus	12±**0.2**	**R**
	Staphylococcus epidermidis	13±**0.3**	**R**
	Enterococcus	9±**0.2**	**R**
Gram negative bacteria	*Proteus mirabilis*	15±**0.2**	**R**
	Pseudomonas aeruginosa	16±**0.1**	**R**
	Klebsiella pneumoniae	12±**0.2**	**R**
	E.coli	12±**0.3**	**R**

R: resistance S: sensitive

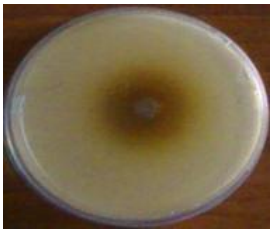
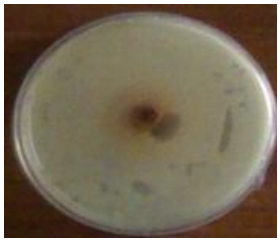
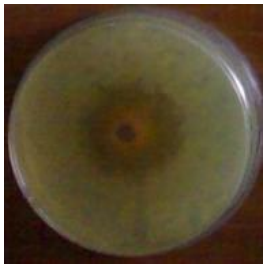
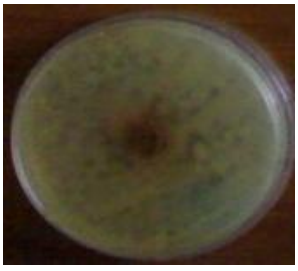
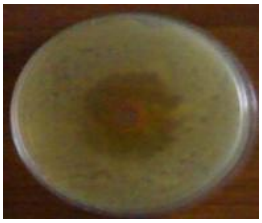
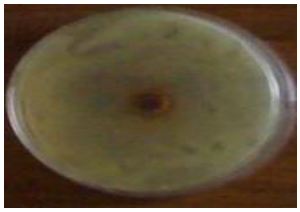
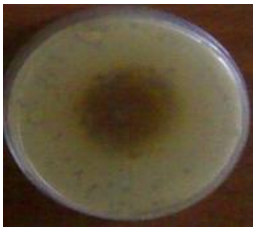
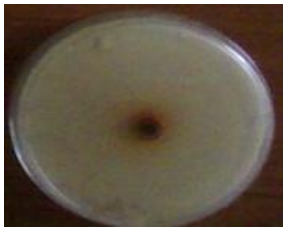
A- **aqueous infusion extract**	B- **aqueous decoction extract**
Streptococcus pneumonia 	*Streptococcus pneumonia* 
Bacillus subtilis 	*Bacillus subtilis* 
Pseudomonas aeruginosa 	*Pseudomonas aeruginosa* 
proteus mirabilis 	*proteus mirabilis* 

Figure 1: (A) An example of visual image of the inhibition zone for the aqueous infusion extract of dried Sidr leaves against some pathogenic microorganisms. (B) Visual image of the inhibition zone for the aqueous decoction extract of dried Sidr leaves against some pathogenic microorganisms

As a comparison between the infusion and decoction of Sidr extract against the tested bacteria, the results are demonstrated in Figure 2. It is clearly shown that aqueous infusion of dried Sidr leaves showed a significantly higher antagonistic effect against all four tested bacteria compared to the decoction extract.

