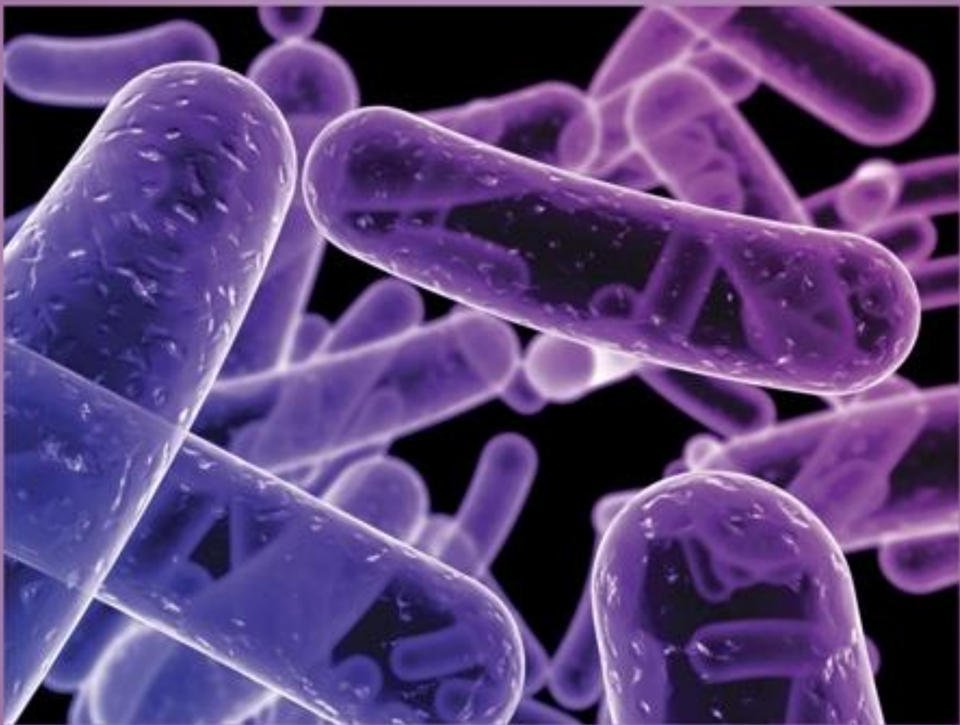
G

EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY



ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 16 No. 1 (2024)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.16 (1) pp.21- 31 (2024)*
DOI: 10.21608/EAJBSG.2024.341864

Egypt. Acad. J. Biolog. Sci., 16(1):21-31(2024)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Exploring the Antimicrobial Activity of Ziziphus spina – christi: A Promising Natural Supply of Antimicrobial Agents

Elhaj, Yagoub HA
Department of Laboratory Medicine, Medical Microbiology, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, Saudi Arabia.
*E.Mail: yelhaj@bu.edu.sa

ARTICLE INFO
Article History
Received:17/1/2024
Accepted:18/2//2024
Available:22/2/2024

***Keywords*:**
Antimicrobial effects, Methanol and ethanol extracts, Ziziphus spina-christi, Pathogenic bacteria.

ABSTRACT

The objective of this study was to evaluate the antimicrobial effects of methanol and ethanol extracts of Ziziphus spina-christi leaves on diverse pathogenic bacteria. Methods: The antimicrobial activity of the leaf extracts was assessed. The extracts were applied to agar plates, and the inhibition zones were measured to determine the extent of bacterial growth inhibition. The MIC values of standard antibiotics were determined using Vitek2 system. Various microorganisms, including *Pseudomonas aeruginosa, Streptococcus agalactiae, Citrobacter koseri, Klebsiella pneumoniae ssp pneumoniae,* and *E. coli*, were tested in the study. Results: The results showed that both the methanol and ethanol extracts of Ziziphus spina-christi leaves exhibited antimicrobial activity against the tested microorganisms. The inhibition zones varied for different microorganisms and extracts. For the methanol extract, the largest inhibition zone of 12 mm was observed for *Pseudomonas aeruginosa*. The ethanol extract showed a maximum inhibition zone of 14 mm for *Citrobacter koseri*. These findings indicated the sensitivity of the tested microorganisms to the extracts and their potential as antimicrobial agents. The MIC values of standard antibiotics against the microorganisms revealed varying levels of sensitivity and resistance.Conclusion: These findings highlight the potential of Ziziphus spina-christi as a natural source of antimicrobial agents.Furthermore, the interpretations of the MIC values contribute to the understanding of the sensitivity or resistance profiles of the microorganisms to standard antibiotics. This information can guide clinicians in selecting appropriate antibiotics for treating infections caused by these microorganisms. Further research is warranted to identify the bioactive compounds responsible for the antimicrobial activity and elucidate their mechanisms of action.

