Antimicrobial and Phytochemical Analysis of Acetone Extract of Trigonellafoenumgrecum Seeds

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***Abstract*- Plants have the ability to synthesize a wide variety of chemical compounds. Some phytochemicals have the ability to inhibit the growth of pathogens. The aim of the present study is to test the antimicrobial activity of *Trigonella foenum grecum*(fenugreek)seeds against seven different bacteria namely,*Escherichia coli, Proteus vulgaris, Klebsheillapnemoniae, Staphylococcus aureus, Psedomonasaeruginosa, Bacillus subtilis, Salmonella paratyphi* and a fungal species *candida albican* by well diffusion method and to screen the phytochemicals present in the acetone fenugreek seed extract. Different concentrations of seed extract ranging from 1mg/ml to 8mg/ml were tested and zone of inhibition were compared against standard antibiotic.Fenugreek seed extract showed maximum activity against *E.coli* and *Proteus vulgaris.*The phytochemical analysis revealed the presence of flavonoids,terpenoids, phenols, proteins, saponins and tannins in fenugreek seed extracts.Results from the present study shows that the acetone extract of *Trigonellafoenum-graecum* seeds has potential antimicrobial activity against harmful pathogenic microorganisms like*E.coli* and *Proteus vulgaris*and thebiologically active compounds can be further studied to developnew antimicrobial drugs.**

Keywords: Antimicrobial activity, Phytochemical,Pathogen, Inhibition

I. INTRODUCTION

From Indian ancient literature like Ayurveda, Unani and Siddha the traditional medicinal values of medicinal plants and there plant parts were evident. From ancient times, plants have been used as primary source of effective and safe medicines. About 80 % of world populations are still dependent on traditional medicines[1]. In India millions of people consume herbal medicine as spices, home remedies, health foods, self-medication andas non-allopathic drug[2]. Various chemical formulations like synthetic drugs and antibiotics have been extensively used to treat many infectious pathogenic diseases. Due to indiscriminate use of these chemical formulations pathogenic organisms have developed resistance[16]. Although there is enormous research going on in the field of herbal medicine and photochemistry, still ethno pharmacologists, botanists, microbiologists and natural-product chemists world over today are constantly in search for the medicinal efficacy of plants and their phytochemicals due to the limited reported data before the vast number of plant population[7].

Trigonellafoenum-graecum Linn. (Fenugreek) is an annual crop belonging to the family fabaceae. It is native to South Europe and Asia and grown in many parts of india[8]. It is used as anti-diabetic, anti-fertility, antimicrobial, antiparasitic and hypocholesterolaemic, antileptic, antibronchitis, carminative, aphrodisiac, analgesic, antipyretic, anticancer, antioxidant[4]. In India, fenugreek powder is also used as a lactation stimulant and protective against ethanol toxicity[13]. Major phyto constituents found in fenugreek seeds include saponins (fenugreekine, diosgenin), alkaloids (trigonelline, gentianine, carpaine), amino acids (4-Hydroxyisoleucin, arginine), flavanoids. and protein[14].

The aim of the present study is to find the inhibitory effect of acetone fenugreek seed extract against the microorganisms and to screen the active phytoconstituent present in the extract.

II. MATERIALS AND METHODS

A. Plant material.

Seeds of *Trigonellafoenum-graecum* (methi) was obtained from the local market, Bangalore. The sample specimen was identified and approved based on the taxonomical characteristics in University of Agricultural science, GKVK, Bangalore. The seeds were washed thoroughly in distilled water and surface sterilized using 1% sodium hypochlorite and the surface water was removed by air drying under shade. The seeds were dried in shade for 48 h, powdered in a grinder and used for further extraction.

B.Test microorganisms.

The test organisms were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India.*Escherichia coli* (MTCC1577),*Bacillus subtilis* (MTCC1789), *Klebsiellapnemoniae* (MTCC3384),*Proteus vulgaris* (MTCC426),*Pseudomonas aeruginosa*(MTCC7732), *Salmonella paratyphi* (MTCC3220), *Staphylococcus aureus*(MTCC3160), and *candida albican*(MTCC854). The bacterial and the fungal cultures were maintained on nutrient agar medium and saborauds dextrose agar (SDA) medium respectively and stored at 4°C.

C.Preparation of solvent extract.