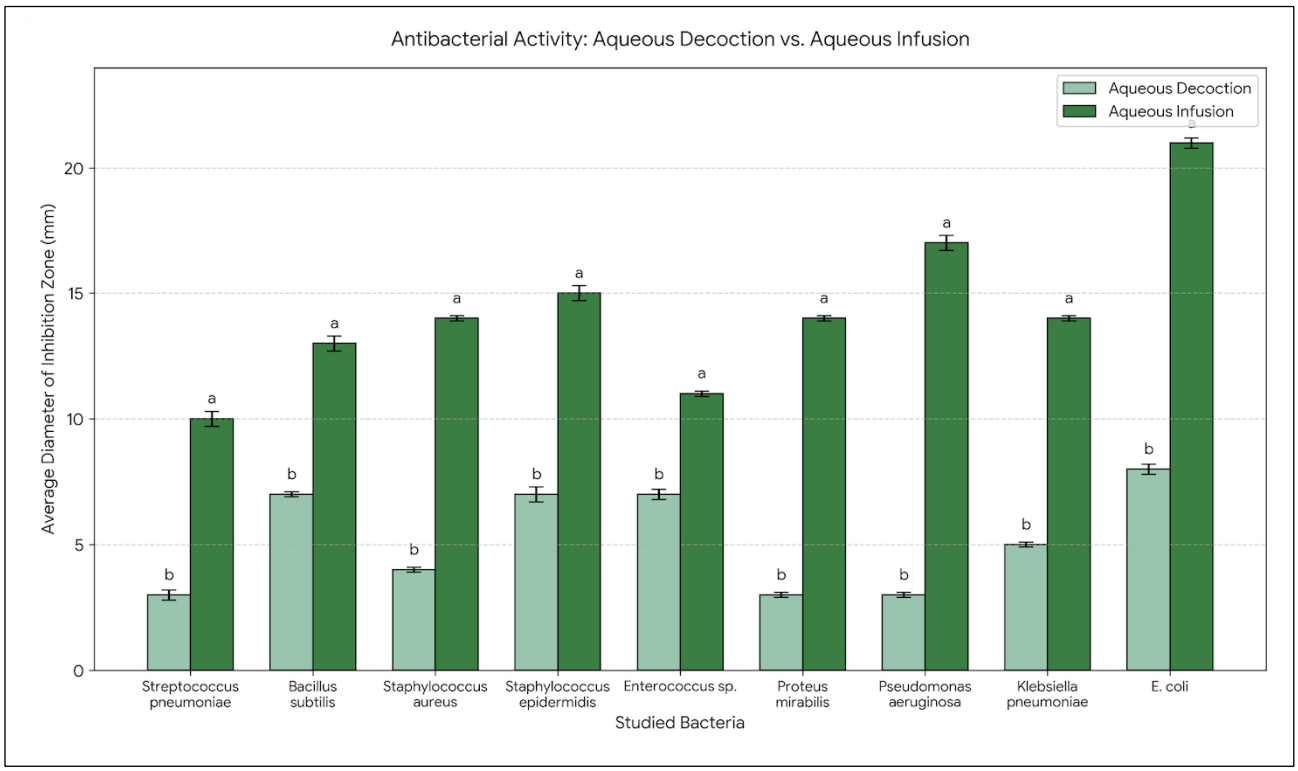


Figure 2: Comparison of the inhibition zone of both aqueous infusion and decoction extract of dried Sidr leaves against some pathogenic microorganisms.

The phytochemical screening of the extract revealed the presence of several major classes of secondary metabolites, including alkaloids, flavonoids, phenols, and saponins. The results are summarized in Table 3.

The Wagner's test confirmed the presence of alkaloids through the appearance of a distinct reddish-brown color, indicating a moderate concentration (++) within the extract. Similarly, the Pb(OAc)2 test for phenols yielded a yellow precipitate, while the ammonia-H2 SO4 test for flavonoids produced a characteristic yellow color. Both phenols and flavonoids were found to be moderately abundant (++) in the sample. Notably, the frothing test demonstrated the highest relative abundance (+++) among the tested groups, characterized by a highly stable froth and subsequent emulsion formation, confirming a strong presence of saponins.

Overall, these results indicate that the extract is a rich source of bioactive compounds, with saponins and alkaloids being the most prominent constituents.

Table 3: The phytochemical screening of liquid extract pf sidr leaves

Phytoconstituent	Test Performed	Intensity / Abundance	Concentration Level	Observation / Positive Result
Alkaloids	Wagner's Test	++	High	Reddish-brown color
Flavonoids	Ammonia-H2 SO4 Test	++	Moderate	Yellow color
Phenols	Pb(OAc)2 Test	++	Moderate	Yellow precipitate
Saponins	Frothing Test	+++	High	Emulsion formation

4. Discussion

To study the effect of the aqueous plant extracts of Sidr (infusion and decoction) on the pathogenic and environmental bacteria, the agar well diffusion method (Egorove, 1985) was applied. The experimental results showed an inhibitory effect depending on two parameters: the type of aqueous extraction (infusion and decoction) and the type of tested bacteria.

The results showed that the aqueous infusion of Sidr leaves had an inhibitory effect against all the pathogenic bacterial species that were tested as showed in Table 1. The main reason could be attributed to the hindering the synthesis of the bacterial cell wall or affecting membrane permeability, which leads to the loss of vital functions. The difference in the intensity of the inhibitory effect was also shown depending on the type of bacteria. For the gram-positive bacteria, The extract showed effectiveness against all tested bacteria. This is explained by the structural composition of the cell wall, where the absence of lipopolysaccharide (LPS) barrier ease the penetrate the active compound inside the cell, hence it disrupted the proteins of the cell membrane (Dahiru *et al*.,2023). For Gram-negative bacteria , the extract also showed an effectiveness activity against all tested bacteria. Meanwhile, Gram -negative bacteria have an outer layer of lipopolysaccharide (LPS) that prevent the permeability of active compounds (Xu *et al*.,2026), but the active compounds in the plant extract have a major role on this activity. This pronounced sensitivity is driven by the abundant water-soluble saponins and polar flavonoids identified during phytochemical testing. Operating as natural surfactants, saponins interact dynamically with the lipid-dense outer membrane of Gram-negative strains, leading to structural instability and increased membrane permeability. Furthermore, the polar phenols present in *Ziziphus* may act as efflux pump inhibitors; by blocking the bacteria's ability to expel these active compounds, they trap the phytochemicals internally and significantly expand the zone of inhibition (Zai *et al*.,2025). Once the outer membrane is compromised, it allows all the other antibacterial flavonoids and phenols to inter inside the cell, killing it efficiently (Ammar-Amin *et al.,*2026). In general, the effectiveness of Sidr plant extracts is attributed to the presence of phenolic compounds, which have an inhibitory effect on both Gram-positive and Gram-negative bacteria. This is consistent with previous studies by (Brito *et al.,* 2015; Sousa *et al.,* 2018), which showed that Sidr leaves are rich in phenolic compounds and flavonoids that have properties against pathogenic bacterial strains. Also , Alkaloids has a significant role in the antibacterial activity , where it can act through multiple targets that harden the bacteria to develop a resistance against the plant extracts including alkaloids (Mohammed *et al*., 2025).