INTRODUCTION

The *Ziziphus spina-christi* tree (ZSC) relates to the family Rhamnaceous. According to studies, this plant contains a high number of phytochemical molecules like polyphenols, and tannins. It has been revealed that those compounds possess significant biological activities like anti-free radicals, anti-microbe, anti-parasitic, anti-inflammatory, anti-obesity, and also anti-cancer properties(Abdulrahman *et al.*, 2022). El-Shahir *et al.*,2022, concluded that gallic acid and ellagic acid were the main components in the leaf extract *of Z. spina-christi* (El-Shahir *et al.*, 2022).

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.16 (1) pp.21- 31 (2024)*
DOI: 10.21608/EAJBSG.2024.341864

Traditionally, it is used as a demulcent, emollient, astringent, and natural remedy for toothaches (Shahat *et al.,* 2001). The leaves and roots are traditionally used to treat local wounds and skin conditions, respectively(Adzu *et al.,* 2001). Furthermore, a decoction of the bark and fresh fruits is used as a body wash to enhance wound healing, while the fruits are used to alleviate dysentery(Adzu and Haruna, 2007). Previous phytochemical investigations of ZSC-L stem bark revealed the existence of alkaloids, flavonoids, sterols, tannins and triterpenoids, saponins and ZSC-L stem bark reported for its antimicrobial and cytotoxic activities (Ads *et al.,* 2017, Ads *et al.,* 2018). A recent study regarding the phytochemical characterization of leaves of ZSC-L resulted in the identification of 10 dammarane-type saponins and 12 phenolic compounds(Bozicevic *et al.,* 2017).

Herbal medicines with their cultural acceptability accompanied by the least injurious effects are being widely used in the world. Additionally, pharmacological activities related to *Ziziphus spina-christi* and its methanolic and ethanolic extract capable of preventing the growth of different species of Gram-negative bacteria are vital for drug development(Alrumman, 2016, Kadioglu *et al.,* 2016). Regarding another activity, the wound-healing properties of *Ziziphus spina-christi* are due to its phenolic compounds. As an important group of phytochemicals and their biological aspects like antimicrobial, anti-inflammatory, and antioxidant properties, it can be said that polyphenolic compounds play strong and effective roles in this plant(Hunter, 2012) Ethnobotanical studies have shown that many cultures rely on traditional medicinal plants for their healing properties (Tounekti *et al.,* 2019). In particular, medicinal plants have been widely used to treat infectious diseases (Borges *et al.,* 2015). The stem bark is particularly known for its various therapeutic properties such as antidiabetic, anticancer, and antimicrobial activities (Makhawi *et al.,* 2020, Tounekti *et al.,* 2019). A study conducted in Morocco showed that the methanolic extract of the plant parts exhibited significant antimicrobial activity against several pathogenic microorganisms(Tounekti *et al.,* 2019).

Phytochemical screening studies showed that the *Ziziphus spina-christi* plant is rich in tannins, saponins, and cardiac glycosides, which are linked to many therapeutic properties (Makhawi *et al.,* 2020).

In addition, a research study by Makhawi *et al.* (2020) evaluated the antimicrobial activity of the methanolic extract of Ziziphus spina-christi stem bark against four standard bacteria species, including *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and one standard fungus species, *Candida albicans*. The results of this study indicate that the extracts inhibited the growth of all the microorganisms, and most of the extracts show several points of antimicrobial activity (Makhawi *et al.,* 2020).

Another study conducted by Ads *et al.* (2017) evaluated the antimicrobial activity of Ziziphus spina-christi stem bark extracts against a variety of pathogenic bacteria, including Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, and Streptococcus pneumonia. The study found that the extracts exhibited significant antimicrobial activity against these bacteria, with the methanolic extract showing the highest activity (Ads *et al.,* 2017).

Medicinal Plants have been attaining great recognition all over the globe (Jamshidi-Kia *et al.,* 2017). They act as very crucial therapeutic agents as well as valuable raw active compounds for manufacturing many traditional and modern treatments (Bamola *et al.,* 2018).

