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Effect of Aqueous Extracts of Leaves and Fruits of *Ziziphus Spina-christi* on Bacterial Isolates

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Abstract

Because of the growing prevalence of pathogenic bacteria that are resistant to commonly prescribed antibiotics, many people are turning to medicinal plants for natural antimicrobials. *Ziziphus spina-christi* has been traditionally used for treating infections, but it still needs scientific studies to validate on its efficacy against bacterial pathogens. To establish scientifically the efficacy of the extract of *Ziziphus spina-christi* on different pathogenic bacteria, this study was carried out. The antibacterial activity of the aqueous and alcoholic extracts of both the leaf extract and the fruit extract of *Ziziphus spina-christi* was measured using agar well diffusion methods against the pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus at* Different Concentrations. The data were analyzed statistically using a One Way ANOVA followed by LSD testing at a significance level of $P \leq 0.05$. Qualitative phytochemical screening was also performed, and GC-MS analysis of the aqueous leaf extract only was performed. The leaf's extract was seen to be significantly more effective than the fruit's extract concerning their antibacterial properties; however, it was found that the leaves' extracts were all concentration-dependent. The leaf's extracts that used alcoholic solvents had a greater effect than those made using aqueous solvents did. Phytochemical screening of the leaves showed the presence of many of the plant's important compounds, including a broad range of phenolic, flavonoid, tannin, and terpenoid compounds. The GC-MS analysis of the aqueous extract from the leaves showed there to be several active molecules, the most abundant of which was Cyclohexanecarbonitrile, 1-(1-piperidinyl). *Ziziphus spina-christi* has a high potential to be a natural source for new antibacterial agents; as such, it will offer many possibilities in future pharmaceutical development.

Keywords: *Ziziphus spina-christi*; Aqueous extract; Antibacterial activity; Phytochemistry; GC-MS.

1. Introduction

Traditionally, plant sources have been known to contain larger numbers of bioactive compounds than any non-plant source. The increasing resistance of bacteria to traditional antibiotics has led to a growing interest in medicinal plant extracts as an alternative for antimicrobial therapy[1,2]. The potential for plant-derived antimicrobial therapies as an alternative to commercial antibiotics and their diverse chemical compositions and biological functions are important factors in microbiological, phytochemical, and plant science research[3].

Ziziphus spina-christi is a perennial tree that occurs in arid and semi-arid regions of the Middle East, North Africa, and Asia[4]. Throughout history, many parts of the plant have been used either directly or indirectly for different reasons, thus creating a scientific interest in its chemical makeup and biological activity[5]. Phytochemical studies conducted to date have described the diverse range of secondary metabolites found in *Ziziphus spina-christi*, including phenolic compounds, flavonoids, tannins, saponins, alkaloids, and terpenoids, many of which exhibit antimicrobial activity[6].

Bioactive compound yield and diversity from plant materials depend significantly on bioactive compound extraction methods and solvent types used[7]. Aqueous extractions have gained increasing interest because they are generally environmentally friendly and cost-effective and play an integral part in traditional use[8]. Since most traditional medicines utilize water as a solvent for the preparation of herbal medicines, testing the antibacterial actions of aqueous extracts has become an important means of determining the biological efficacy of plant-based compounds[9].

Several bacterial species, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, are considered to be model organisms for microbiological research owing to the ecological relevance and differential sensitivities exhibited by them to different classes of antimicrobial agents[10]. By measuring the responses of these bacteria to extracts of plants, we can further our understanding of the antimicrobial action of natural products[11].

The chemical characterization of plant extracts, in addition to biological evaluation, is necessary to determine which particular chemical compounds are responsible for establishing biological activity[12]. Because it provides information that can be used to identify volatile and semi-volatile phytochemicals, gas chromatography/mass spectrometry (GC-MS) has been used extensively as an analytical technique to identify compounds in plant materials[13]. The combination of phytochemical screening with GC-MS analysis, followed by testing the plant's antibacterial properties, represents a complete assessment framework for plant extracts' biological activity[14].

The current study aims to assess the antibacterial capacity of water-soluble extracts from *Ziziphus spina-christi*, derived from leaves and fruits, on a variety of isolated microorganism strains identified as having clinical relevance using the agar well diffusion technique. Additionally, qualitative screening procedures were conducted to examine the presence of active constituents related to antibacterial activity, and a further analysis through GC/MS of the phytochemicals present in these extracts was utilized to identify which phytochemical constituents are most likely responsible for the antibacterial activity noted in this study.

