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ORIGINAL ARTICLE

Evaluation the Antibacterial Activity of *Cornulaca moncantha* Extract Against Multidrug-resistant Coagulase-negative Staphylococci

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Abstract

Background: **Emergence of anti-microbial resistance represents a serious challenge to public health and the global economy, creating an urgent need for searching of new anti-microbial agents.**

Objective: **To investigate the antibacterial activity of *Cornulaca moncantha* ethanolic root extract against multidrug resistant coagulase-negative Staphylococci.**

Material and methods: **The ethanolic extract of *Cornulaca moncantha* roots was prepared using a Soxhalet apparatus. Phytochemical screening was performed via standard qualitative tests (alkaloids with Mayer's test, tannins with ferric chloride, flavonoids with Shinoda test, and saponins with foam test). The plant antibacterial activity as well as susceptibility of antibiotic against bacteria were evaluated using agar well diffusion besides disc diffusion methods.**

Results: **Phytochemical analysis revealed the presence of alkaloids, tannins and flavonoids in Cornulaca *moncantha* extract. The extract demonstrated potent antibacterial activity against bacteria compared to all tested antibiotics except vancomycin, exhibiting an inhibition zone of 22 mm versus vancomycin 20 mm. Notably, the antibiotic susceptibility test revealed that the bacteria were highly sensitive to vancomycin and resistant to other antibiotics.**

Conclusions: ***Cornulaca moncantha* exhibited antibacterial activity and might serve as a potential candidate for controlling and treating infections caused by coagulase-negative Staphylococci.**

Keywords: Antibacterial activity, Antimicrobial resistance, *Cornulaca moncantha* extract, Coagulase-negative staphylococci, Multidrug-resistant

Introduction

Antimicrobial resistance (AMR) is a worldwide prominent cause of morbidity and mortality. It arises from the overuse and misuse of commercially available antimicrobial agents, which facilitates the development of resistant pathogens and endanger public health. This resistance increases among several *Staphylococcus* species such coagulase-negative staphylococci (CoNS) (Tang et al., 2023).

Coagulase-negative staphylococci categorized as an opportunistic pathogen which found in the skin and mucous membranes of human which responsible for a variety of infections like; urinary tract infections, endocarditis, blood infections, heart valve infections, peritonitis, mediastinitis, pyoderma, catheter-related infections, prosthetic device-related infections (Asante et al., 2020). These bacteria have become a major concern to the medical community because they have an extraordinary ability to adapt rapidly to antibiotics resulting in multidrug resistance toward commonly used antimicrobials (Goulart, 2023). Hence, it is essential to explore novel antimicrobial agents to treat resistant bacterial infections.

Cornulaca monacantha (*C. monacantha*) is a flowering plant species from the genus *Cornulaca*, belonging to the Amaranthaceae family. It grows in Mediterranean and Arabic regions and is commonly known as "Had". It contains several biologically active

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compounds mainly alkaloids, flavonoids, tannins, coumarin, triterpenoid and polyphenolic compounds (Mhiri et al., 2020). It possesses various biological and therapeutical activities including, antioxidant, antimicrobial, antidiabetic, anti-arthritic, cytotoxicity, purgative and hepatoprotective activities (Badawya et al., 2023). The aerial parts of *C. monacantha* have been employed in traditional medicine for treating skin infections, wounds, scabies and even fungal infections due to their ability to inhibit the growth of harmful microorganisms, also it has been used to alleviate inflammation and swelling associated with infections (Hussien et al., 2023; Boussadia et al., 2024). This study carried out to assess the antimicrobial activity of *C. monacantha* root extract against multidrug resistant coagulase-negative *Staphylococci*. It also aimed to compare its efficacy with that of standard antibiotics and to identify the bioactive phytochemicals contributing to its antimicrobial effects.