World Health Organization (WHO) recently reported that there is an unprecedented increase in the occurrence of multidrug-resistant (MDR) infections worldwide (Mendelson and Matsoso, 2015, Unemo *et al.,* 2019). Currently, efforts are being focused on proposing new drugs for multidrug-resistant bacterial strains. Natural products, especially those obtained from medicinal plants, have been demonstrated to be remarkable compounds with exceptional properties, such as rupturing the membrane of bacteria, enzyme activity inhibition and bacterial biofilm formation, which makes them perfect applicants for these MDR bacteria (Mulat *et al.,* 2019, Khatri *et al.,* 2016, Barbieri *et al.,* 2017, Ayaz *et al.,* 2019, Shin *et al.,* 2018, Borges *et al.,* 2015).

Ziziphus Spina-christi is widespread in tropical and subtropical regions. The genus *Ziziphus Spina-christi* is widely distributed in the Middle East. Since ages extracts of *Ziziphus spina –christi* have been used as inflammatory treat toothache, analgesic, pectoral, astringent, antirheumatic, purgative, for stomach pain, anti-helminthic (Tounekti *et al.,* 2019, Al Mutair *et al.,* 2021, Shakiba *et al.,* 2021).

Significance of Study:

This study highlights the potential of Ziziphus spina-christi leaves as a natural source of antimicrobial agents. The study's findings may contribute to the development of new therapeutic interventions and help address the pressing issue of antimicrobial resistance.

Objectives:

The objective of this study was to evaluate the antimicrobial effects of methanol and ethanol extracts of Ziziphus spina-christi leaves on diverse pathogenic bacteria.

Study Question:

What is the antimicrobial activity of methanol and ethanol extracts of *Ziziphus spina*-christi leaves against various microorganisms, and what are the minimum inhibitory concentration (MIC) values of standard antibiotics against these microorganisms?

MATERIALS AND METHODS

Sample Collection and Preparation:

Fresh leaves of *Ziziphus spina-christi* had been gathered from a local botanical tree in December 2023. The leaves have been carefully washed with water to cast off any surface contaminants and air-dried underneath the shade. The dried leaves have been then floor into a satisfactory powder.

Microbial Strains:

Various bacterial strains were obtained from clinical isolates and were used to assess the antimicrobial interest of the Ziziphus spina-christi methanol and ethanol extracts. The bacteria species identity and antibiotic susceptibility have been completed via the usage of the Vitek2 implement system. These species included *Streptococcus agalactiae, Escherichia coli, Citrobacter koseri, Klebsiella pneumoniae spp pneumoniae and Pseudomonas aeruginosa.*

Antimicrobial Activity Assay:

Extraction with Methanol and Ethanol:

About 20 grams and 10 grams of powdered Ziziphus spina-christi leaves were transferred into 2 separate glass boxes. A one hundred ml quantity of ethanol was delivered to the container comprising 20 grams of Ziziphus spina-christi leaves A forty ml quantity of methanol was turned into introduced to the box comprising 10 grams of Ziziphus spina-christi leaves. Ensuring that the plant cloth is absolutely submerged. The box is sealed tightly with aluminum foil to save you from evaporation and contamination. Then the box becomes located on the rotatory apparatus in a groovy, dark area and permits it to macerate for a particular duration (3 days) for methanol and (four days) for ethanol to facilitate the extraction of bioactive compounds.

Filtration and Concentration:

After the maceration period, the extract uses filter paper to dispose of stable debris and plant particles. After that collect the filtrate in a smooth glass box. The filtrate allowed it to air-dry in a well-ventilated and dark vicinity.

Storage:

Transfer the filtered extract into glass vials and garage. Store the extracts in a cool, darkish vicinity to reduce degradation and keep their balance. Containers became categorized with vital data, such as the plant supply, extraction solvent, and date of instruction.

Measurement of Antimicrobial Activity:

The antimicrobial activity of the methanol extract of Ziziphus spina-christi leaves turned into decided the use of the agar nicely diffusion technique. Muller-Hinton agar plates had been prepared, and every plate was inoculated with a standardized suspension of the check microorganism. Wells were then made within the agar using a sterile cork borer, and 100µL of the methanol and ethanol extracts were delivered to each properly. Plates were incubated at appropriate temperatures overnight for microorganisms.

Measurement of Zone of Inhibition:

After the incubation length, the plates have been tested for the presence of clean zones around the wells, indicating inhibition of microbial increase. The diameter of the area of inhibition becomes measured with the usage of a calibrated ruler. The experiment was executed in reproduction, and the mean region of inhibition changed into calculated for every microbial strain.

Measurement of MIC:

Regarding standard antibiotics, the minimum inhibition concentration was measured by using Vitek2 machine system based on MIC cut-off the microbial strains were identified as resistant/susceptible using VITEc antimicrobial susceptibility test card (AST card) for Gram-positive and Gram-negative bacterial strains.

