

Antibacterial Activities of Fenugreek Leaves and Stems against the Pathogenic Bacteria *E. Coli*

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ABSTRACT

In the present study antibacterial activity of *Trigonella foenum graecum* leaves and stems in three different solvents such as methanol, acetone and water extracts were carried out. The antibacterial activity of various extracts was screened using standard protocol of disc diffusion method and methanol extract was found to be more potent. The antibacterial activities were assessed by the presence or absence of inhibition zones and Minimum Inhibitory Concentration (MIC) values. Fenugreek leaves methanol extract determined by broth dilution method these results recommended that methanol extract being the most potent at MIC of 2µg/disc. This finding formed a basis for further studies on screening of local medicinal plant extracts for antibacterial properties.

Keywords: Antimicrobial activity, Fenugreek plant's leaves and stem parts, methanol, acetone, water, human pathogen-E.coli.

1. INTRODUCTION

T. foenum-graecum is greatly used as medicinal plant, particularly in North Africa, the Middle East and India. It contains a broad spectrum of therapeutic properties. The seed is extremely nutritious and are suggested to improve body weight gain, particularly in anorexia nervosa. It also improved feed intake and protein utilization in rats (Chevallier, 1996). Moreover, the seeds inhibit cancerous cells of the liver and decrease blood cholesterol level. It also has an antidiabetic property.

The discovery of medicinal plants in different parts of the world is important both to the agriculture and medicine sectors, in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits. Medicinal plants do plays an important role in the treatment of ailments.(Zaidan *et.al.*,2005) The use of plant preparation for such purposes has been documented (Herbal Medicine Research Centre, 2002).

Furthermore, it is used to cure late-onset diabetes, digestive problems and inadequate lactation (Bown D., 1995). Powdered seed can be applied as a poultice to treat abscesses, boils, ulcers and burns (Chevallier, 1996). The seeds extract displayed a positive effect as cardio tonic, hypoglycaemic, diuretic, and antiphlogistic and hypotensive agents (Duke *et al.*, 1985). Alkaloid derived from fenugreeks seed showed high capacity in cancer treatment. Seeds are known as a potential approach to control glucose and prevent hyperlipidaemia and atherosclerosis in diabetic cases (Raghuram *et al.*, 1994).

All extracts (acetone 80%, methanol 100%, and water) of the fenugreek leaves and stems exhibited antimicrobial activities and the extract ability of bioactive compounds in the presence of water was higher in germinated seeds (Noziah *et al.*, 2015). Based on disc diffusion method, exhibited highest antimicrobial activity against all tested bacterial pathogens (*Escherichia coli*).

2. MATERIAL AND METHOD

- i. **Plant material: Plant material:** Fresh and healthy leaves of *T. foenum graecum* were obtained from local market of Barwaha (West Nimad)dist Khargone (Madhya Pradesh state, India) in the year 2016. The leaves and stems were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves and stems were subsequently dried under shade for 1 week, powered and used for extraction. Three different solvents (methanol, water and acetone)of fenugreek (*Trigonella foenum graecum*) leaves and stems of fenugreek plant were selected for analysis.
- ii. **Test microorganisms:** The test organisms used were clinical isolates causing Urinary Tract

Infections (UTI) namely, *Escherichia coli*, were obtained from Department of Bioscience, Christian Eminent College, Indore. The bacterial cultures were maintained on nutrient agar medium.

iii. **Preparation of aqueous extract:** Fifty grams of powdered leaves of *T. foenum graecum* was macerated with 100 mL sterile distilled water in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and centrifuged at 4000 rpm for 30 min. The supernatant was filtered through Whatman No.1 filter paper and heat sterilized at 120°C for 30 min. The extract was preserved aseptically in a brown bottle at 4°C until further use.