Materials and methods

Plant collection and extraction

Roots of *Cornulaca monacantha* were collected in August from Karbala city, Iraq and identified by Dr. Neepal Imtair Al-Garaawi, Professor of Plant taxonomist, University of Karbala. The plant roots were thoroughly cleaned, rinsed with water, then dried at 25 °C and ground into a fine powder. Powder extracted (30 g) with 300 ml of 95% ethanol in a Soxhlet apparatus for 5 hrs. The final extract filtered through sterile Whatman No. 1 filter paper. By a rotary evaporator, the filtrate concentrated under reduced pressure at 40 °C until a crude extract obtained. Prior to use, the extract dissolved in 25% dimethyl sulfoxide (DMSO) to prepare 550 mg/mL solution. DMSO alone was used as negative control to verify the absence of any antibacterial activity on its own (Anwar et al., 2023).

Phytochemical analysis of plant extract

Phytochemical analysis performed on the *Cornulaca monacantha* extract to identify the existence of alkaloids, tannins, flavonoids and saponins using phytochemical screening procedures (Badawya et al., 2023; Dubale et al., 2023).

Collection and identification of bacteria

Isolates of coagulase-negative Staphylococci were collected from the burned patients' skin, in the burn unit at Imam Hussein Medical City, Karbala province, Iraq. Isolates were identified based on their cultural, morphological, as well as microscopical & biochemical tests. Besides, the Vitek automated bacterial identification instrument (Acquaviva et al., 2021; Fernandes Queiroga Moraes et al., 2021).

Antibiotic susceptibility test

The antibiotic susceptibility of all isolates was determined by Kirby–Bauer disc diffusion method using antibiotic discs including; vancomycin, oxacillin, ciprofloxacin, rifampicin, tetracycline, gentamicin, ampicillin, imipenem, and erythromycin (Bauer et al., 1966). Freshly grown bacteria were swabbed onto the Mueller-Hinton agar plates and allowed to air dry/30 min. Antibiotic discs were placed on the inoculated medium and then incubated at 37 °C/24 hrs. After that, the diameters of the inhibition zones were measured. Results categorized as sensitive (S) and resistant (R) according to the "Clinical and Laboratory Standards Institute" (Wayne and Clinical and Laboratory Standards Institute, 2023).

Evaluation the antimicrobial activity of plant extract

Cornulaca moncantha's antimicrobial activity was evaluated by agar well diffusion method. A 0.1 mL of bacterial inoculum was spread on the Muller Hinton agar then left for 30 min. Wells of 8 mm diameter were then created on the agar surface using a sterile borer. The wells were filled with 50 μL of plant extract and 50 μL of DMSO separately, then left at 25 °C for 30 min to facilitate diffusion. The plates were subsequently incubated at 37 °C for 24 hrs. The antimicrobial effect of extract and solvent were determined by measuring the diameter of the inhibition zones (Ashour & Alsuwayt, 2019). All experiments were performed in triplicate on bacterial isolates to ensure consistency and reliability of results.

Statistical analysis

Data were expressed as mean ± standard deviations (SD) and analyzed by one-way analysis of variance (ANOVA) using (SPSS, version 20). P value < 0.05 was considered statistically significant.

Results

The phytochemical analysis of *Cornulaca moncantha* extract identified the presence of alkaloids, flavonoids and tannins (Table 1).

The extract demonstrated potent antibacterial activity with an inhibition zone of (22 mm) which was

Table 1. Phytochemical screening of the ethanolic extracts of Cornulaca moncantha.

Phytochemical	Procedures	Result
Alkaloids	Mayer's test	+ ve
Tannins	Ferric chloride test	+ ve
Flavonoids	Shinoda test	+ ve
Saponins	Foam test	- ve

+ ve: indicates presence of phytochemicals; - ve: indicates absence of phytochemicals.

Table 2. Antibacterial activity of plant extract and standard antibiotics against Coagulase-negative staphylococci.