Statistical Analysis:

The data obtained from this study was subjected to statistical analyses to provide meaningful insights into the antimicrobial activity of Ziziphus spina-christi leaf extracts and the minimum inhibitory concentration (MIC) values of standard antibiotics. Descriptive statistics was used to summarize the characteristics of the inhibition zones observed for the methanol and ethanol extracts. The results were interpreted the significance of the findings and contributed to the scientific knowledge in the field of antimicrobial research.

RESULTS

The results chapter provides a comprehensive analysis of the antimicrobial consequences of methanol and ethanol extracts of Ziziphus spina-christi leaves, as well as the minimal inhibitory concentration (MIC) values of standard antibiotics against various microorganisms. The findings shed light on the sensitivity/resistance patterns of the tested microorganisms to the extracts and antibiotics, offering valuable insights into their potential as antimicrobial agents.

Table 1, presents the properties and extractive value of *Ziziphus spina-christi* leaves using different solvents for extraction, namely methanol and ethanol. The table provides information on the characteristic of the leaves, the weight of the sample used for extraction, and the percentage yields obtained for each solvent.

The characteristic of the leaves is described as "powder," which suggests that the leaves were dried and ground into a fine powder before extraction. This is a common practice to increase the surface area and facilitate the extraction process.

For the methanol extraction, a sample weighing 10 grams of the powdered Ziziphus spina-christi leaves was used. The extraction process using methanol resulted in a yield of 5%, indicating that 5% of the total weight of the leaves was extracted using this solvent.

Similarly, for the ethanol extraction, a larger sample weighing 20 grams of the powdered leaves was used. The yield obtained with ethanol as the solvent was 10%, indicating that 10% of the total weight of the leaves was extracted using ethanol.

The extractive value of a plant material is the percentage of desirable compounds or constituents obtained through extraction. In this case, the extractive value is represented by the percentage yields, indicating the efficiency of each solvent in extracting compounds from the Ziziphus spina-christi leaves.

Table 1: Properties and extractive value of Ziziphus spina –christi leave:

Solvents of extraction	Characteristic	Weight (g)	Yields (%)
Methanol	Powder	10	5
Ethanol	Powder	20	10

Table 2, provides the effects of testing the antimicrobial consequences of methanol and ethanol extracts of *Ziziphus spina-christi* leaves towards diverse microorganisms. The inhibition zone is a measure of the diameter of the clear area across the wells where the extract becomes implemented, indicating the extent to which the extract inhibits bacterial growth.For the methanol extract, the inhibition zone for *Pseudomonas aeruginosa* became 12 mm, for *Citrobacter koseri* and *Streptococcus agalactiae* it turned to 10 mm, for *E.Coli* and *Klebsiella pneumoniae ssp pneumoniae* it changed into 8 mm.

For the ethanol extract, the inhibition zone for *Citrobacter koseri* was 14 mm, *Pseudomonas aeruginosa* turned to 13 mm, *Klebsiella pneumoniae ssp pneumoniae* it was 12 mm, *Streptococcus agalactiae* it became 11 mm and for *E.Coli* it turned into 10 mm.In each extract, the sector of inhibition values implies the sensitivity of the tested microorganism to the extracts. A larger area of inhibition shows a stronger inhibitory impact on bacterial growth.

Based on the important thing supplied, all tested organisms have been observed to be touchy (S) to each of the methanol and ethanol extracts of *Ziziphus spina-christi* leaves. In this manner the extracts exhibited antimicrobial pastime against the tested bacteria, inhibiting their growth.

These findings recommend that *Ziziphus spina-christi* leaves comprise bioactive compounds with potential antimicrobial residences. Further investigation into the unique compounds chargeable for the antimicrobial hobby and their mechanisms of movement might be beneficial in information on the entire capability of *Ziziphus spina-christi* as a natural source of antimicrobial agents.

Table 2. Antimicrobial results of methanol and ethanol extracts of Z. Spina-christi Leaves (zone of inhibition).

Tested bacteria	Mean diameter zone of inhibition (mm)			
	Methanol (50%)	Interpretation	Ethanol (50%)	Interpretation
Pseudomonas aerugenosa	12	S	13	S
E.coli	8	S	10	S
Citrobacter koseri	10	S	14	S
Streptococcus agalactiae	10	S	11	S
Klebsiella pneumoniae ssp pneumoniae	8	S	12	S

Key: S: Sensitive

Table 3, gives the minimal inhibitory concentration (MIC) values of fashionable antibiotics in opposition to one-of-a-kind isolated microorganisms, inclusive of *Pseudomonas aeruginosa, Streptococcus agalactiae, Citrobacter koseri, Klebsiella pneumoniae ssp pneumoniae, and E. Coli.* Each row represents a specific antibiotic, and every column represents a specific microorganism. The MIC values imply the lowest concentration of the antibiotic required to inhibit the growth of the respective microorganism.

