Article

Investigation into the Impact of Aqueous Fenugreek Seed Extracts on Bacterial Isolates from a Burn Hospital

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Abstract: Background: Fenugreek, a historically significant medicinal herb, contains various beneficial compounds such as alkaloids, flavonoids, and saponins. This study aimed to investigate the antimicrobial activity of fenugreek seed aqueous extract against gram-negative bacteria, particularly those isolated from burn patients in Mosul city.
Materials and Methods: A total of 300 samples were collected from burn patients and hospital environments in Mosul hospitals. Gram-negative bacterial strains were isolated and identified using standard methods. Sensitivity testing was performed, and the aqueous extract of fenugreek seeds was analyzed using high-performance liquid chromatography (HPLC).
Results: Among the samples, Acinetobacter baumannii, Citrobacter freundii, and Enterobacter hormaechei were identified. Resistance was observed in all Acinetobacter, Citrobacter, and Enterobacter isolates to pipraciillin/tazobactam, with varying degrees of resistance to imepenem and meropenem. HPLC analysis revealed the presence of active compounds in fenugreek seeds. Plant extracts exhibited significant inhibitory effects, surpassing the effects of antibiotics.
Conclusion: The study concludes that the aqueous extract of fenugreek seeds demonstrates superior antimicrobial and anti-inflammatory activities compared to standard antibiotics and anti-inflammatory drugs. This suggests that fenugreek seed extract could be a promising alternative for treating inflammation resulting from burns.

Keywords: fenugreek, gram negative bacteria, HPLC, Antibiotic resistance, Antimicrobial activity

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1. Introduction

Infection stands as a leading cause of mortality in burn patients, contributing to multi-organ failure in 46-51% of cases. Critically ill patients, facing changes in host metabolic and immune defenses, are at an elevated risk of developing nosocomial infections [1].

Acinetobacter baumannii, a significant opportunistic pathogen within Gram-negative bacteria, is accountable for 2–10% of nosocomial illnesses and recognized as one of the six major multidrug-resistant hospital pathogens by the Infectious Diseases Society of America. Patients with A. baumannii bacteremia exhibit high mortality rates (56.2%).

Additionally, Enterobacteriaceae, encompassing Enterobacter, Escherichia, Citrobacter, and others, pose threats with various antibiotic resistance mechanisms, including extended-spectrum $beta-lactamase (ESBL) genes [2].

Global bacterial resistance to antibiotics is escalating, influenced by factors such as inappropriate clinical antibiotic usage, extensive application in the food production sector, and the unrestricted availability of antibiotics in many countries without prescriptions [3]. Multidrug resistance (MDR), where microbes resist

multiple drugs, is a growing concern, manifesting through natural resistance, genetic mutation, or acquired resistance from other species [4].

As the need for antibiotic alternatives intensifies, exploring naturally occurring botanicals becomes crucial. Medicinal plants, historically and contemporarily, have played pivotal roles in medicine, offering bioactive secondary metabolites that facilitate healing [5]. Trigonella foenum-graecum, or fenugreek, belonging to the Fabaceae family, is a medicinal herb with a rich therapeutic history, including anti-diabetic, anti-hyperlipidemic, and gastroprotective properties [6,7]. Fenugreek seeds contain diverse bioactive compounds such as galactomannan, steroidal saponins (diosgenin), amino acids (4-hydroxyisoleucine), flavonoids, and phenolic acid derivatives.

High-Performance Liquid Chromatography (HPLC) is employed to detect antimicrobial substances in fenugreek, allowing for the separation, identification, and quantification of compounds in liquid samples. Recognized as a powerful analytical tool, HPLC is widely used for the qualitative and quantitative analysis of drug products and determining their stability [8].

The study aimed to determine the effect of aqueous extracts of fenugreek seeds on Gram-negative bacterial species isolated from burn patients hospitalized in the Specialized Burn Center in Mosul and to compare that with the effect of some antibiotics.

