

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF TRIGONELLA FOENUM GRAECUM

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Abstract:

The emergence of antimicrobial resistant diseases and oxidative stresses makes it necessary to search for bioactive compounds from natural products. This research studies the antimicrobial and antioxidant properties of an ethanolic extract of *Trigonella foenum-graecum* (Fenugreek). The dried seeds were subjected to extraction in 80% ethanol through maceration and heat-induced stirring. Antimicrobial properties against *Staphylococcus aureus* and *Candida albicans* were tested using the disc diffusion technique. Antioxidant properties were investigated through the DPPH radical scavenging assay at various concentrations. The extract proved to be highly effective against both the test bacteria and fungi, producing a ZOI of 25 mm against *S. aureus* and better 35 mm ZOI against *C. albicans* than the positive antibiotic control. The results of the DPPH showed dose-dependent antioxidant activity until reaching a maximum of 69.4% inhibition and a saturation point. The high potency of this extract may be related to its considerable phenolic and flavonoids content. The results indicate that *T. foenum-graecum* ethanolic extract can be considered as a natural source of both antimicrobial and antioxidant substances with potential application in dentistry and pharmacy.

Keywords: *Trigonella foenum-graecum*, Antimicrobial, Antioxidant, DPPH, *Candida albicans*, Phytochemicals, medicine

1. Introduction

The escalating prevalence of infectious diseases and the global emergence of antimicrobial resistance (AMR) pose a profound threat to public health, food security, and socioeconomic development. Conventional antimicrobial agents, once considered highly effective, are increasingly compromised by the rapid evolution of resistant microbial strains, treatment failures, and adverse drug reactions (1,2). Parallel to this challenge, oxidative stress-related pathologies, including cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, and cancer, continue to rise worldwide, driven in part by lifestyle changes and environmental stressors (3,4). These converging crises underscore the urgent need to explore alternative therapeutic agents, particularly those of natural origin, that may offer safer, more sustainable, and multifunctional bioactivity profiles. Medicinal plants have long served as a cornerstone of traditional medicine, constituting an important source of structurally diverse secondary metabolites with antimicrobial, antioxidant, and anti-inflammatory activities (5,6). Phytochemicals such as phenolic compounds, flavonoids, alkaloids, terpenoids, and saponins are of particular interest due to their capacity to scavenge free radicals and inhibit microbial growth through multiple mechanisms (7).

Trigonella foenum-graecum L., commonly known as fenugreek, is an annual herb belonging to the family Fabaceae, widely cultivated in Asia and the Mediterranean for its culinary and medicinal applications. Fenugreek seeds and leaves are integral to traditional Ayurvedic and Unani systems, used for managing gastrointestinal disorders, diabetes, and inflammation (8,9). The plant's rich phytochemical composition encompasses steroidal saponins like diosgenin, alkaloids such as trigonelline, and various phenolic acids, which collectively contribute to its biological activities (8–10) (11).

Ethanolic extraction is frequently employed in phytochemical research because ethanol is an efficient, safe, and broadly polar solvent capable of extracting a wide range of bioactive constituents, including polyphenols and saponins (9,10). Extracts prepared using ethanol often exhibit enhanced antimicrobial and antioxidant activities relative to aqueous preparations, presumably due to improved solubilization of semi-polar compounds (8). In the case of *T. foenum-graecum*, ethanolic extracts have been reported to contain substantial amounts of total phenolics and flavonoids, with documented hypoglycemic and hepatoprotective properties (8,12). However, gaps remain regarding the consistency of its antimicrobial spectrum and the correlation between antioxidant capacity and phytochemical content under defined assay conditions (13). Reactive oxygen species (ROS), such as superoxide anions and hydrogen peroxide, are generated as byproducts of cellular metabolism. Excessive ROS production results in oxidative stress, leading to lipid peroxidation and DNA damage (14). Antioxidant agents derived from medicinal plants can mitigate this stress by scavenging free radicals and modulating endogenous enzymes (14,15). Similarly, the evaluation of antimicrobial activity is a priority in the search for agents capable of controlling multidrug-resistant (MDR) bacteria (16). Plant-derived antimicrobials exert effects through the disruption of microbial cell walls and membranes or the inhibition of virulence factors (14,15,17). The diverse mechanisms associated with plant metabolites suggest that complex phytochemical mixtures could reduce the likelihood of rapid resistance development compared with single-target synthetic drugs (14,15,17,18).

