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# Fenugreek (*Trigonella foenum-graecum* L.):Antibacterial Activity Of Its Phytochemical Constituents

***Anbumalarmathi.J*, Aruna Sharmili.S and Esther Sharon***

Department of Biotechnology, Stella Maris College, Chennai -600 086
* Corresponding author:amalarmathi@gmail.com

**ABSTRACT**

Phytochemical analysis of leaves and shoot tips of *Trigonella foenum-graecum* L showed the presence of alkaloids, steroids, carbohydrates, terpenoids, flavonoids and quinones. The antibacterial activity of *Trigonella foenum-graecum* L. leaves and shoot tips on *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were evaluated by agar well diffusion method. Zones of inhibition against *Staphylococcus aureus* and *Salmonella typhi* were observed only in ethanol and methanol leaf and shoot tip extracts of fenugreek. The MIC values ranged from 2.73 and 87.5 mg/ml.

Keywords: *Trigonella foenum-graecum* L., phytochemical screening, antibacterial activities, Minimum Inhibitory Concentration, *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus.*

## INTRODUCTION

Plant extracts are known for their ethno medical traditions, that is they possess various pharmaceutical and nutraceutical properties. Fenugreek is one such plant that posesses phytoconstituents such as flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanin and tannins (Sumayya *et al*., 2012). With the emergence of multiple antibiotic resistant strains of microorganisms, great interest has been shown in the search of potential plant compounds for therapeutic, medicinal, aromatic and aesthetic uses (Gurinder and Daljit, 2009; Newman and Cragg, 2007). Phytochemicals are natural and non-nutritive bioactive compounds produced by plants, which act as protective agents against external stress and pathogenic attack. It could be used as single therapeutic agents or as combined formulations in drug development (Rashmi *et al*., 2011).
Fenugreek seeds and sprouts was found to be effective against a variety of Gram negative (*Escherichia coli* and *Salmonella typhi*) and Gram positive bacteria such as *Staphylococcus aureus* (Thomas *et al*., 2006).

## MATERIALS AND METHODS

### PLANT MATERIAL

Leaves and shoot tips of *Trigonella foenum-graecum* L. were collected from pot grown plants and were used for the extraction of bioactive compounds.

### PREPARATION OF EXTRACTS

Leaves and shoot tips of *Trigonella foenum-graecum* L. were thoroughly washed thrice in sterile distilled water, shade dried and ground separately to a fine powder using a mechanical blender. The powdered leaves and shoot tips were subjected to extraction in a Soxhlet apparatus using ethanol and methanol as solvents. The extracts were then filtered using Whatman no. 1 filter paper and the filtrates were air dried at 37$^{o}$C in a fume hood. A constant dry weight of each extract was obtained and the residues were stored at 4$^{o}$C

### PHYTOCHEMICAL ANALYSIS

Phytochemical analysis obtained from the leaves and shoot tip extracts were analysed as follows:

**Test for alkaloids - Dragendorff's test** (Waldi, 1965)

To a few millilitre of the filtrate, 1 or 2 ml of Dragendorff's reagent was added. A prominent red or orange precipitate indicated a positive result for alkaloids.

**Test for tannins** (Mace, 1963)

To 1 ml of the plant extract, 2 ml of 5% ferric chloride were added. A dark blue or greenish black colour change indicated the presence of tannin.

**Test for steroids** (Sathish Kumar *et al*., 2007)

To 2 ml of acetic anhydride, 0.5 ml of the plant extract and 2 ml of $H_2SO_4$ were added. Colour change from violet to blue or green indicated the presence of steroids.

**Test for carbohydrates** (Onyegbule *et al*., 2011)

To 2 ml of the plant extract, 1 ml of Molisch's reagent was added. A purple or reddish change in colour indicated the presence of carbohydrates.

**Test for terpenoids** (Egwaikhide *et al*., 2007)

The plant extract of 0.5 ml was mixed with 2ml of chloroform followed by the addition of 3 ml of concentrated $H_2SO_4$ to form a layer. Formation of a reddish brown interface indicated the presence of terpenoids.