2. Material and methods

2.1. Sample Collection:

A total of 300 samples were acquired from hospitals in Mosul. Specifically, 100 samples were obtained from burn injuries under medical supervision at the Specialized Burns Center in Mosul between September 21, 2023, and January 1, 2024. Immediate attention was given to the handling of the samples to ensure their integrity.

2.2. Ethics Statement:

Ethical approval for this research was obtained from the Ministry of Health and Environment, Nineveh Health Directorate, with reference number (37160) on September 20, 2023.

2.3. Cultivation and Diagnosis of Samples:

The collected samples were directly cultured on MacConkey medium and incubated at 37°C for 24 hours. Subsequently, isolates were purified using the streak plate method on MacConkey medium, followed by incubation at 37°C for an additional 24 hours. A single pure colony was initially subjected to standard bacteriological tests, encompassing assessments of colony morphology on MacConkey agar, Gram stain, catalase, and oxidase tests [9].

Phenotypic and microscopic characterization, along with chemical tests, were performed. Further diagnosis was carried out using the Vitek2 system, followed by molecular diagnosis through 16S rRNA analysis.

Collection and Preparation of Plant Extract: Fenugreek seeds were procured from stores, dried, and ground using a household mixed.

2.4. Antibiotic susceptibility test

The antibiotic susceptibility test was conducted using the disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines from the year 2023. Acinetobacter baumannii, Citrobacter ferundii and Enterobacter hormaechei isolates were cultured on brain heart infusion agar. After an incubation period at 37°C for 18-24 hours, single colonies, approximately 3-5 in number, were selected and transferred into a test tube containing 4 ml of normal saline to produce a bacterial suspension. Activated the bacteria and were incubated at 37 close to tube (0.5) of the standard McFarland tubes for 24-18 hours [10].

On the next day, a bacterial suspension was prepared, in which the bacterial count was equivalent to bacterial culture and placed in a plate containing the1.5 x 810 cells/cm² [11].

A sterile cotton swab was immersed in the adjusted suspension, rotated several times firmly on the inside wall of the tube above the fluid level to eliminate excess inoculum, and then used to streak the entire

surface of a Mueller-Hinton agar plate. This streaking process was repeated two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculum. The plates were left to dry at room temperature for 15-20 minutes.

Subsequently, antibiotic discs were placed on the agar using sterile forceps and pressed firmly. The plates were then incubated at 37°C for 18-24 hours. Following incubation, the diameter of the inhibition zones around the discs was measured using a metric ruler (mm), and the results were interpreted as sensitive, intermediate, or resistant based on the CLSI guidelines from 2023.

The MICs of Antibiotic for the bacteria were determined by broth microdilution method as described by Clinical and Laboratory Standard Institute-CLSI 2023, For each strain, duplicate sets10 tubes for double dilutions each were with Concentration (5000,2500.1250,625,312.5,156,78,39,19.5 µg/ml)and the first tube was a control,. The MIC was defined as the lowest concentration of antibiotic that inhibits the visual growth of bacteria after 18 hours of incubation. The final inoculum was5 × 105 CFU/mL. The tubes were incubated for 18 h – 24 h at 37°C.

The results of meropenem showed that the minimum Inhibitory concentration for bacteria they showed higher MIC concentrations, so they were 1250 µg/ml for Acinetobacter baumannii and Citrobacter freundii while the Enterobacter hormaechei bacteria were 10.000µg/ml.

2.5. Aqueous Extract:

The Seeds taken and ground in an electric grinder, then 10 grams were taken from them and placed in a Soxhelt device to extract a water extract. This extract was filtered with Whitman filter paper and this extract was concentrated using a rotary evaporator under reduced pressure at a 40C [12]. The aqueous extract powder was stored at 4°C in closed bottles until use [13].