2. Materials and Methods

2.1. Plant material collection and preparation

Samples of leaves and fruits of the *Ziziphus spina-christi* were collected from the trees available in the garden of the Ibn Al-Haitham College of Education in September and October 2025. Aqueous extraction was performed by soaking a set weight of powdered leaf and fruit material in distilled water (1:10 g/ml) for 24 hours while stirring continuously in a room-temperature environment. After completion of the 24-hour soak, the entire mixture was then passed through Whatman No. 1 filter paper to remove the solid plant material, and the liquid extract was then concentrated to a low volume through evaporation via the use of a water bath at 40°C of temperatures[15].

The same plant material was then soaked in ethanol according to the same above procedure, and the dried extracts were stored frozen until needed. Various dilutions of the dried extracts were created with sterile distilled water at varying concentrations (40%, 20%, 10%, and 5%)[16].

2.2. Qualitative Phytochemical Screening

The qualitative analysis of the phytochemical constituents was carried out using both aqueous and alcoholic extracts of the leaves and fruits to identify the presence of major secondary metabolites. The detection of glycosides (Benedict's Test), alkaloids (Mayer's Reagent), tannins (1% ferric chloride), resins, and sapogenins (foam test and mercuric chloride) was tested by using established chemical procedures[17]. The presence of coumarins, flavonoids, phenolic compounds, terpenoids, and steroids was determined by using standard chemical methods, including sodium hydroxide and UV light (coumarins), ethanol and potassium hydroxide (flavonoids), and ferric chloride (phenolic); plus, the intensity of all responses was graded as weak (+), moderate (++), and strong (+++).[18]

2.3. GC-MS analysis

Chemical components found within *Ziziphus spina-christi* leaves were identified by gas chromatography-mass spectrometry (GC-MS) analysis. Using a GC-MS model QP2010Plus supported by SHIMADZU–Japan, with a capillary column (Optima 5HT-0.25 mm for 30m) and standard operating parameters, the identity of individual compounds was determined by comparing both the retention times and the mass spectral data against reference data available in the NIST library database[19].

2.4. Bacterial isolates

For this experiment, the bacterial species used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Laboratory stock cultures were utilized to provide the isolates with a source of bacteria and kept under refrigeration at a temperature of 4 °C on nutrient agar. Before performing the experiments, fresh bacterial suspensions were created using sterile saline, and the concentrations were standardized by measuring turbidity to ensure accuracy.

2.5. Antibacterial activity assay

The antibacterial properties of the extracts from the tested plants were assessed by the agar well diffusion method[20]. To ensure even distribution of bacterial inoculum, sterile cotton swabs were used to inoculate sterile nutrient agar plates with the bacterial suspensions. Using a sterile cork borer, uniform diameter wells were cut into the agar, which were subsequently filled with a constant volume of the plant extract at varying concentrations (40%, 20%, 10%, and 5%). Following inoculation and filling of the wells, plates were incubated at 37 °C for 24 hours. Upon completion of the incubation period, diameters of inhibitory zones surrounding each well were measured in millimeters (mm). The negative control in the agar well diffusion assay was sterile distilled water, which did not produce an inhibitory zone surrounding the control wells[21]. All experiments were conducted in duplicate.

2.6. Statistical analysis

Experiments in this research were replicated three times to ensure soundness of the data, which were presented as the average (Mean) and the standard error (SE). In the analysis of data from all treatments (extract concentrations, plant parts, and bacterial species), the following statistical analyses were performed: One-Way ANOVA using SPSS version 26 with Fisher's least significant difference (LSD) testing at the α-level of 0.05 to evaluate whether any significant differences existed between any of the treatments[22].

3. Results

3.1. Phytochemical screening results

Several bioactive groups of varying degrees of accordance were detected as a result of qualitative analysis for phytochemicals. Leaves were found to contain many bioactive groups, with the highest amount being produced by the alcoholic leaf extract, having higher levels of phenolics, flavonoids, tannins, terpenoids, and coumarin compounds compared with fruit. The aqueous fruit extract has relatively little phytochemical composition diversity (**Table 1**). That such a high degree of phenolic and flavonoid content exists within leaves is likely the reason for the unique level of antibacterial activity exhibited.