Antibiotics and plant extract	Inhibition zone (mm) (mean ± SD)	Category
Ampicillin 30 µg	1 ± 0.01	R
Oxacillin 1 µg	0 ± 0.00	R
Ciprofloxacin 10 µg	2 ± 0.01	R
Rifampicin 15 µg	1 ± 0.02	R
Tetracycline 30 µg	3 ± 0.02	R
Gentamycin 10 µg	4 ± 0.02	R
Imipenem 10 µg	2 ± 0.02	R
Erythromycin 25 µg	3 ± 0.02	R
Vancomycin 30 µg	20 ± 0.06	S
DMSO (25%)	4 ± 0.03	R
Plant extract 550 mg/mL	22 ± 0.05	S

S: Sensitive; R: Resistant.

comparable to that of vancomycin (20 mm) and outperforming all tested antibioticsNotably, the antibiotic susceptibility test revealed that the bacteria were highly sensitive to vancomycin but resistant to dimethyl sulfoxide and most commonly used antibiotics (Table 2; Figs. 1 and 2).

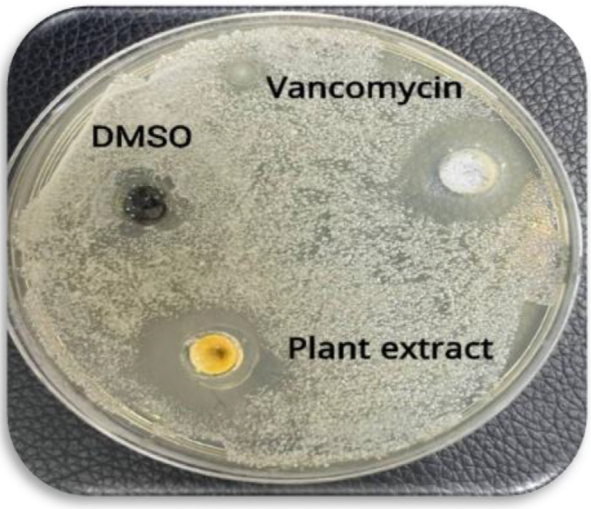

Fig. 2. Antibacterial activity of Cornulaca moncantha *extracts against Coagulase-negative* staphylococci.

Discussion

Antimicrobial resistance caused by *coagulase-negative staphylococci* is a major challenge in infection control and a cause of increased mortality. Therefore, it is necessary to find natural products with antibacterial activity to treat infections caused by these bacteria (França, 2023).

In present study, the phytochemical analysis showed that *Cornulaca moncantha* contains several biological active components including, Alkaloids, tannins and flavonoids, which are consistent with

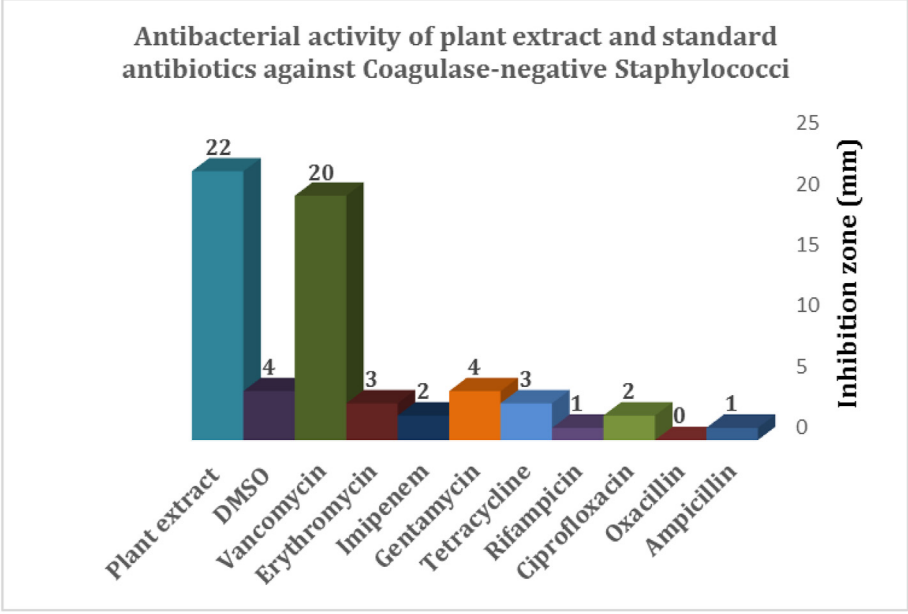

Fig. 1. Antibacterial activity of plant extract and standard antibiotics against Coagulase-negative staphylococci.

previous studies (Ashour & Alsuwayt, 2019; Badawya et al., 2023).