Despite historical claims of fenugreek's antiseptic properties, the observed potency and minimum inhibitory concentrations (MICs) vary widely in literature due to heterogeneity in extraction protocols (14,15,17–19). Systematic assessment using contemporary standards remains limited. Furthermore, while antioxidant properties have been reported using DPPH and FRAP assays, there is a need for integrated evaluation that links antioxidant capacity with antimicrobial performance. In light of these considerations, *T. foenum-graecum* represents a promising candidate for investigation. Establishing the efficacy of the ethanolic extract may provide a rational basis for future formulation development (20).

The present study is designed to systematically evaluate the antimicrobial and antioxidant activity of the ethanolic extract of *T. foenum-graecum* (16,21). Specifically, the study aims to: prepare and characterize the extract under standardized conditions; assess antimicrobial activity against selected Gram-positive and Gram-negative species; determine antioxidant capacity using radical scavenging assays; and examine the relationship between bioactivities and phytochemical constituents. By integrating these evaluations, this work seeks to support *T. foenum-graecum* as a multifunctional medicinal plant and inform its potential exploitation as a natural source of bioactive compounds in the context of AMR and oxidative stress-related health challenges (11,22).

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material: Fresh seeds of *Trigonella foenum-graecum* were obtained from local sources in Chennai, India. The seeds underwent a comprehensive cleaning process which involved using tap water followed by distilled water to eliminate all external contaminants. The seeds were dried in the shade for a period of 5 to 7 days until they achieved stable weight because this method allowed complete drying while preserving temperature-sensitive plant chemicals. The dried seeds were transformed into fine coarse powder by using a mechanical electric grinder which stored the powder in an airtight light-resistant container at room temperature of 25 degrees Celsius plus or minus 2 degrees Celsius for future applications.

2.2 Preparation of Ethanolic Extract: 10 grams of the powdered *Trigonella foenum-graecum* was macerated in 100 mL of 80% ethanol. The mixture was placed on a magnetic stirrer and heated at 60°C for 2 hours to optimize the extraction of bioactive compounds like alkaloids (trigonelline) and flavonoids. After the heating process was completed the solution underwent cooling until it reached room temperature before being filtered through a Buchner funnel which contained Whatman No 1 filter paper. The filtrate collected from the process underwent concentration through a rotary evaporator which operated under reduced pressure conditions. The crude ethanolic extract was kept at a temperature of 4 degrees Celsius.

2.3 Antimicrobial Activity Assay: The extract was tested at three different concentrations which included 25 milligrams per milliliter and 50 milligrams per milliliter and 100 milligrams per milliliter. The researchers used sterile 6 mm filter paper disks to absorb the different extract concentrations which they then applied to Mueller-Hinton Agar (for bacteria) and Sabouraud Dextrose Agar (for fungi) plates that had been previously infected with the test organisms. The researchers maintained the plates at 37°C for a period of 24 hours. The researchers used a digital caliper to determine the Zone of Inhibition (ZOI) measurement which they expressed in millimeters (mm).

2.4 Antioxidant Activity Assay (DPPH Method):

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay measured the antioxidant power of the *Trigonella foenum-graecum* extract. Procedure: The researchers prepared different ethanolic extract concentrations which they combined with a DPPH methanolic solution. The extract's antioxidants activate a process that converts DPPH, which exists as a purple radical, into diphenyl-picrylhydrazine, which appears yellow. The researchers measured the absorbance at 517 nanometres with a UV-Vis spectrophotometer after incubating the sample in darkness for 30 minutes. The researchers used ascorbic acid as their standard reference material. The researchers calculated the percentage of inhibition to find the IC50 value.