However , it was observed a clear differences in the sensitivity of bacterial species to the natural extracts (infusion and decoction) at varying rates. The results for the aqueous infusion was effective against all the bacteria that used in the study, compared to the aqueous decoction as demonstrated in Figure 1 and Table 2. The Sidr leaf infusion was the most effective and serves as a natural source for an antiseptic, which is consistent with the findings of (Elhaj ., 2024). This indicates that the active ingredients in the plant are more effective, and that temperature affects their potency. The reason for the variation in these results is that the antibacterial activities affected by several factors, such as different temperatures and an increase in pH. It also depends on the effect of the extraction method on the plant's active compounds. It was found that the antagonistic activity of the volatile type of active substances may be weakened or reduced by using heat and the type of solvent used in the extraction (Awlqadr *et al.,*2025). Moreover, the extraction efficiency, differences in the quality or composition of the same plant species, or even the variations in environmental conditions and genetic differences altogether affected on the rate of antibacterial efficacy (Al-Bayatti *et al*., 2011). Another important factors that effect the potential antibacterial activity of plant extract is the polarity of the solvent, and the time of preparation of the extract, as keeping it for a long time may cause the active substance to lose its properties (Ramesh *et al*.,2024).

In this study, an aqueous (water-based) extraction method was intentionally selected over an alcoholic extraction for *Ziziphus*. This choice was guided by the principles of green and eco-friendly chemistry, aiming to eliminate the toxic chemical waste and environmental footprint associated with organic solvents. Furthermore, utilizing a liquid water extract ensures high practical feasibility and accessibility, allowing the preparation to be easily replicated outside of a specialized laboratory environment–such as for personal or traditional daily use–without the safety hazards of handling volatile or flammable alcohol. So to make sure that Sidr leaf extract could have a broad-spectrum activity, Gentamyicin at a concentration of 0.01 mg/ml was used as a control to compare the extract with, as both have the same mode of action. Interestingly, the tested antibiotic showed less effectiveness compared to both the infusion and decoction extracts as demonstrated in Table 3.

The problem of widespread bacterial infections acquired in hospitals and from the community, especially after the spread of the coronavirus, has made the use of 70% medical alcohol a very quick alternative for hand sanitisation. However, the excessive use of medical alcohol has shown many side effects on the skin, such as allergies, dermatitis, and eczema, which cause itching and pain. Alcohol also causes the drying of the upper layer of the skin over time, leading to its peeling (Reichel *et al*., 2009; Hübner *et al*., 2011). It's also known that no disinfectant can remain effective for a long time. Therefore, from a practical standpoint, we recommend using the aqueous infusion of *Zizyphus spina-christi* (Sidr) leaves as a natural hand sanitiser to replace 70% medical alcohol. This is due to its inhibitory activity against *Pseudomonas aeruginosa*, a bacterium known for its resistance to disinfectants and antibiotics, especially amid current health and environmental challenges from increasing bacterial resistance to most synthetic antibiotics. Adding such natural plant products to the manufacturing of antiseptic and disinfectant preparations is a sustainable and safe alternative solution.

Using the aqueous extract alone may limit its effectiveness in some applications, so it is preferable to combine it with another natural

agent to increase its potency or to load it nano-chemically to enhance its antibacterial activity. At the same time, there should be an intensive search of Iraqi plants to examine their biologically active compounds for use as therapeutic agents for treating inflammatory and infectious diseases. This effort would confidently support the fact that herbal treatments play an essential role in traditional medicine.

In summary, dried Sidr leaves demonstrate potent antimicrobial efficacy across a diverse range of microorganisms, highlighting their potential for integration into modern healthcare. Notably, the aqueous infused extract outperformed boiled preparations in bacterial inhibition, offering a simple, energy-efficient method ideal for resource-limited settings. By matching the performance of 70% medical alcohol, Sidr extract emerges as a viable natural disinfectant. It provides a safer, plant-based alternative that effectively eliminates pathogens without the adverse side effects often linked to synthetic chemical agents.

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