In this study, antibiotic susceptibility test showed that coagulase-negative staphylococci were sensitive to vancomycin, while showing resistance against the most commonly used antibiotics. Similar findings were observed in previous studies reported that the CoNS exhibited high sensitivity to vancomycin and high resistant to penicillin G, gentamicin and oxacillin (Mashaly & El-Mahdy, 2017; Qu et al., 2010).

In the current study, the extracts of *Cornulaca moncantha* indicated antimicrobial activity against CoNS, and the current results agreed with other studies revealing that alcoholic *Cornulaca monacantha* extract had variable antimicrobial activities against *Staphylococcus aureus* due to presence of several bioactive compounds mainly alkaloids, tannins and flavonoids (Ashour & Alsuwayt, 2019; Badawya et al., 2023).

The antibacterial activity of alkaloids against multidrug-resistance *Staphylococcus aureus* due to their ability to intercalate DNA and inhibiting or destroying the action of β -lactamase, alkaloids also cause marked shrinkage of the bacterial cytoplasm, creating noticeable gaps between the cell membrane and cytoplasm. This disruption damages the cell membrane, leading to the intracellular leakage and eventually death of bacteria (Aini et al., 2016). Additionally, alkaloids can exert antibacterial effects by inhibiting pyruvate kinase, an enzyme necessary for the production of pyruvate. Inhibition of this enzyme would likely reduce ATP production, disrupting the bacterial metabolic processes and ultimately causing bacterial cell death (Yan et al., 2021).

The tannins present in *Cornulaca moncantha* exert antibacterial activity due to their potential to penetrate the bacterial wall and reach the inner membrane, where they interfere with cellular metabolism, ultimately leading to bacterial destruction. In addition, tannic acid inhibits the bacteria attachment to the surfaces, leading to the bacterial death. Moreover, tannic acid also inhibits bacterial growth by preventing bacteria from uptaking sugar and amino acids (Kaczmarek, 2020).

Plant flavonoids have been shown to produce antibacterial activities against multidrug-resistance *Staphylococcus aureus* via various mechanisms of action, mainly by disrupting nucleic acid synthesis, impairing of cytoplasmic membrane function, and interfering with energy metabolism (Shamsudin et al., 2022). Additionally, flavonoids can reduce bacterial adhesion, biofilm formation, cell membrane porin expression, membrane permeability, and pathogenicity, all of which are crucial for bacterial growth. Moreover, certain flavonoids have also been shown to counteract antibiotic resistance, thus enhancing the effectiveness of existing antibiotics (Biharee et al., 2020).

In conclusion, *Cornulaca monacantha* extract exhibits potent antibacterial activity against multidrug-resistant coagulase-negative staphylococci, surpassing most tested antibiotics and matching vancomycin in efficacy. Phytochemical analysis revealed the presence of alkaloids, flavonoids, and tannins, which likely contribute to its antibacterial activity through membrane disruption and biofilm inhibition. These findings highlight its potential as promising natural source for managing and treating infections caused by these bacteria. Further studies are warranted to explore its synergistic effects with conventional antibiotics and evaluate its in vivo efficacy to determine its suitability for clinical and pharmaceutical applications.

Ethics information

The study was approved by the Scientific and Ethical Committee at the University of Al-Ameed.

Author contribution

All authors participated in selecting the research topics and were actively involved in the writing and revision of the manuscript.

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This study was self-funded.

Conflict of interest

The authors declare that there are no conflicts of interest.

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