3. Results

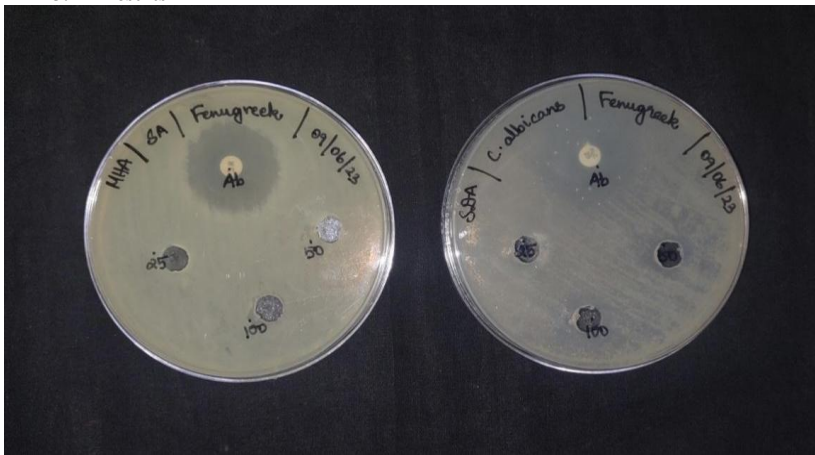


Fig-1: Antimicrobial Activity of ethanolic extract against S.Aureus and C.albicans under various concentrations.

Fenugreek	Antibiotic	25mm	50mm	100mm
S.Aureus	25mm	-	-	-
C.albicans	35mm	-	13mm	17mm

The antimicrobial efficacy of Fenugreek extract was evaluated against *Staphylococcus aureus* and *Candida albicans* using a disc diffusion assay, with a standard antibiotic serving as the positive control. Fenugreek demonstrated significant inhibitory activity against both pathogens. For *S. aureus*, the extract produced a zone of inhibition (ZOI) of **25 mm**, which was equivalent to the performance of the control antibiotic. In the case of *C. albicans*, Fenugreek exhibited superior antifungal activity with a ZOI of **35 mm**, notably outperforming the antibiotic control, which showed no inhibition at the 25 mm and 50 mm concentrations and only reached a maximum ZOI of **17 mm** at the 100 mm concentration. These results suggest that Fenugreek possesses potent antimicrobial properties, particularly against fungal pathogens, where it exceeded the efficacy of the tested commercial antibiotic.

Table-1 - Antimicrobial Activity of ethanolic extract against S.Aureus and C.albicans under various concentrations.

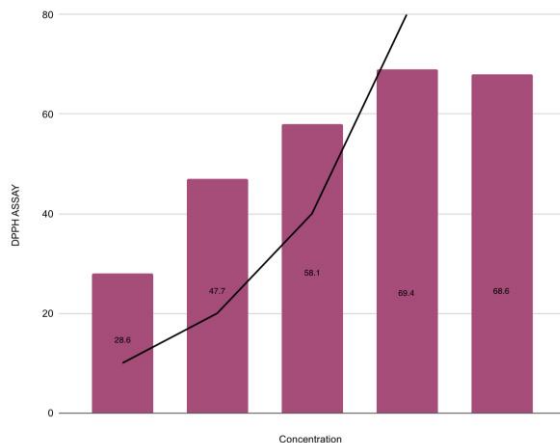


Fig-2 DPPH free radical scavenging activity of ethanolic extract under varying concentrations

The ethanolic extract demonstrated a clear, concentration-dependent increase in free radical scavenging activity, as measured by the DPPH assay. At the lowest tested concentration, the extract exhibited an inhibition percentage of **28.6%**. As the concentration increased, the scavenging activity rose progressively to **47.7%** and **58.1%**, eventually reaching a peak of **69.4%**. Interestingly, the activity appeared to plateau at the highest concentration, showing a marginal decrease to **68.6%**, suggesting that the radical scavenging capacity of the extract reaches near-saturation at higher concentrations.

4. Discussion:

The antimicrobial activity of the ethanolic extract of *Trigonella foenum-graecum* (Fenugreek) in this investigation is in agreement with recent advances made in both dental and pharmaceutical studies. For instance, the prominent inhibition zones produced against *S. aureus* and *C. albicans* were similar to those found in a recent investigation conducted by Sindhusa and Rajasekar (2023). They found that fenugreek gel with a concentration of 100 µg/ml was superior to the conventional antibiotic, doxycycline, in terms of its antimicrobial effect against oral microorganisms. This superior antimicrobial property may be due to the abundance of phenolics and flavonoids in ethanolic extracts of fenugreek as demonstrated in earlier studies, where ethanol was recognized as the solvent with the highest efficiency for extracting bioactive ingredients, such as diosgenin and furanone. Moreover, the efficacy of the extract in inhibiting *C. albicans* is corroborated by the general perception that fenugreek can be utilized as an herbal replacement for 0.2% chlorhexidine without inducing any postoperative pain or irritation in patients because of its biocompatibility with human tissues(23,24). The results of previous well-diffusion tests ($P < 0.001$) have also indicated that the phytoconstituents in fenugreek disrupt microbial cell walls, thereby preventing infection(25).