2.6. Preparation of Concentrations:

Concentrations of 1000, 500, 250, and 125 µg/ml of the aqueous extract were prepared by dissolving it in sterile distilled water (1g/10ml), following the method described by (Abdalah et al.,2011) [14], resulting in a descending range of extract concentrations from 1000 µg/ml to approximately 125 µg/ml.

2.7. Antibacterial Activity:

Agar dilution method used for determining antimicrobial susceptibility, was employed. The antimicrobial agent was incorporated into a series of agar plates containing increasing concentrations of the agent to be tested. Inoculums of various microorganisms were simultaneously applied to the agar surface and incubated for 24-48 hours. A standardized inoculum was prepared, achieving 0.5 turbidity on the McFarland scale (1 × 108 colony-forming units (CFU) mL-1). Growth was measured and compared with the control. Mueller Hinton medium was used for its proven efficacy in routine susceptibility testing of non-fastidious bacteria [15].

3. Result

Isolation and Diagnosis: One hundred samples were obtained from burn patients using swabs, and MacConkey medium revealed bacterial growth. Following isolation and diagnosis through the Vitek2 system and molecular methods, 22 samples of Acinetobacter baumannii, 4 samples of Enterobacter hormaechei, and 3 samples of Citrobacter freundii were identified.

3.1. Antibiotic susceptibility test

The sensitivity of the bacterial isolates obtained in this study was tested against 10 types of antibiotics that are used to treat infection with Acinetobacter baumannii, Enterobacter hormaechei, and Citrobacter freundii, which included Amikacin, Azithromycin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, Levofloxacin, Meropenem and piperacillin-tazobactam.

The diameter of the Inhibition zone was measured in millimeters, and isolates were divided as either resistant, intermediate, or sensitive to antibiotics compared to the standard inhibition zone. All isolates from burn cases were resistant to all antibiotics, as shown in the table 1

Table 1. All isolates from burn cases were resistant to all antibiotics

Antibiotic name	**Antibiotic group**	**susceptibility**
Amikacin- AK10	Aminoglycosides	R
Azithromycin -AZM15	Macrolides	R
Ciprofloxacin -CIP10	Fluoroquinolones	R
Ceftazidime-CAZ30	Cephems	R
Ceftriaxone -CRO10	Cephems	R
Gentamicin -CN10	Aminoglycosides	R
Imepenem -IPM10	Carbapenems	R
Levofloxacin -LEV5	Fluoroquinolones	R
Meropenem -MEM10	Carbapenems	R
Piperacillen _tazobactam-P/T 30/6	B_lactam+inhibitors	R

3.2. Qualitative and Quantitative Detection of Fenugreek Extract:

Following both qualitative and quantitative detection, the fenugreek seed extract was subjected to High-Performance Liquid Chromatography (HPLC) analysis.

The HPLC separation profile displayed various chromatographic peaks in the examined sample extract. It was observed that the fenugreek extract contained glycosides, tannins, phenols, and flavones in the aqueous extract, as depicted in Figure No. 1. The study investigated the effect of the plant extract on bacterial growth, comparing its inhibitory effect with antibiotics. The results indicated that the Minimum Inhibitory Concentration (MIC) of anti-meropenem for Citrobacter ferundii ,Acinetobacter baumannii , and Enterobacter hormaechei bacteria was, 625,1250 and 5000µg/ml, respectively, as shown in Figure No. 2. When fenugreek was introduced using the repeated dilution method, the MIC became 250,500,500µg/ml as illustrated in Figure No. 3. This observation suggests that the inhibitory capacity of fenugreek is comparable to that of meropenem.

