



Effect of Fenugreek Oil on Escherichia coli that causes Urinary Tract Infection

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Abstract

Introduction: A study was conducted to investigate the antibacterial activity of fenugreek (*Trigonella foenum-graecum*) oil against Escherichia coli one of the most important organisms causing urinary tract infections. Fenugreek contains several bioactive compounds that may kill the growth of microbes.

Methods: In the current study alcoholic and aqueous extracts of fenugreek have been prepared and tested for the biocontrol of E. coli isolated from clinical samples. The action the activity of the extracts in inhibiting the bacterial growth was determined. Wells were bored on agar plates seeded with overnight culture of bacteria and 5ml of each fenugreek extract (at different concentrations) was pipetted inside the wells. Plates were incubated for 28 h and inhibition zones were measured with the measurement of a ruler. Diameters of inhibitor against bacterial growth were compared with diameters of inhibition caused by standard antibiotics to compute relative antibacterial activity.

Results: The oil extract showed an inhibitory effect against G+ve strains and the highest zone of inhibition of 20mm was observed at 200 mg/ml concentration while the least inhibitory zone was found at 100 mg/ml concentration of 8 mm in inhibitory diameter. Preliminary phytochemical analysis found presence of certain active components in extract like flavonoids, alkaloids, tannins and terpenoids which have already been reported to have an antimicrobial activity. The effect was better with the alcoholic extract than with the hot and cold extracts in aqueous solution because relatively better active constituents were solved with the alcohol. Inhibition zone increased with the increased concentration of extract and thus there was a proportional effect of extract concentration of antibacterial activity.

Conclusion: The present results show that fenugreek oil does have a remarkable antibacterial effect against E. coli and it can be used as an alternative natural source to treat infections caused by antibiotic-resistant strains. This study underscores the fact that plants-derived sources have a potential for treatment and further study is needed to enhance values for the underlying mechanism of the inhibitory action of fenugreek and its active elements.

Keywords: Fenugreek oil, Plant extracts, UTI, Antibacterial

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Introduction

A great challenge of modern medicine is resistance of antibiotics, which has made many pathogenic species resistant to commonly used treatments (1). Escherichia coli is the most prevalent community and nosocomial pathogen found in UTIs (2). In recent years, as a result of increased resistance mechanisms of E. coli, the incidence of infection and treatment failure has significantly increased (3).

Due to the increasing problem of resistance, however, attention has been brought back to the search for identifying natural antimicrobial agents that could be used as safer and more sustainable treatment options. Several medicinal plants have been identified as containing compounds with inhibitory activity against detrimental microorganisms among the most extensively studied for their medicinal potential is fenugreek (*Trigonella foenum-graecum*). Its seeds have been found to have several biological activities such as blood glucose lowering effect in those with

diabetes, hormone modulation, and improvement of the urinary system disorders (4, 5). Fenugreek is also known to have constituents that may boost testosterone levels in men, as well as potent antioxidants, including an array of extremely vital fatty acids and other active elements (6).

UTI due to E. coli is more common in females and pregnant women, with potential urinary retention and reflux due to pregnancy-related changes. While E. coli is a normal resident of the intestine, it can become an opportunistic pathogen when it colonizes other regions of the body. It has been linked to diarrhea, neonatal meningitis, and septicemia as well as different types of urinary tract infection, being responsible for up to 90% of UTI cases (7, 8).

E. coli is a Gram-negative, motile bacterium that ferments lactose with the production of acid; it can grow readily on MacConkey agar, producing pink colonies. GS produces no H₂S and has an optimal pH range for growth of 6-8 (9). It possesses several virulence factors



that contribute to its pathogenic potential, including hemolysin production, lipopolysaccharide (LPS) endotoxins, and adhesion mechanisms that enable colonization of the urinary epithelium (10). Other key factors include capsule production, biofilm production, and bacteriocin secretion, as well as the potential for β -lactam miogenesis via β -lactophore production that inactivates penicillin-type antibiotics such as methicillin and amoxicillin (11, 12).

Given the difficulty of treating UTIs caused by multidrug-resistant *E. coli*, there is a need for plant-derived antibacterial compounds. The purpose of this work was to analyze the inhibitory effects of different fenugreek extracts on pathogenic bacteria, focusing on *E. coli*, and to assess the extent to which these extracts could serve as a natural alternative to antibiotics.

Materials and Methods

Sample Collection

A total of 80 urine samples were obtained from male and female patients with an age distribution receiving diagnostic services at Al-Diwaniyah Teaching Hospital during the period from January to the end of April 2023. Specimens were collected in sterile plastic containers for diagnostic, culture, and antibiotic susceptibility testing.