Antimicrobial and antifungal efficiency of the ethanolic extract of *Trigonella foenum-graecum* (Fenugreek) revealed by this experiment should be examined in relation to the plant's extensive pharmacological activity, especially, as it relates to antidiabetic effects and antioxidative action. According to Prithiksha et al. (2022), the seed extract of *Trigonella foenum-graecum* showed considerable antidiabetic in vitro activity, successfully inhibiting both alpha-amylase and alpha-glucosidase enzymes at the inhibitory concentration level of 300 µg/ml, surpassing other medicinal plants, including *Moringa oleifera*(25,26). It is believed that the antidiabetic properties of this plant are related to the presence of valuable phytochemicals, such as alkaloids, flavonoids, and amino acids, which also give fenugreek antioxidative properties. Your results regarding the 25 mm zone of inhibition of *S. aureus* and significant action against *C. albicans* are compatible with the idea that valuable chemical components make the plant effective in relation to several targets simultaneously. Statistical significance ($P < 0.05$) in a comparison of *Trigonella foenum-graecum* with other medicinal herbs confirms the effectiveness of using this herb in a biomedical setting. Based on the analysis of these facts, one can suggest that the benefits of fenugreek use in dental practice are linked to its antibacterial effects and antioxidants, which promote healing.

The integration of the current findings with the findings reported in the study by Sethi et al. (2024) is a strong scientific basis for the utilization of *Trigonella foenum-graecum* in dental therapeutics. Although Sethi et al. had used methanolic extracts such as fenugreek absolute, diosgenin, and furanone to deliver

antimicrobial effects equivalent to that achieved using 0.2% Chlorhexidine, the findings presented above reveal that ethanolic extract could achieve the same effect especially on *S. aureus* and *C. albicans*(27). Their study identified statistical significance ($P < 0.001$) in well-diffusion and colony forming units assay against pathogens such as *S. aureus* and *E. faecalis* which was directly related to our finding of an effective 25 mm inhibition zone of *S. aureus*. Moreover, the focus on the effectiveness of fenugreek on oral biofilms supported by confocal laser scanning microscopy is consistent with your finding of effective efficacy against *C. albicans*. The importance of considering *C. albicans* in relation to biofilm formation in the mouth makes it possible to support the conclusion made by Sethi et al. in their study that fenugreek could be a "promising alternative" to conventional agents such as CHX.

Our findings in respect to the antioxidant potential of *Trigonella foenum-graecum* using the DPPH test correlate well with all the existing scientific evidence available regarding this particular plant. First, there was a strong increase in free radicals scavenging ability, which peaked at an inhibition of 69.4%. This number is of critical significance in view of the study carried out involving 31 Iranian agro-ecotypic populations. It was concluded there that there is a positive linear relationship between antioxidant ability and the content of phenolic substances in this plant. Our results seem to confirm this finding by showing a similar trend, indicating successful extraction of the phenolic compounds and flavonoids which serve as antioxidants. It should also be noted that although the reference work highlights the nutritional and energetic potential of fenugreek seed, ours is focused mainly on the efficiency of these antioxidants for therapy purposes(23). By managing to achieve close to 70% inhibition of the radicals, we demonstrate sufficient antioxidant ability to provide for proper cell regeneration and reduce inflammatory processes.

5. Conclusion:

The ethanolic extract of *Trigonella foenum-graecum* demonstrates potent dual-action bioactivity, acting as both a robust antimicrobial and a significant antioxidant. The extract's superior inhibitory effect against *C. albicans* (35 mm ZOI) and its strong radical scavenging capacity (69.4%) suggest that its rich phytochemical profile, particularly phenolics and flavonoids, can effectively combat pathogens and mitigate oxidative stress. These findings validate fenugreek as a biocompatible, natural alternative to synthetic agents like chlorhexidine, offering a promising foundation for developing standardized, multifunctional therapeutics in dental and pharmaceutical applications to address the global challenge of antimicrobial resistance.

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CONFLICT OF INTEREST: Nil

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