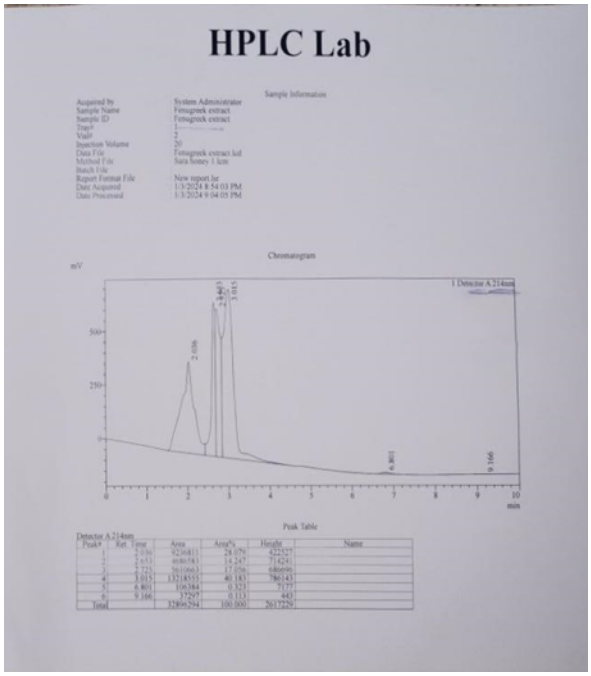

Figure 1. HPLC chromatogram of the studied fenugreek seeds recorded at 214nm by Shimadzu