**Test for flavonoids** (Trease and Evans, 1989)

To 2ml of the plant extract, 1ml of 2N sodium hydroxide solution was added. The resulting yellow solution indicated the presence of flavonoids.

**Test for starch** (Khandelwal, 2005)

To a small amount of the powdered plant part (seed), a few millilitre of concentrated sulphuric acid was added. A prominent colour change to black indicated the presence of starch.

**Test for catechol** (Khandelwal, 2005)

To 2 ml of the plant extract in Erlich's reagent a few drops of concentrated hydrochloric acid were added, making it blackish green indicated the presence of catechol.

**Test for quinones** (Peach and Tracy, 1955)

To 1 ml of the plant extract, 1 ml of concentrated sulphuric acid was added. A prominent red colour indicated the presence of quinones.

## ANTIBACTERIAL ACTIVITY

### MICROORGANISMS AND GROWTH CONDITIONS

Stock cultures of Gram negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC14028) and Gram positive bacteria (*Staphylococcus aureus* ATCC25923) were obtained from the Department of

Biotechnology, University of Madras. Pure cultures of bacteria were maintained on nutrient agar slants in the laboratory. Bacteria were grown in Muller Hinton broth at 37$^{o}$C for 18 hours and adjusted to a concentration of 1x10$^{8}$ colony forming units per millilitre (CFU/ml) using 0.5 McFarland standard.

The agar well diffusion method for antimicrobial susceptibility testing was carried out according to the standard method of Cheesbrough (2006) to assess the antibacterial activity of the plant extracts. Muller Hinton agar plates were seeded with 100 µl of the test organisms. The varying concentrations (10mg, 20mg and 40mg) of plant extracts were added into the wells made using a sterile cork borer. The control wells contained 5% (v/v) dimethyl sulfoxide (DMSO). The plates were incubated at 37$^{o}$C for 18 - 24hours. Antibacterial activities of the extracts were determined by measuring the diameter of inhibitions zone (mm) against each test bacteria. The tests were performed in triplicate.

**Minimum Inhibitory Concentration (MIC)**

The MIC (Chen, 2011) was determined by macro-broth dilution method. The extract (350 mg/ml) was serially diluted and added to a nutrient broth (10 ml) in separate test tubes inoculated with the respective bacterial cultures adjusted to a concentration of 1x10$^{8}$ colony CFU/ml. The tubes were incubated at 37ºC overnight. After incubation, the tubes were examined for microbial growth by observing for turbidity. Broth tubes that appear turbid indicated bacterial growth tubes that remain clear indicate no growth. The MIC of the extract is the lowest concentration that does not show active growth.

## RESULTS AND DISCUSSION

## PHYTOCHEMICAL ANALYSIS

Phytochemical analysis showed the presence of alkaloids, tannins, terpenoids, catechol and quinones in methanolic leaf extract; alkaloids, tannins, terpenoids and catechol in ethanolic leaf extract; alkaloids, steroids, carbohydrates, terpenoids, flavonoids and quinones in methanol and ethanol extracts of shoot tips (Table 1).

**Table 1. Qualitative analysis of the phytochemical constituents present in extracts of *Trigonella foenum-graecum* L.**

| PHYTOCHEMICAL CONSTITUENTS | LEAF EXTRACT (Methanol) | LEAF EXTRACT (Ethanol) | SHOOT TIP EXTRACT (Methanol) | SHOOT TIP EXTRACT (Ethanol) |
|---|---|---|---|---|
| Alkaloids | + | + | + | + |
| Tannins | + | + | - | - |
| Steroids | N.D. | N.D. | + | + |
| Carbohydrates | N.D. | N.D. | + | + |
| Terpenoids | + | + | + | + |
| Flavonoids | - | - | + | + |

| | | | | |
|---|---|---|---|---|
| Starch | N.D. | N.D. | - | - |
| Catechol | + | + | - | - |
| Quinones | + | - | + | + |

'+' = Positive, '-' = Negative, 'N.D.' = Not Determined

The presence of these phytochemical constituents is thought to be responsible for antibacterial activity. Alkaloids and their derivatives have activities against *Staphylococcus aureus* and methicillin - resistance *Staphylococcus aureus.* The mechanism of action of the highly aromatic planar quartenary alkaloids is attributed to their ability to intercalate with DNA (Kumar *et al*., 2007) Similar findings were reported by Devi and Kumar (2011) and Rashmi *et al*. (2011).