iv. **Preparation of solvent extract:** Fifty grams each of the powdered material was extracted initially with 300 mL of methanol and acetone separately for 24 h. The extract was filtered with sterile whatman filter paper into a clean conical flask. Second extraction was carried out with same amount of solvent for another 24 h and filtered. The extracts were later pooled and transferred into the sample holder of the rotary flash evaporator for the evaporation of the solvents. The evaporated extract so obtained was weighed and preserved at 4°C in airtight bottle until further use. Ten milligram each of dried solvent extract was dissolved in 1 mL of respective solvent and used for the antibacterial assay.

v. **Antibacterial screening:** The antibacterial activity was carried out by disc diffusion method. Antimicrobial activity of fenugreek phytochemicals was determined by disc diffusion method. Species of bacteria *E.coli* were spread on nutrient agar medium. Different concentration of different solvent of fenugreek leaves and stem extract was loaded onto the disc of 4mm diameter in the ratio of 100%, 50% and control (pure culture) using PBS. The plates were then incubated at 37°C for 24hrs. The zone of inhibition was measured with the help of standard scale (Bauer *et al.*, 1966).

vi. **Determination of Minimum Inhibitory Concentration (MIC):** The MIC was carried out by broth dilution method. Microdilution method was used to evaluate the MIC (Wiegand *et al.*, 2008). MIC has been explained to be the lowest concentration, which can attain complete growth inhibition. The nutrient broth (100 µl) of each bacterium was loaded on sterilized microplates. 50µl of the fenugreek leaves, stems extracts were added to each disc and then incubated at 37°C for 24 hrs. The disc which had no bacterial growth was regarded as MIC. (Brantner and Grein, 1994)

3. RESULT

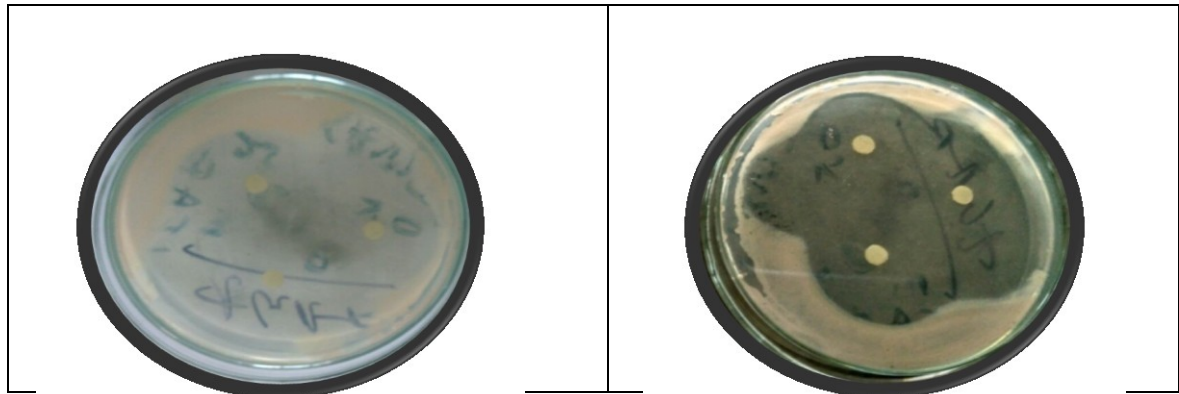
Evaluation of antimicrobial activity of these phytochemicals against *E.coli* of fenugreek leaves and stems.

In the present study, fenugreek contains antimicrobial activity and preliminary phytochemical analysis revealed the presence of various phytochemicals qualitatively in methanol, acetone and aqueous extracts of fenugreek. The present study also correlated with the aforesaid studies. These phytochemicals present in leaves extracts might be responsible for the antibacterial activity. It is not surprising that there are differences in the antimicrobial effects of different solvent extracts due to the phytochemical properties and differences among species. Their antibacterial potency was assessed by the presence of inhibition zones and zone diameters.

The result of antibacterial activity of Fenugreek leaves extract was evaluated against gram negative bacteria *E. coli*. The antimicrobial activity was determined by using disc diffusion method. Among different extract of leaves methanol extracts of *Fenugreek* leaves showed maximum antimicrobial activity. It shows 100% activity against *E.coli*. Zone of inhibition was observed with 4.9 mm respectively. *E. coli* displayed the highest susceptibility to methanol leaves extract. These data indicated that methanol extract of leaves exhibited strong antibacterial activity compared to aqueous extract and acetone extract.