Part of the urine specimens were analyzed by imaging to identify bacteria. Samples with no bacteria were excluded from the study. Blood Agar and MacConkey Agar were meanwhile inoculated with the remaining samples using a sterile inoculating loop, streaking the plates in the exclusion method. The plates were then incubated at 37°C for 24 h. The isolates were morphologically characterized by colony shape and color, biochemically tested, and subsequently serotyped using an API 20E test strip.

Preparation of Alcoholic Extract of Fenugreek Seeds

The alcoholic extract of fenugreek (*Trigonella foenum graecum*) seeds was prepared using a Soxhlet apparatus, following the method described by (13). Then, 100 g of fenugreek seeds were extracted with 500 ml of 95% ethanol. The resulting extract was poured into Petri dishes and incubated at 70°C until the solvent had evaporated entirely. The crude extract was harvested, stored for further dilution, and used in antibacterial screening.

Effect of Fenugreek Oil on Bacterial Isolates

Agar plate surfaces were inoculated with bacterial isolates using sterile cotton swabs. Agar wells were perforated with a sterile cork borer, and various concentrations of fenugreek extract were applied to the wells. After that, the plates were incubated at 37°C for 24 h. The antibacterial activity was determined by measuring the diameters of inhibition zones (in millimeters) after incubation.

Well diffusion assay of the extract activity

The activity of the extracts in inhibiting bacterial growth was determined using the method described by (14). Wells were bored on agar plates seeded with an overnight culture of bacteria, and 5 ml of each fenugreek extract (at different concentrations) was pipetted into the wells. Plates were incubated for 28 h, and inhibition zones were measured using a ruler. Diameters of inhibition were compared with those caused by standard antibiotics to determine relative antibacterial activity.

Statistical Analysis

All statistical analyses were performed with SPSS. Significant differences were determined by analysis of Variance (ANOVA) and were considered statistically significant at $P < 0.05$.

Results and Discussion

Isolation and Identification

Three hundred specimens were examined from patients with diverse diseases, including swabs from the ear, pharynx, vagina, and wounds, as well as stool specimens. The yield of bacterial culture \pm growth on differential Blood Agar and selective MacConkey Agar media was 52% in middle ear infections, 22% in pharyngeal swabs, 17% and 14% from burns and wounds, respectively, and was least from vaginal swabs (12%) though the number of samples did not correspond to that obtained for stool specimens which produced a rate of only 10%.

Of these, 51 samples (71%) were negative for bacterial growth, and 249 samples (83%) were positive. After isolation and identification, 52 isolates of the bacterial species under investigation were isolated, containing 12 *E. coli*, 15 *Proteus mirabilis*, 10 *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Qualitative and Quantitative Phenolic Analysis of Fenugreek Extracts

The qualitative analysis showed that glycosides, tannins, phenols, flavonoids, and sterols are present in aqueous and alcoholic extracts of fenugreek ingredients, as described in Table 1. The concentration level of each active principle in the hot and cold watery extracts, as well as the alcoholic extract of fenugreek, was estimated using some chemical reagents, which are depicted in Table 2.

Effect of plant extracts on bacterial inhibition

There were very significant differences in the effects of extract source, extraction method, and extract concentration on bacterial growth inhibition across all studied species, as indicated by the results. The highest antibacterial activity against *P. mirabilis*, *S. aureus*, *P. aeruginosa*, and *E. coli* was demonstrated by the fenugreek oil extract, with average inhibition zones of 21.34 mm, 19.3 mm, 18.31 mm, and 15.64 mm, respectively (Table 3).

Table 1. Qualitative analysis of aqueous and methanolic extracts of fenugreek

Active Compounds	Alcoholic Extract	Hot Aqueous Extract	Cold Aqueous Extract
Volatile oils	+	-	-
Resins	+	-	-
Tannins	+	+	+
Glycosides	+	+	+
Alkaloids	+	-	-
Saponins	-	+	+
Phenols	+	+	+
Flavonoids	+	+	+

(+ indicates the presence of the active compound; - indicates its absence.)

This intense inhibitory action may be due to the presence of all active compounds in fenugreek oil, which exerts greater inhibition on both aqueous and alcoholic extracts, and to the oil extract's significantly higher concentration of active ingredients compared with other plant extracts (15).