Based on the important thing furnished, the interpretations of the MIC values are as follows: Benzylpenicillin: It exhibited a MIC of 0.12 mg/ml, indicating sensitivity (S) in opposition to *Pseudomonas aeruginosa*. Ampicillin: It confirmed a MIC of 0.25 mg/ml, indicating sensitivity towards *Pseudomonas aeruginosa* and resistance (R) in opposition to *Streptococcus agalactiae and Citrobacter koseri*. Levofloxacin: It exhibited a MIC of 8 mg/ml, indicating resistance in opposition to *Pseudomonas aeruginosa and sensitivity* in opposition to *Streptococcus agalactiae.* Moxifloxacin: It confirmed a MIC of 0.25 mg/ml, indicating sensitivity against *Pseudomonas aeruginosa.* Clindamycin: It exhibited a MIC of 0.25 mg/ml, indicating resistance, Quinupristin/dalfopristin: It showed a MIC of 0.25 mg/ml, indicating sensitivity. Linezolid: It exhibited a MIC of 2 mg/ml, indicating sensitivity. Vancomycin: It confirmed a MIC of 0,5 mg/ml, indicating sensitivity. Tetracycline: It exhibited a MIC of 16 mg/ml, indicating resistance. Tigecycline: It showed variable interpretations, with a MIC of 8 mg/ml for *Pseudomonas aeruginosa* and resistance, sensitivity, and intermediate (I) interpretations against different microorganisms. Nitrofurantoin: It exhibited a MIC of 16 mg/ml, indicating sensitivity against all tested microorganisms. Amoxicillin/clavulanic acid: It showed variable interpretations, with resistance, sensitivity, and intermediate interpretations in opposition to distinct microorganisms. Piperacillin/tazobactam: It exhibited a MIC of 4 mg/ml, indicating sensitivity towards all examined microorganisms. Cefotaxime, Cefalotin, Cefoxitin, Ceftazidime, Ceftriaxone, and Cefepime: These antibiotics showed variable interpretations of sensitivity, resistance, and intermediate against exclusive microorganisms. Imipenem, Meropenem, and Amikacin: These antibiotics exhibited sensitivity in opposition to most of the tested microorganisms. Gentamicin, Ciprofloxacin, Trimethoprim/ sulfamethoxazole, and Colistin: These antibiotics confirmed variable interpretations of sensitivity and resistance in opposition to one-of-a-kind microorganisms. Ertapenem, Norfloxacin, Fosfomycin, Ticarcillin/clavulanic Acid, and Tobramycin: These antibiotics exhibited sensitivity against precise microorganisms.

These findings offer valuable information approximately the susceptibility/resistance patterns of the tested microorganisms to diverse antibiotics. The interpretations can manual clinicians in selecting suitable antibiotics for treating infections because of these microorganisms, deliberating their sensitivity or resistance profiles.

Table 3. Antimicrobial effects of standard antibiotics (MIC):

Antibiotics	MIC of isolated microorganisms' mg/ml				
	P. aeruginosa[1]	*Strep. Agalactiae*[2]	*C. koseri*[3]	*K.p.p*[4]	*E. coli*[5]
Benzylpenicillin		0.12 S			
Ampicillin		0.25 S		32R	32R
Levofloxacin	8 R	1S			
Moxifloxacin		0.25 S			
Clidamycin		0.25 R			
Quinupristin/dalfopristin		0.25S			
Linezolid		2S			
Vancomycin		0.5S			
Tetracycline		16 R			
Tigecycline	8R	0.12S		0.5S	0.5S
Nitrofurantoin		16S	16S	16S	16S
Amoxicillin/clavulanic acid			32R	2S	16I
Piperacillin/tazobactam	4S		4S	4S	4S
Cefotaxime			16I		
Cefalotin				2S	64R
Cefoxitin				4S	4S
Ceftazidime	4S		64R	1S	4S
Ceftriaxone					64R
Cefepime	1S		1S	1S	2S
Imipenem	16R		1S	0.25S	0.25S
Meropenem	0.25I		0.25S	0.25S	0.25S
Amikacin	2S		2S	2S	8I
Gentamicin	1S		1S	1S	16R
Ciprofloxacin	4R			0.25S	4R
Trimethoprim/sulfamethoxazole			20S	20S	20S
Ertapenem			0.5S		
Norfloxacin			1R		
Fosfomycin			16S		
Ticarcillin/clavulanic Acid	32S				
Tobramycin	1S				
Colistin	0.5S				

Key: 1= Pseudomonas aerugenosa 2. Streptococcus agalactiae, 3=Citrobacter koseri, 4=Klebsiella pneumoniae spp pneumoniae, 5= E. coli
S: Sensitive, R: Resistance, I: Intermediate.