Table 1. Qualitative phytochemical screening of aqueous and alcoholic extracts of *Ziziphus spina-christi* leaves and fruits**.**

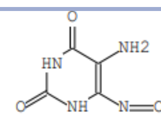
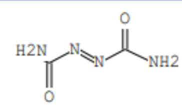
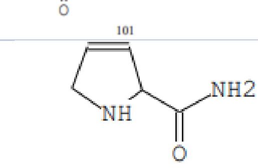
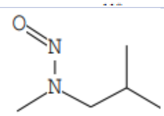
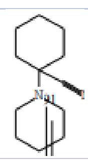
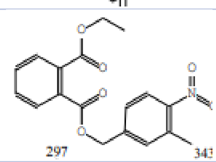
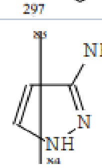
Phytochemical group	**Test reagent**	**Aqueous leaves**	**Aqueous fruits**	**Alcoholic leaves**	**Alcoholic fruits**
Glycosides	Benedict's test	+	−	+	+++
Alkaloids	Mayer's reagent	+	+	−	++
Tannins	$FeCl_3$ (1%)	+++	−	+++	−
Resins	Ethanol + HCl	−	+	−	++
Saponins	Froth / $HgCl_2$	+++	−	−	−
Coumarins	NaOH + UV	+	−	++	+++
Flavonoids	Ethanol + KOH	++	−	+++	+
Phenols	$FeCl_3$ (1%)	+++	−	+++	−
Terpenoids	Chloroform + Acetic anhydride + H_2SO_4	−	−	+++	+++
Steroids	Same as above	−	−	−	−

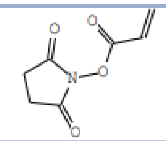
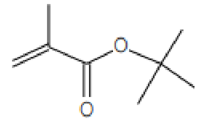
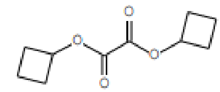
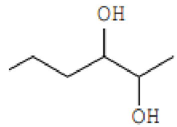
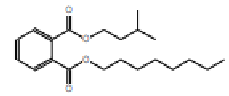
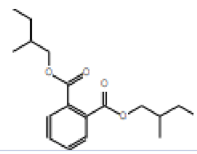
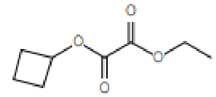
\+ weak, ++ moderate, +++ strong, − absent.

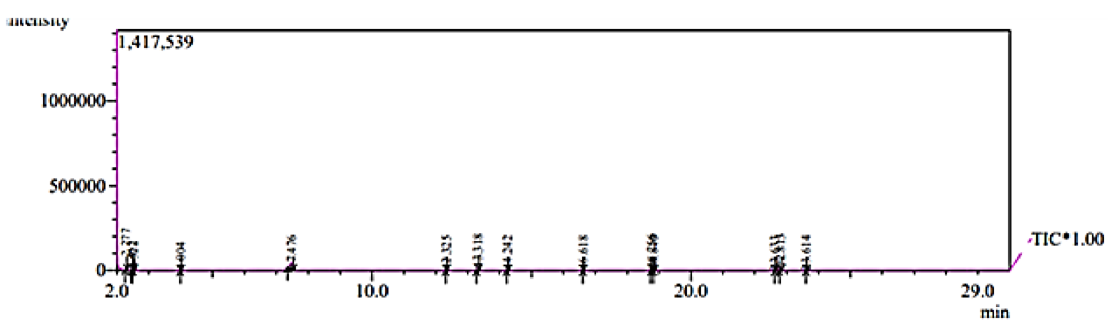
3.2. GC-MS Analysis (leaves – aqueous extract)

Analyses of aqueous leaf extracts using GC-MS showed that there were 14 different compounds present, with cyclohexanecarbonitrile, 1-(1-piperidinyl) being the most abundant at 70.09%. The presence of nitrogen-containing compounds, organic acids, and ester derivatives indicates that the leaves have potential for alternative medicinal use (**Table 2** and **Figure 1**).