## ANTIBACTERIAL ACTIVITY

The ethanolic extract had various zone of inhibition (10 - 40 mm) for *Staphylococcus aureus* and *Salmonella typhi* (Table 2).

**Table 2. Antibacterial activity of leaf and shoot tip extracts of *Trigonella foenum-graecum* L.**

| **Solvent** | **Concentration (mg/ml)** | **Zone of inhibition (mm)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***Escherichia coli*** | | ***Staphylococcus aureus*** | | ***Salmonella typhi*** | |
| | | **Leaf** | **Shoot tip** | **Leaf** | **Shoot tip** | **Leaf** | **Shoot tip** |
| Methanol | 50 mg/well<br>60 mg/well<br>70 mg/well<br>80 mg/well | - | N.D | - | N.D | - | N.D |
| Ethanol | 50 mg/well<br>60 mg/well<br>70 mg/well<br>80 mg/well | - | - | - | 10<br>12<br>10<br>10 | 12<br>16<br>12<br>12 | 40<br>12<br>14<br>12 |

Ethanolic extract of leaf and shoot tip have shown antibacterial activity against *Salmonella typhi* at a concentration of 50mg/ml (Fig 1).

**Plate 1. Antimicrobial activity of ethanol extract of leaf of *Trigonella foenum-graecum* l. against**

***Salmonella typhi* showing zones of inhibition**

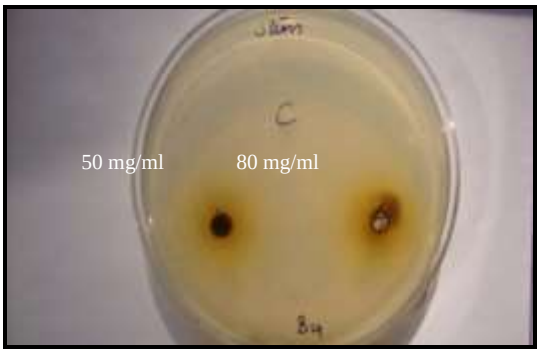


**Plate 2. Antimicrobial activity of ethanol extract of shoot tip of *Trigonella foenum-graecum* l. against *Salmonella typhi* showing zones of inhibition**

The ethanolic extract of shoot tip alone had an antimicrobial activity against *Staphylococcus aureus* at 50 mg/ml whereas the leaf extract had no activity against *Staphylococcus aureus*. The methanol extracts had no zones of inhibition against all the three test organisms. All plant extracts were ineffective against *Escherichia coli*. Similarly, Ritu *et al*. (2010) has reported antibacterial activity in the ethanolic extract of fenugreek.

**Minimum Inhibitory Concentration (MIC)**

The MIC values ranged between 2.73 and 87.5 mg/ml. The ethanolic leaf extract had a MIC of 87.5 mg /ml against *Escherichia coli* and *Salmonella typhi*, whereas it was 2.73 mg/ml against *Staphylococcus aureus*. In contrast the shoot tip extracts had a MIC of 2.73 mg/ml for *Escherichia coli* and *Staphylococcus aureus*, but for *Salmonella typhi*, the MIC was 10.94 mg/ml, and the methanolic leaf extract had an MIC of 2.73 mg/ml for *Staphylococcus aureus* (Table 3).

**Table 3. Minimum Inhibitory Concentration (MIC) activity of leaf and shoot tip extracts of *Trigonella foenum-graecum* L.**

| **Solvent** | **Concentration** | ***Escherichia coli*** | | ***Staphylococcus aureus*** | | ***Salmonella typhi*** | |
|---|---|---|---|---|---|---|---|
| | | **Leaf** | **Shoot tip** | **Leaf** | **Shoot tip** | **Leaf** | **Shoot tip** |
| Methanol | 350 mg/ml | N.D. | N.D. | 2.73 mg/ml | N.D. | 87.5 mg/ml | N.D. |
| Ethanol | 350 mg/ml | 87.5 mg/ml | 2.73 mg/ml | 2.73 mg/ml | 2.73 mg/ml | 87.5 mg/ml | 10.94 mg/ml |

N.D. = Not Determined

From the results obtained, it is evident that the methanol and ethanol extracts of *Trigonella foenum-graecum* L. possess both antimicrobial and inhibitory activities.