The results reveal that the alcoholic extract of fenugreek was always more effective in preventing the growth of bacteria as compared to the aqueous extracts. In the case of *E. coli*, the alcoholic extract gave an inhibition zone of 25.28 mm and this was considerably greater than the 18.39 mm with the hot aqueous extract and the 16.22 mm with the cold aqueous extract. This trend was also similar in *P. aeruginosa* with the alcoholic extract recording 22.23 mm which was higher than the 17.77 mm and 14.46 mm that the hot extract and cold extract had respectively. The same was observed in *S. aureus* (21.52, 15.90, and 13.76 mm) and *P. mirabilis* (19.30, 14.20 and 12.23 mm). These differences highlight alcohol's superior ability to extract and retain active compounds that contribute to antibacterial activity.

The greater inhibitory effect of the alcoholic extract may be due to its active substances being more soluble in ethanol than in water, as well as to the higher level of bioactive ingredients found in this type of extract rather than in the aqueous extracts (16).

A clear positive linear relationship was observed between extract concentration and inhibition zone diameter. At the highest concentration (200 mg/ml), the extracts produced the largest inhibition zones, measuring 27.87 mm for *E. coli*, 25.23 mm for *P. aeruginosa*, 24.72 mm for *S. aureus*, and 22.81 mm for *P. mirabilis*. This increase in antibacterial activity is likely due to the greater availability of active compounds at higher concentrations, which enhances the cumulative inhibitory effect. At the lowest concentration tested (25 mg/ml), the inhibition zones were noticeably smaller, reaching 13.13 mm for *E. coli*, 11.23 mm for *P. aeruginosa*, 10.40 mm for *S. aureus*, and 8.48 mm for *P. mirabilis*.

Interaction between the source of the extract and its concentration was also important. To illustrate, the *E.*

Table 2. Quantitative estimation of aqueous and alcoholic extracts of fenugreek (mg/ml)

Active Compounds	Alcoholic Extract	Hot Aqueous Extract
Volatile oils	2.5	-
Resins	12.5	-
Tannins	1.8	1.2
Glycosides	16	14
Alkaloids	5.5	-
Saponins	-	14
Phenols	3	7
Flavonoids	5.5	3.5

coli, *P. aeruginosa*, *S. aureus*, and *P. mirabilis* had the inhibition zones of 29.77 mm, 26.48 mm, 25.95 mm, and 23.21 mm with a 100 mg/ml of the fenugreek oil extract. The same pattern was seen in case of comparing extraction methods: the alcoholic extraction at a concentration of 100 mg/ml had more potency, with the corresponding bacterial species giving an inhibition zone of 36.82 mm, 30.82 mm, 34.22 mm and 31.88 mm.

In general, the alcoholic extract of fenugreek at 200 mg/ml demonstrated the strongest antibacterial activity, and the *E. coli*, *P. aeruginosa*, *S. aureus* and *P. mirabilis* were inhibited with the diameter of 40.06 mm, 31.44 mm, 34.88 mm and 33.55 mm, respectively. These results prove that the extraction process and the concentration of the extract are the decisive factors that determine the antibacterial efficacy with the alcoholic fenugreek extract being the most effective preparation.

Additional comparative analysis of anti-bacterial efficacy of the tested antibiotics and alternative fenugreek extracts (hot- and cold-water-soluble; alcoholic extracts of fenugreek oil and seeds) also indicated dissimilar patterns of inhibition of the tested bacterial strains. The 100 per cent sensitive isolates were with Imipenem and it was also the best antibiotic in our study. This aligns with the established mechanism of action of imipenem which interacts with and suppresses PBPs involved in bacterial cell wall production and stability. Imipenem inhibits the formation of cell walls and block bacterial growth by inhibiting these proteins, leading to the lysis of bacteria (17, 18).

High inhibition effects were also generated with Amikacin and Ciprofloxacin respectively, with imipenem in the descending order of activity. They affect the growth of bacteria by the prevention of protein synthesis, which is a necessary step in the cell wall formation and other ways of bacterial survival. Protein synthesis inhibition causes the cell wall to lose its strength, thus, being unable to withstand the osmotic pressure, causing bacterial death (19).

The efficacy of fenugreek extracts was much lower than that of those antibiotics, but their inhibitory properties also depended on the extraction process and concentration,

Table 3. Antibacterial Comparison between inhibitory effect of antibiotics with aqueous and alcoholic extracts of fenugreek oil- Fenugreek seeds on the isolates

Bacterial species	GM	AMC	ATM	CAZ	PRL	AMP	AK	CIP	IMP
<i>E. coli</i>	13.7	14.6	18.5	18.66	17.7	0	28	29	33.5
<i>P. mirabilis</i>	14.1	15	18.6	0	0	0	29	29	33
<i>P. aeruginosa</i>	14.4	14	18.6	21	17.4	0	30	30	31
<i>S. aureus</i>	13.8	14.3	18	18	0	0	30	26	32.5

Table 4. Mean diameter of inhibition zone (mm) of fenugreek extracts.