Table 2. GC-MS identified compounds in the aqueous leaf extract of *Ziziphus spina-christi.*

Peak	**R.Time (min)**	**Compound**	**Area (%)**	**Molecular formula**	**Structural formula**
1	2.277	Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitroso	1.41	$C_4H_4N_4O_3$	
2	2.462	Azodicarbonamide	1.17	$C_2H_4N_4O_2$	
3	2.522	3,4-Dehydroproline amide	1.26	$C_5H_8N_2O$	
4	4.004	N,2-Dimethyl-N-nitrosopropanamine	0.36	$C_5H_{12}N_2O$	
5	7.476	Cyclohexanecarbonitrile, 1-(1-piperidinyl)	70.09	$C_{12}H_{20}N_2$	
6	12.325	Phthalic acid, ethyl 3-methyl-4-nitrobenzyl ester	1.20	$C_{18}H_{17}NO_6$	
7	13.318	Aminopyrazole-3	8.99	$C_3H_5N_3$	

Peak	R.Time (min)	Compound	Area (%)	Molecular formula	Structural formula
8	14.242	1-[(1-Oxo-2-propenyl)oxy]-2,5-pyrrolidinedione	1.10	$C_7H_7NO_4$	
9	16.618	2-Propenoic acid, 2-methyl-, tert-butyl ester	0.52	$C_8H_{14}O_2$	
10	18.756	Oxalic acid, dicyclobutyl ester	4.47	$C_{10}H_{14}O_4$	
11	18.840	RS-2,3-Hexanediol	0.59	$C_6H_{14}O_2$	
12	22.633	Phthalic acid, 3-methylbutyl octyl ester	0.19	$C_{21}H_{32}O_4$	
13	22.813	Phthalic acid, di(2-methylbutyl) ester	8.30	$C_{18}H_{26}O_4$	
14	23.614	Oxalic acid, cyclobutyl ester	0.36	$C_8H_{12}O_4$	



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	0.000	0.000	0.000	0	0.00	0	0.00	0.00	V	
2	2.277	2.258	2.308	4349	1.41	3032	3.18	1.54	MI	
3	2.462	2.442	2.492	3617	1.17	2100	2.21	1.76	MI	
4	2.522	2.500	2.542	3914	1.26	2843	2.99	1.38	MI	
5	4.004	3.992	4.017	1120	0.36	1148	1.21	0.98	MI	
6	7.476	7.358	7.542	216910	70.09	35088	36.85	6.18	MI	
7	12.325	12.308	12.342	3700	1.20	2655	2.79	1.39	MI	
8	13.318	13.275	13.350	27835	8.99	15368	16.14	1.81	MI	
9	14.242	14.217	14.267	3402	1.10	1684	1.77	2.02	MI	
10	16.618	16.608	16.633	1619	0.52	2203	2.31	0.73	MI	
11	18.756	18.733	18.775	13821	4.47	10065	10.57	1.36	MI	
12	18.840	18.825	18.858	1828	0.59	1402	1.47	1.30	MI	
13	22.633	22.625	22.642	577	0.19	1153	1.21	0.50	MI	
14	22.813	22.775	22.842	25677	8.30	15265	16.03	1.68	MI	
15	23.614	23.600	23.625	1113	0.36	1209	1.27	0.92	MI	
				309482	100.00	95215	100.00			

Figure 1. GC-MS chromatogram of bioactive compounds in aqueous extracts of *Ziziphus spina-christi* leaves**.**

3.3. Antibacterial activity

Similar to the results shown in **Table 3**, antibacterial activity was observed through aqueous extracts from both leaves and fruits of *Ziziphus spina-christi* against all bacterial species tested. However, the aqueous extracts from leaves were significantly more effective than those from fruits ($P \leq 0.05$). The increasing extract concentrations were also correlated with larger inhibition zones of growth. These suggested a clear dose-dependent response to the extracts (**Figures 2** and **3**). All bacterial species were tested, with *Pseudomonas aeruginosa* being the most affected, while *Klebsiella* spp. were the least affected. The most potent antibacterial effects from both leaf extracts and fruit extracts were yielded from 40% concentration, with a noticeable reduction in inhibition at lower concentrations. On the whole, the leaves of *Ziziphus spina-christi* were more potent against the selected bacterial strains than the fruit was, and there were also significant differences detected in the data presented ($P \leq 0.05$).