## REFERENCES


1. Chen, X. 2011. Determination of the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations(MBCs) for a novel antibiotic. *Afr. J. Microbial. Res.* 5(5): 572-575.
2. Cheesbrough M (2006). District laboratory practical in tropical countries, Part 2, Cambridge University Press, Cambridge, UK, pp 137-150.
3. Devi M.S. and Kumar, B.S.2011. Evaluation of anti-diarrhoel activity of fenugreek seed (vendhayam) extracts in rats. *International Journal of Universal Pharmacy and Life Sciences.* 2(1): 17-27.
4. Egwaikhide P.A., Okeniyi, S.O. and Gimba, C.E. 2007. Screening for anti-microbial activity and phytochemical constituents of some Nigerian medicinal plants. *Advances in Biological Research.* 1(5-6): 155-158.
5. Gurinder, J.K. and Daljit, S.A. 2009. Antibacterial and phytochemical screening of *Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. BMC Complement Altern Med*. 9: 30
6. Khandelwal, K.R. 2005. Practical pharmacognosy. 27-35.
7. Kumar, G.S, Jayaveera, K.N, Kumar, A.C.K, Sanjay, U.P, Swamy, B.M.V and Kumar, D.V.K 2007. Antimicrobial effects of acne-inducing bacteria. Tropical journal pharmaceutical research 6, 717-723.
8. Mace, M.E. 1963. Histochemical localization of phenols in healthy and diseased tomato roots. *Phytochem.*16: 915-925.
9. Newman, D.J. and Cragg, G.M. 2007. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* 70: 461-477.
10. Onyegbule, F.A, Sunday Adelusi, A. and Chukwudi Onyegbule, E.2011. Extractive visible spectrophotometric determination of chloroquine in pharmaceutical and biological materials. *IJPSR.* 2(1): 56-62.
11. Peach, K. and Tracy, M.V. 1955. Modern Methods of Plant Analysis, Vol. III. German Edition, Springer Verlay, Berlin: 04, 464.
12. Rashmi Yadav, Richa Tiwari, Partha Chowdhary and Chandan Kumar Pradhan. 2011. A pharmacognostical monograph of *Trigonella foenum-graecum* seeds. *Int. J. Pharm Pharm Sci*. 3(5): 442-445.
13. Ritu, K., Chatterjee Atanu, Singh Bhupender, Goel Ashutosh, and Sen Kalyan. 2010. Antimicrobial activity of ethanolic extract of *Trigonella foenum-graecum* Linn. *IRJP*. 1 (1): 181-183.
14. Sathish Kumar, T., Rajesekaran, P., Shanmugam, S. and Bharathi Kumar V.M. 2007. Phytochemical constituents and antibacterial activivties of *Elaeo carpus ganitrus* Roxb. and *Canthium parviflora* Linn. leaves.
15. Sumayya, A.R., Sivagami Srinivasan and Nabeelah Amatullah. 2012. Screening and biochemical quantification of phytochemicals in fenugreek (*Trigonella foenum-graecum*). *RJPBCS*. 3(1): 165-169.
16. Trease, C.E and Evans, W.C. 1989. Pharmacognosy. Bailliere Tindal, London.
17. Thomas, J.E., Basu, S.K., and Acharya, S.N. 2006. Identification of *Trigonella* accessions which lack antimicrobial activity and are suitable for forage development.*Can. J. Plant Sci*., 86: 727-732.
18. Waldi, D. 1965. Spray Reagents for Thin-Layer Chromatography. In: Egon Stahl (Ed.). Thin Layer Chromatography- A Laboratory Handbook. Acadmic press Inc., Publishers, New York, U.S.A.