Comparative study on antibacterial activity of fenugreek leaves & stems in different extract

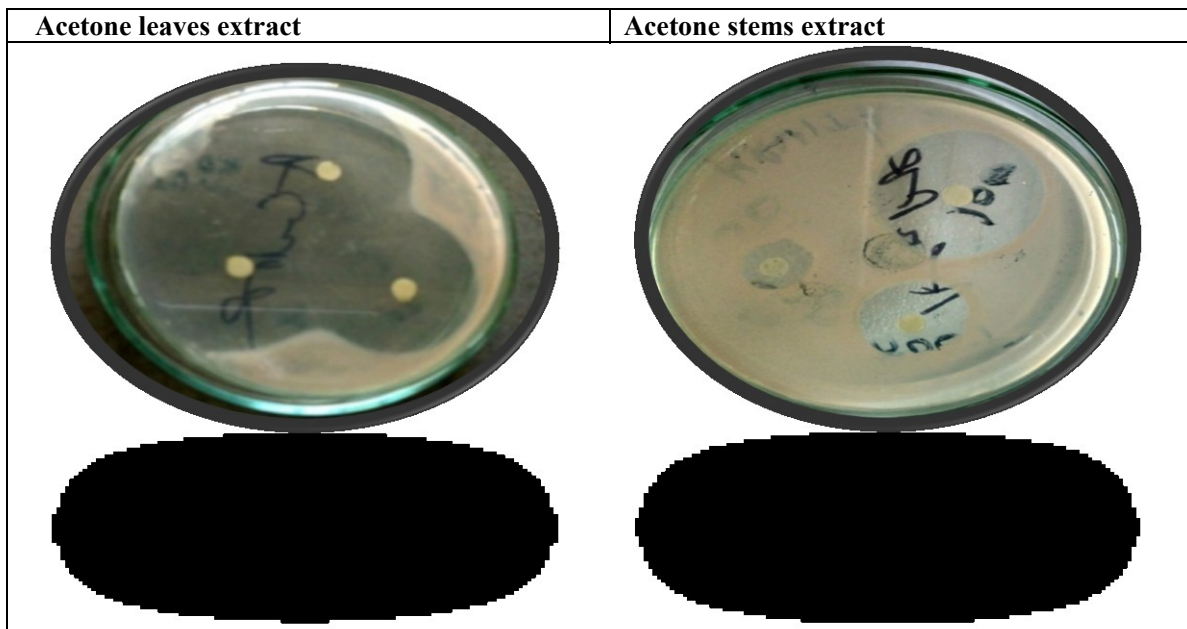
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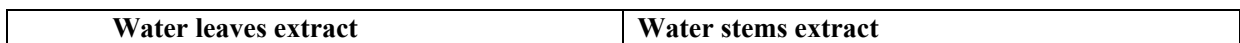
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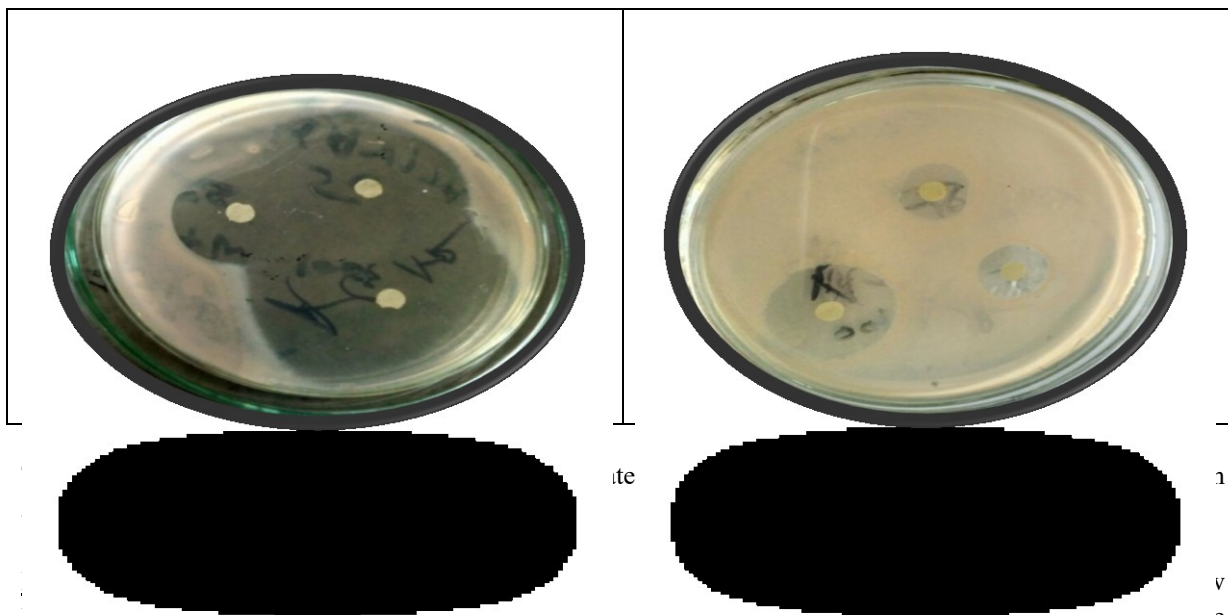
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In acetone leaves extract a clear zone of inhibition was also observed with 2.5-4mm diameter.