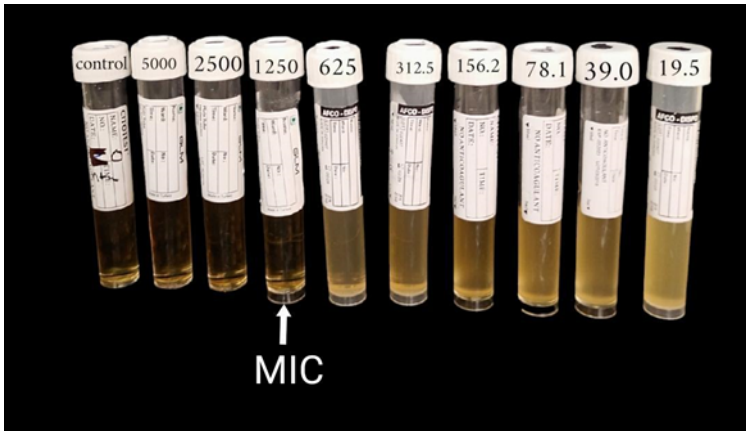

Figure 2. minimum inhibition concentration of bacteria and antibiotic

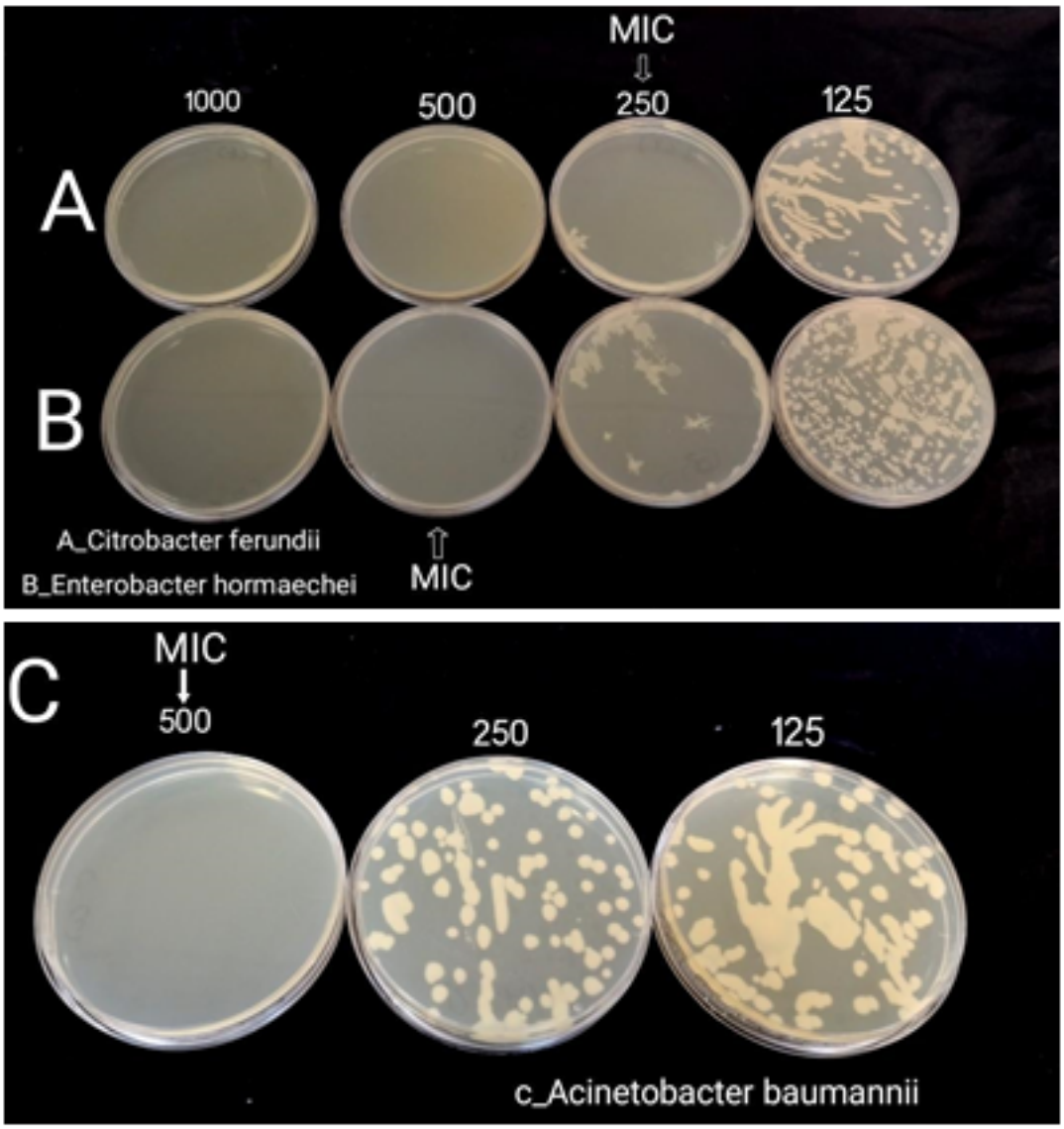

Figure 3. antibiotic+bacteria+fenugreek minimum inhibition concentration

4. Discussion

The indiscriminate use of antibiotics and synthetic antibacterial agents has led to the emergence of multidrug-resistant microbial strains, and some microbial strains exhibit reduced susceptibility to antibiotics [16].

Natural therapeutic medications from various plant extracts have been developed to treat antimicrobial resistance [17].

Traditional plant-based medicines have demonstrated high effectiveness in providing antimicrobial compounds. Phytochemical compounds help combat various infections caused by microorganisms. Plants are known to be rich sources of secondary metabolites, including terpenoids, tannins, alkaloids, and flavonoids, and these secondary metabolites are often responsible for plants' medicinal properties [18].

Broth dilution techniques were used to determine the effect of plant compounds in inhibiting the growth of specific microorganisms [19].

Aqueous extracts of fenugreek seeds are found to have significant antibacterial activity against several bacterial strains, including Acinetobacter, Enterobacter, Citrobacter, In another study, fenugreek extract was found to have great antimicrobial properties [20].

5. Conclusions

This study concludes that the aqueous extract of fenugreek seeds exhibits superior antimicrobial and anti-inflammatory activities compared to standard antibiotics and anti-inflammatory drugs. Consequently, the fenugreek seeds' aqueous extract holds potential as an alternative for treating inflammation resulting from burns. However, further investigations are essential to assess its viability for in vivo applications.

Conflicts of Interest: None declare

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