Table 3. Antibacterial activity of aqueous extracts of *Ziziphus spina-christi* leaves and fruits.

Bacterial species	Extract	40%	20%	10%	5%
E. coli	Leaves	21.0 ± 9.0 a	15.0 ± 5.0 b	10.0 ± 1.0 c	7.5 ± 0.5 d
	Fruits	25.0 ± 7.5 a	18.0 ± 7.0 b	11.5 ± 1.5 c	7.5 ± 0.5 d
P. aeruginosa	Leaves	28.0 ± 3.0 a	16.5 ± 1.5 c	14.5 ± 1.5 b	11.0 ± 0.0 d
	Fruits	26.0 ± 3.0 a	16.5 ± 1.5 c	14.0 ± 2.0 b	12.0 ± 1.0 d
Klebsiella spp.	Leaves	25.5 ± 0.5 a	17.0 ± 7.0 b	15.0 ± 0.0 c	11.0 ± 1.0 d
	Fruits	25.5 ± 0.5 a	16.0 ± 2.0 b	14.0 ± 2.0 c	10.0 ± 1.0 d
Staphylococcus aureus	Leaves	19.0 ± 1.0 a	11.0 ± 1.0 c	15.5 ± 1.5 b	9.5 ± 1.5 d
	Fruits	19.0 ± 1.0 a	11.0 ± 1.0 c	12.0 ± 3.0 b	9.5 ± 1.5 d

LSD values ($P \leq 0.05$), LSD (concentrations) = 2.10, LSD (plant part) = 1.85, LSD (bacterial species) = 2.30. (Mean ± SE, inhibition zone in mm; LSD test at $P \leq 0.05$) Values are expressed as mean ± standard error (SE). Means followed by different letters within the same row are significantly different according to the Least Significant Difference (LSD) test at $P \leq 0.05$.

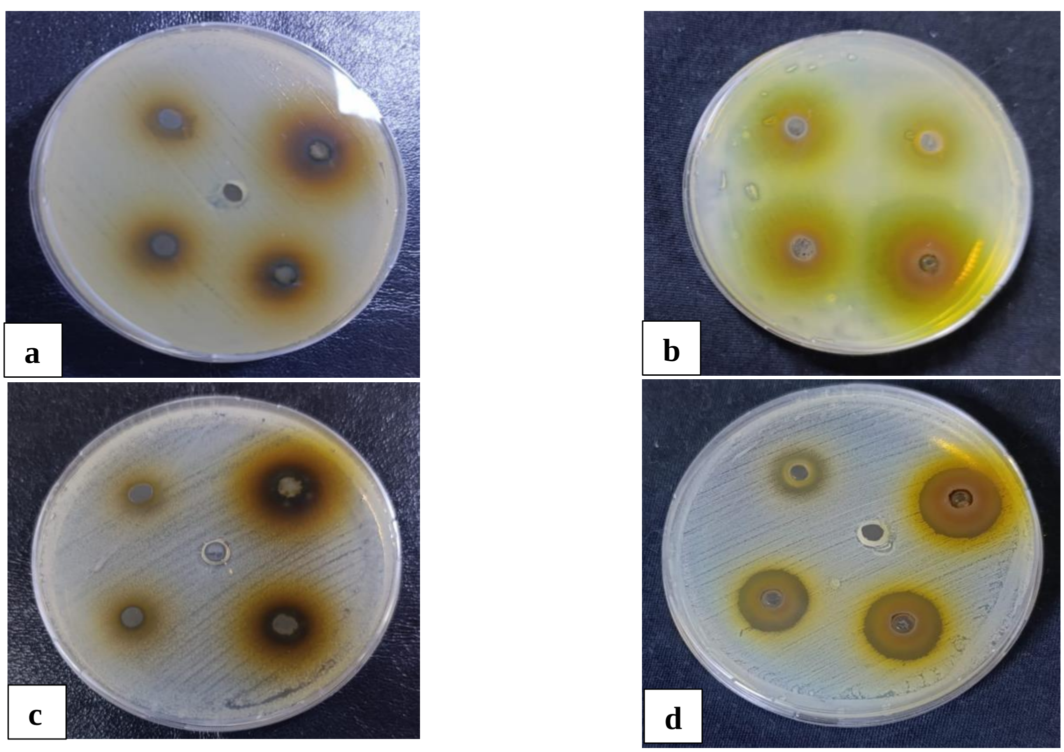


Figure 2. Antibacterial activity (agar well diffusion) of the aqueous leaf extract showing clear inhibition zones against the tested bacteria a. *E. coli*, b. *P. aeruginosa*, c. *Klebsiella* spp. d. *Staphylococcus aureus*.