Bacterial species	Alcoholic Extract	Hot Aqueous Extract	Cold Aqueous Extract
<i>E. coli</i>	33.59	23.98	20.34
<i>P. mirabilis</i>	30.21	18.89	16.33
<i>P. aeruginosa</i>	30.21	21.55	20.21
<i>S. aureus</i>	33.56	19.33	17.59

most notably on the alcohol concentration. Alcoholic extracts also exhibited a regular increase in the area of inhibition in comparison to the aqueous extracts, which implies that ethanol is better than water in extracting and preserving bioactive compounds with anti-bacterial effects. This enhanced antibacterial action indicates that there are naturally active compounds in fenugreek seeds which prevent the growth of bacteria, and in some cases they are stronger than some antibiotics (20).

These findings suggest that Fenugreek extracts can be a useful complementary or alternative therapeutic agent especially in the face of the development of antibiotic-resistant strains. Even though such antibiotics like imipenem are very effective, the impressive antibacterial efficacy exhibited by fenugreek extracts could be useful and serve as possible alternatives with pathogenic bacteria, particularly when resistance patterns are strong or the already existing ones aggravate (21, 22).

Other antibiotics had varied resistance profiles across species with the most active (only in several instances) being other than C153. Some of the isolates were partially sensitive, some with an apparent resistance, which was evidenced by their diversity of (and the existence of more than one) mechanisms of resistance that tend to evolve in clinical pathogenic agents (23-27).

When identifying the antibacterial activity of plant extracts, both aqueous and alcoholic fenugreek extracts inhibited all of the bacteria tested under all levels of concentration assessed. This general mode of action implies that the fenugreek could be having more than one active agent, which can attack different bacterial structures and metabolic pathways. Amongst the plant preparations, best inhibition was seen with the fenugreek oil extracts. This enhanced action can be linked to the fortification of lipophilic bioactive compounds found in the oil with the specifically those which are known to have antimicrobial property (23, 28).

The effectiveness of the alcoholic fenugreek oil extract is also realized in a complete comparison between the

extracts and the antibiotics. This inhibitory transfer even in most instances surpassed that of the antibiotics in the study thereby showing that it has a colossal antibacterial potential. The antimicrobial properties of the alcoholic extract of fenugreek seeds were found comparable to the standard antimicrobials thus proving that fenugreek could be a prospective source of antimicrobials. These remarks are well justified by the figures in Table 3 which enlists the zones of inhibition achieved on the one hand around the antibiotics or on the other around the plant extracts against the tested bacterial species.

Altogether, the results provided herein suggest that the version of the fenugreek alcohol extract oil, in specific, might turn out as an effective alternative or adjuvant therapeutic intervention, especially, as the cases of classical antibiotics resistance grow. This highlights the possible usefulness of antimicrobials of plant-derived origin in future plans to combat multidrug-resistant pathogens.

Conclusion

Multidrug resistance (MDR) was present in all *E. coli* isolates of urinary tract infections in the current study as it is an indication that uropathogenic-resistant strains are increasing in difficulty and there is no use of the old-fashioned antibiotics to cure the infection. The effective antimicrobial properties of fenugreek were also supported by the results regardless of whether it is in extract/essential oil form. In addition to this, their potential to prevent the proliferation of bacteria that cause urinary tract infections implies that fenugreek can be a viable alternative source of therapy especially with the growing resistance to antibiotics.

Recommendations

1. It is suggested that further studies should be conducted to clarify the genetic grounds of increased antibiotic resistance in these *E. coli* isolates. These would be beneficial to studies, especially in the discovery of certain genes of resistance to antibiotics, which would lead us a step closer to knowing how these strains not only evolve but also move.
2. There is a need to increase the degree of public health concern regarding the abuse of antibiotics. Learning ought to be directed towards the role played by the inappropriate use of antibiotics in the faster onset and spread of antibiotic-resistant strains and therefore citizens are held accountable to the responsibility of proper prescribing of antibiotics as well as taking the

prescribed medications correctly.

Competing Interests

The author states that there is no conflict of interest.

Ethical Approval

Not applicable.

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