Figure 2: Antibacterial Activity Of Fenugreek Leaves And Stems In Acetone Solvent By Disc Diffusion Method.





Diffusion Method.

The following results show that the extract possessed some antimicrobial activity against gram negative bacteria, depending upon the nature of the active ingredients present in the extracts and their capacity for diffusion into agar medium. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism. These compounds contain major therapeutic application adjacent to human pathogens. The encouraging or highest inhibitory results indicate that the methane leaves and stem extracts of Fenugreek might be exploited as a natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications. With this study we found that methane extract provides more consistent antibacterial activity. The analysis of Phytochemicals constituents of Fenugreek has been proved to contain alkaloids, tannins, saponins, flavonoids, phenols etc. The presence of these compounds might help to explain the observed antibacterial activity of Fenugreek leaves extracts. The presence of bioactive compounds has been known to show medicinal as well as some physiological activity.

Numerous naturally occurring antimicrobials are present in animal and plant tissues where they probably evolved as part of the defence mechanisms of the host against microbial

originate application in the food industry while antibacterial and antifungal agents.

4. DISCUSSION

Therefore, fenugreek could be an important source of biologically active compounds useful for developing better new antibacterial drug. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products (Srichamroen *et. al.*, 2005).

With accumulation of more experimental evidence in support of the nutraceutical properties of fenugreek, there is growing interest in marketing fenugreek as a natural health product. Therefore, there is a need to evaluate the biochemical productivity of fenugreek to allow for selection of suitable that may be further developed into cultivars specific for the natural health product industry.

This study presents the first attempt to evaluate the differences in biological activities among leaves and stems of fenugreek. The results showed that extracts from leaves had higher antioxidant molecules and antimicrobial activities compared to the extracts from stem and root, regardless of the solvent used. In addition, this study revealed that methanol had superior efficacy as an extracting solvent for recovering potent antioxidant components from fenugreek relative to other solvents, indicating an

admirable potential of the related extracts for isolation of natural antioxidant and antimicrobial agents. Furthermore, the presented data would certainly help to ascertain the potency of the tested parts of fenugreek, especially the leaves, for medicinal health functions and functional food and nutraceutical applications.

The antibacterial activity resided mainly found in leaves methanol fraction and the minimum inhibitory concentration (MIC) of methanol fraction which caused total inhibition if *E.coli* was 0.5µg/ml. Results of current study suggested that the constituents of *Trigonella foenum-graecum* have potential against harmful pathogenic bacteria.

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