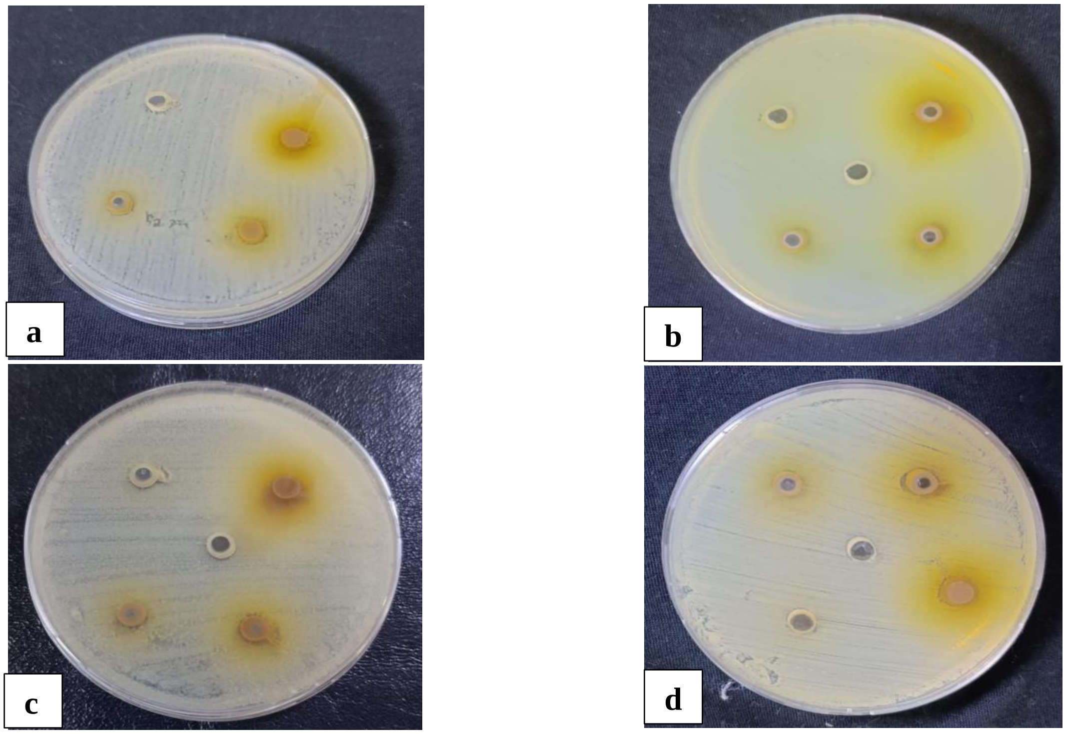


Figure 3. Antibacterial activity of the aqueous fruit extract showing smaller inhibition zones compared with leaves a. *E. coli*, b. *P. aeruginosa*, c. *Klebsiella* spp. d. *Staphylococcus aureus*.

4. Discussion

This current research paper examined the antibacterial effects of both water- (aqueous) and ethanol- (alcoholic) based extracts of the leaf and fruit of the plant *Ziziphus spina-christi* using the agar-well diffusion technique. Qualitative phytochemical analysis and GC-MS analysis were also performed on the most effective extract, which consisted of leaves. Leaf extracts consistently demonstrated a much greater antibacterial effect than extracts from the fruits, and the results indicated that the extracts' effectiveness depended on three factors: extraction solvent, concentration, and type of bacteria[23].

The inhibition zones observed in the antibacterial assay increased with the increasing concentration of extracts used. The 40% concentration had the strongest antibacterial effect, as seen from the inhibition zone. This phenomenon is consistent with previous studies that found greater antibacterial effects when increasing the concentration of the extract due to the increased amount of diffusion of bioactive compounds into the agar medium that inhibited bacterial growth[24, 25]. These findings were confirmed statistically through the results of one-way ANOVA followed by the least significant difference (LSD) test ($P \leq 0.05$), demonstrating that the results were strong evidence.