DISCUSSION

This article evaluates the antimicrobial effects of methanol and ethanol extracts of Ziziphus spina-christi leaves, also known as Christ's thorn, on diverse pathogenic bacteria. The research demonstrates that the plant extract has broad inhibitory effects against numerous microorganisms and may have potential as a natural source of antimicrobial agents.

The findings of this study indicate that both methanol and ethanol extracts exhibit significant inhibitory activity against the tested organisms, as indicated by the suggest area of inhibition values . These findings are consistent with previous studies. For example, a study conducted by Makhawi *et al.* in 2020 demonstrated that methanol extraction showed high activity at all concentrations (100%,50%,25%), against clinical isolates of *E. coli* (22,21,20mm) respectively and *Pseudomonas arginosa*(24,23,19mm), while ethanol also exhibited activity against these bacteria *E. coli* (20,18,17mm), *pseudomonas aeruginosa* (19,17,16mm) (Makhawi *et al.,* 2020).

Furthermore, the present findings align with assays reported by El-kamali and Mahjoub in 2009. Their study found that methanol extract of Ziziphus spina-christi stem bark was effective against all tested Gram-negative and Gram-positive bacteria (range between 20-30 mm), while ethanol extract showed efficacy against all tested bacteria except *Escherichia coli* (El-Kamali and Mahjoub, 2009).

Similarly, in a study conducted by Al-Kaabi *et al.* in 2021, Ziziphus spina-christi leaf extracts dissolved in distilled water, ethanol, and methanol exhibited variable antibacterial activity against various bacteria. The methanol extract showed greater efficacy at both 50 µg/ml and 100 µg/ml concentrations, while ethanol exhibited limited inhibitory effects (Al-Kaabi *et al.,* 2021).

The results of these studies collectively demonstrate the potential inhibitory effects of methanol and ethanol extracts of Ziziphus spina-christi on a wide range of tested bacteria. The findings suggest that the extracts may contain different antimicrobial agents, with methanol extraction showing greater efficacy. Ethanol and methanol are identified as better solvents for extracting the active ingredients of this plant (Al-Kaabi *et al.,* 2021).

Additionally, the study by Al-Bayatti *et al.* in 2011 reported the effectiveness of methanol and water extracts of Ziziphus spina-christi seeds and leaves against various bacterial species isolated from skin infections (Al-Bayatti *et al.*, 2011). Another study by Al-Saimary in 2009 investigated the effects of aqueous extracts of Ziziphus leaves on *Staphylococcus aureus* and found inhibitory effects at higher concentrations (Alsaimary, 2009).

Overall, these studies collectively highlight the antimicrobial activity of Ziziphus spina-christi and its potential as a natural source of antimicrobial agents. The reported findings underscore the importance of further exploration to identify the specific bioactive compounds responsible for the observed antimicrobial effects and elucidate their mechanisms of action.

In conclusion, while previous studies have focused on exploring the antimicrobial activity of Ziziphus spina-christi, the present article provides a comprehensive review and evaluation of these studies, highlighting the plant extract's potential as an alternative source of antimicrobial agents.

To compare the antimicrobial activity of standard antibiotics and *Ziziphus spina-christi* leaves similar to the same organism was used. Methanol and Ethanol Extracts give the suggested area of inhibition values for the tested extracts, with the interpretation of "S" (Sensitive) against all tested organisms, whilst standard Antibiotics provide the MIC values for one-of-a-kind antibiotics, with the diverse interpretations of "S" (Sensitive), "R" (Resistance), and "I" (Intermediate) based totally at the susceptibility of microorganisms to the antibiotics.

Conclusion:

In conclusion, the findings presented in this article demonstrate the significant antimicrobial effects of methanol and ethanol extracts of Ziziphus spina-christi leaves. The extracts exhibited broad inhibitory activity against diverse pathogenic bacteria, indicating their potential as natural sources of antimicrobial agents. These results align with previous studies that have reported similar antimicrobial properties of Ziziphus spina-christi extracts.

The observed inhibitory effects of the extracts highlight the importance of further exploration to identify and characterize the bioactive compounds responsible for their antimicrobial activity. Understanding the mechanisms of action of these compounds can provide valuable insights for the development of novel therapeutic interventions, particularly against drug-resistant pathogens.

It is worth noting that ethanol extraction generally showed higher efficacy compared to methanol extraction. However, both extraction methods demonstrated significant antimicrobial activity, suggesting that different antimicrobial agents may be present in each extract.

The findings of this study contributed to the growing body of evidence supporting the antimicrobial potential of Ziziphus spina-christi. Utilizing these plant extracts as alternative sources of antimicrobial agents can have implications for the development of new treatment strategies against infectious diseases.

Further research is warranted to explore the therapeutic applications of Ziziphus spina-christi extracts, including in vivo studies and clinical trials. Additionally, investigating the safety profile and potential side effects of these extracts is essential for their future use in medical and pharmaceutical settings.

Recommendations:

Perform comparative studies between Ziziphus spina-christi extracts and commonly used antimicrobial agents to assess their efficacy, safety, and potential synergistic effects. This will provide a better understanding of the comparative advantages and limitations of the plant extracts as alternative antimicrobial agents.

Conduct rigorous studies to evaluate the toxicity profile and potential side effects of Ziziphus spina-christi extracts. This is crucial for determining the safety of these extracts and establishing appropriate dosage guidelines for clinical applications.

Declarations:

Ethical Approval: It is not applicable.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Authors Contributions:I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

Funding: No funding was received.

Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

Acknowledgements: Not applicable.

REFERENCES

Abdulrahman, M. D., Zakariya, A. M., Hama, H. A., Hamad, S. W., Al-Rawi, S. S., Bradosty, S. W. & Ibrahim, A. H. 2022. Ethnopharmacology, biological evaluation, and chemical composition of Ziziphus spina-christi (L.) Desf.: A Review. *Advances in Pharmacological and Pharmaceutical Sciences*, 2022, 1-36.

Ads, E. N., Rajendrasozhan, S., Hassan, S. I., Sharawy, S. M. S. & Humaidi, J. R. 2017. Phytochemical, antimicrobial and cytotoxic evaluation of Ziziphus spina-christi (L.) stem bark. *Biomedical Research,* 28**,** 6646-6653.

Ads, E. N., Rajendrasozhan, S., Hassan, S. I., Sharawy, S. M. S. & Humaidi, J. R. 2018. Phytochemical screening of different organic crude extracts from the stem bark of Ziziphus spina-christi (L.). *Biomedical Research,* 29**,** 1645-1652.

Adzu, B., Amos, S., Wambebe, C. & Gamaniel, K. 2001. Antinociceptive activity of Zizyphus spina-christi root bark extract. *Fitoterapia,* 72**,** 344-350.

Adzu, B. & Haruna, A. K. 2007. Studies on the use of Zizyphus spina-christi against pain in rats and mice. *African Journal of Biotechnology,* 6 (11), 1317-1324

Al-Bayatti, K. K., Fitua, M. A. & Abdalah, M. E. 2011. A study of antibacterial activity of Cidar (Zizyphus spinachristi L.) on bacterial pathogens isolated from skin infections. *Al Mustansiriyah Journal of Pharmaceutical Sciences,* 9**,** 13-20.

Al-Kaabi, H. K. J., Hmood, B. A., Jebur, L. S., Abdullah, S. M. & Al-Qraghi, S. A. A. 2021. Determination of Ziziphus spinachristi leaves extracts antibacterial activity against some pathogenic bacteria. *Al-Kufa University Journal for Biology,* 13**,** 14-20.

Al Mutair, A., Alhumaid, S., Al Alawi, Z., Zaidi, A. R. Z., Alzahrani, A. J., Al-Tawfiq, J. A., Al-Shammari, H., Rabaan, A. A., Khojah, O. & Al-Omari, A. 2021. Five-year resistance trends in pathogens causing healthcare-associated infections at a multi-hospital healthcare system in Saudi Arabia, 2015–2019. *Journal of global antimicrobial resistance,* 25**,** 142-150.

Alrumman, S. A. 2016. Phytochemical and antimicrobial properties of Tamarix aphylla L. leaves growing naturally in the Abha Region, Saudi Arabia. *Arabian Journal for Science and Engineering,* 41**,** 2123-2129.

Alsaimary, I. E. 2009. Efficacy of some antibacterial agents on Staphylococcus aureus isolated from various burn cases. *International Journal of Medicine and Medical Sciences,* 1**,** 110-114.

Ayaz, M., Ullah, F., Sadiq, A., Ullah, F., Ovais, M., Ahmed, J. & Devkota, H. P. 2019. Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. *Chemico-biological interactions,* 308**,** 294-303.