Pseudomonas aeruginosa exhibited the greatest sensitivity to both aqueous and ethanolic leaf extracts compared to the other bacterial species tested. Previous studies have also reported the sensitivity of *Pseudomonas aeruginosa* to *Z. spina-christi* extracts, even though it is typically known to be resistant to a large number of synthetic antibiotics[21,26]. On the other hand, *Klebsiella* spp. had a lower sensitivity when compared to other test microorganisms as a result of the presence of a thick polysaccharide capsule that protects against the action of antimicrobial agents[27, 28]. Therefore, the findings of this study support the existence of distinct types of bacteria based on their differences in their respective cell envelope structures[29].

The leaves, especially when extracted with alcohol, contain a considerable number of phenols, flavonoids, tannins, terpenoids, and coumarins, according to qualitative phytochemical profiling[30]. Previous studies have revealed that the same types of phytochemical compounds in *Z. spina-christi* were associated with antibacterial activity via different methods, including disrupting membranes, inhibiting enzymes, causing proteins to precipitate, and inhibiting nucleic acid synthesis[6, 31]. The relatively large number of bioactive compounds found in the leaves versus the fruit likely explains the greater antibacterial activity seen in this study.

The analysis using GC-MS of the aqueous extract from *Z. spina-christi* leaves revealed that a total of fourteen mixed compounds were identified, with cyclohexanecarbonitrile (1-(1-piperidinyl)) being the most prevalent. The other components included nitrogen-containing substances, organic acids, and esters that were similar to those documented in previous GC-MS assessments of this plant's leaves, which have been linked to having antimicrobial activity[32]. The combination of major and minor components in the extract enables synergistic interactions between compounds, leading to improved antibacterial potency, as reported in other studies related to the use of plants for antimicrobial purposes[33, 34].

The findings of this study demonstrate that alcoholic extracts exhibited significantly greater antibacterial activity than aqueous extracts. In previous studies, the greater efficiency of extraction of non-polar and semi-polar secondary metabolites from plant material when using organic solvents as opposed to water was cited as a possible explanation for the observed differences in antibacterial activities[35,36]. Additionally, alcohol facilitates the extraction of many secondary metabolites (flavonoids, terpenoids, and phenolic compounds) that cannot be efficiently extracted from plants using only water[36]. While the performance of the aqueous leaf extract was lower than that of the other extracts, it is suggested that the extract had potential as a low-cost antimicrobial formulation and could be incorporated into traditional medicine[38].

Therefore, the research supports the notion that *Ziziphus spina-christi* has antimicrobial benefits (particularly from the leaves) by reiterating what past studies have shown regarding its antimicrobial effects[39]. The development of a microbial assay and the use of GC-MS and other phytochemical analysis techniques were a good way to understand the factors that affect the biological activity of *Ziziphus spina-christi*[40]. Further study that focuses on identifying and isolating specific active components, determining their mechanisms of action, and understanding the antimicrobial benefits of *Ziziphus spina-christi* is needed[25].

5. Conclusion

The results of the study showed that the leaf extract of *Ziziphus spina-christi* had greater antibacterial efficacy than that of the fruit at multiple levels of concentration. Differences between the concentration of the extracts, type of extract, and bacterial species were statistically significant. The results of the phytochemical screening confirm that the leaf extracts contain higher quantities of phenolic compounds, flavonoids, tannins, and terpenes, thereby providing greater abilities to inhibit the growth of bacteria .Several bioactive compounds were found in the aqueous leaf extract by GC-MS analysis. Cyclohexanecarbonitrile, 1-(1-piperidinyl), was the largest component of this extract but was significantly less than that of the alcohol extracts. Alcohol extracts had a stronger antibacterial activity than aqueous extracts. The data also suggest that the choice of solvent in which an extract was prepared influences the effectiveness of the antibacterial activity .Therefore, *Ziziphus spina-christi* leaves have commercial potential as an antibacterial agent.

The promising preliminary results of this research on the antimicrobial effectiveness of plant extracts could lay the groundwork for future studies focused on isolating and purifying compounds with antimicrobial properties, instead of relying on antibiotics for resistant bacterial strains. This is particularly important since most of the active ingredients are safe for human use.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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No founding.

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