Bamola, N., Verma, P. & Negi, C. 2018. A review on some traditional medicinal plants. *International Journal of Life-Sciences Scientific Research,* 4**,** 1550-1556.

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F. & Nabavi, S. M. 2017. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological research,* 196**,** 44-68.

Borges, A., J Saavedra, M. & Simoes, M. 2015. Insights on antimicrobial resistance, biofilms and the use of phytochemicals as new antimicrobial agents. *Current medicinal chemistry,* 22**,** 2590-2614.

Bozicevic, A., De Mieri, M., Di Benedetto, A., Gafner, F. & Hamburger, M. 2017. Dammarane-type saponins from leaves of Ziziphus spina-christi. *Phytochemistry,* 138**,** 134-144.

El-Kamali, H. H. & Mahjoub, S. A.-T. 2009. Antibacterial activity of Francoeuria crispa, Pulicaria undulata, Ziziphus spina-christi and Cucurbita pepo against seven standard pathogenic bacteria. *Ethnobotanical Leaflets,* 2009**,** 6. 722-33.

El-Shahir, A. A., El-Wakil, D. A., Abdel Latef, A. A. H. & Youssef, N. H. 2022. Bioactive Compounds and Antifungal Activity of Leaves and Fruits Methanolic Extracts of Ziziphus spina-christi L. *Plants,* 11**,** 746.

Hunter, P. 2012. The inflammation theory of disease: The growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment. *EMBO reports,* 13**,** 968-970.

Jamshidi-Kia, F., Lorigooini, Z. & Amini-Khoei, H. 2017. Medicinal plants: Past history and future perspective.

Journal of herbmed pharmacology, 7**,** 1-7.

Kadioglu, O., Jacob, S., Bohnert, S., Naß, J., Saeed, M. E., Khalid, H., Merfort, I., Thines, E., Pommerening, T. & Efferth, T. 2016. Evaluating ancient Egyptian prescriptions today: anti-inflammatory activity of Ziziphus spina-christi. *Phytomedicine,* 23**,** 293-306.

Khatri, S., Kumar, M., Phougat, N., Chaudhary, R. & Kumar Chhillar, A. 2016. Perspectives on phytochemicals as antibacterial agents: an outstanding contribution to modern therapeutics. *Mini Reviews in Medicinal Chemistry,* 16**,** 290-308.

Makhawi, A. M., Mustafa, M. I. & Uagoub, H. A. 2020. Phytochemical screening and antimicrobial activity of Ziziphus spina-christi stem barks. *bioRxiv***,** 2020.02. 24.963157.

Mendelson, M. & Matsoso, M. P. 2015. Guest Editorial: The World Health Organization Global Action Plan for antimicrobial resistance. *South African medical journal,* 105**,** 325-325.

Mulat, M., Pandita, A. & Khan, F. 2019. Medicinal plant compounds for combating the multi-drug resistant pathogenic bacteria: a review. *Current pharmaceutical biotechnology,* 20**,** 183-196.

Shahat, A. A., Pieters, L., Apers, S., Nazeif, N. M., Abdel-Azim, N. S., Berghe, D. V. & Vlietinck, A. J. 2001. Chemical and biological investigations on Zizyphus spina-christi L. *Phytotherapy Research,* 15**,** 593-597.

Shakiba, R., Nilforoushzadeh, M. A., Hashem-Dabaghian, F., Minaii Zangii, B., Ghobadi, A., Shirbeigi, L., Aliasl, J., Shakiba, M. & Ghods, R. 2021. Effect of Cedar (Ziziphus spina-christi) topical solution in mild to moderate acne vulgaris: a randomized clinical study. *Journal of Dermatological Treatment,* 32**,** 197-202.

Shin, J., Prabhakaran, V.-S. & Kim, K.-S. 2018. The multi-faceted potential of plant-derived metabolites as antimicrobial agents against multidrug-resistant pathogens. *Microbial pathogenesis,* 116**,** 209-214.

Tounekti, T., Mahdhi, M. & Khemira, H. 2019. Ethnobotanical study of indigenous medicinal plants of Jazan region, Saudi Arabia. *Evidence-based complementary and alternative medicine,* 2019.

Unemo, M., Lahra, M. M., Cole, M., Galarza, P., Ndowa, F., Martin, I., Dillon, J.-A. R., Ramon-Pardo, P., Bolan, G. & Wi, T. 2019. World Health Organization Global Gonococcal Antimicrobial Surveillance Program (WHO GASP): review of new data and evidence to inform international collaborative actions and research efforts. *Sexual health,* 16**,** 412-425.