100gms of seed powder was weighed. 80% Acetone was added (in the ratio 1:10) to the seed powder and kept in dark brown bottle for 2days[12]. The crude acetone extracts was then filtered by passing the extracts through

Whatmann No. 1 filter paper. The extract was concentrated under vacuum at 40°C by using a rotary evaporator. The obtained extract was lyophilized and the viscous mass that was obtained was stored at 4°c.

D. Antimicrobial screening of acetone extracts of Trigonellafoenum-graecum seed..

The antibacterial activity was carried out by Kirby bauer,well diffusion method.

For solvent extract, different concentrations from 1mg-8mg was taken per ml of DMSO.Pure Bacterial and fungal cultures were used to lawn Muller Hilton agar plates evenly using a sterile cotton swab (Mueller Hinton Agar for bacteria, 2% Glucose with Methylene blue for fungal culture). Wells were punched using the well borer (5mm) and 50µl of sample was added in each well.Tetracyclin standard antibiotic disc (10 µg/disc) served as the positive control (for bacteria). Nystatin standard antibiotic disc (100 units/disc) served as the positive control (for fungi). DMSO served as negative control. The experiment were performed in duplicates.The plates were then incubated at 37°C for 18-24 h. After overnight incubation the plates were examined for the zone of inhibition.

E. Phytochemical screening.

Phytochemical analysis of acetone seed extract of *Trigonellafoenum-graecum* was carried out to test for the presence of proteins, tannins, phenols, terpenoids, flavonoids, saponins and alkaloids[16].

1) *Test for flavonoids*: 1ml of extract was taken in a test tube and 5ml of diluted Ammonium solution was added followed by few drops of concentrated sulphuric acid. Formation of yellow colour indicates the presence of flavonoids.

2) *Test for tannins*: Ferric chloride test,1 ml of extract was treated with 1% ferric chloride solution. Formation of reddish brown colour indicated the presence of tannins.

3) *Test for Terpenoids*: Salkowski test,1 ml of extract was dissolved in chloroform and few drops of concentrated sulphuric acid were added to it. Formation of reddish brown colour on the inner face suggested the presence of Terpanoids.

4) *Test for alkaloids*: Dragendorffa's test,To 1 ml of extract, 1 ml of Dragendroffa's reagent (potassium bismuth iodide solution) was added. An orange-red precipitate indicates the presence of alkaloids.

5) *Test for saponins*: Frothing test, 1ml of extract was vigorously shaken with distilled water and was allowed to stand for 10 min. Stable froth indicated the presence of saponins.

F.Determination of total phenol content.

The amount of total phenol content, in acetone seed extract was determined by Folin-Ciocalteu's reagent method.0.5ml of extract and 0.1 ml (0.5N) Folin-Ciocalteu's reagent was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm.Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compounds).

G. *Determination of total protein content.*

Seeds were dried and ground to fine powder. Mixed with ice cold extraction buffer (phosphate buffer pH 7.2) and kept at 4°C with continuous stirring for 2hrs. This mixture was centrifuged at 10000rpm at 4°c for 10mins. The Supernatant which is the crude extract was collected and stored at 4°c.

The protein concentration was measured usingBenchtop spectrophotometer (BioPhotometer® D30) at 280nm.

III. RESULTS AND DISCUSSIONS.

A. *Antimicrobial screening of acetone extracts of Trigonellafoenum-graecum seed.*

All zone of inhibition sizes were measured in millimetre. Acetone extract of fenugreek showed maximum activity against *Escherichia coli* and *Proteus vulgaris.*The figure below shows the antimicrobial activity of acetone extract of fenugreek seed for *Escherichia coli* and *Proteus vulgaris.*A clear zone of inhibition of 24mm was obtained for concentration 8mg/ml, 22mm was obtained for concentration 4mg/ml and 2mg/ml, 20mm was obtained for concentration 1mg/ml for *Escherichia coli*. A clear zone of inhibition of 24mm was obtained for concentration 8mg/ml, 22mm was obtained for concentration 4mg/ml, 21mm was obtained for concentration 2mg/ml and 1mg/ml for *Proteus vulgaris.* A clear zone of inhibition of 15mm was obtained for concentration 8mg/ml, 14mm was obtained for concentration 4mg/ml, 9mm and 8mm was obtained for concentration 2mg/ml and 1mg/ml,correspondingly for *Bacillus subtilis.* A clear zone of inhibition of 10mm was obtained for concentration 8mg/ml, 9mm was obtained for concentration 4mg/ml, 8mm and was obtained for concentration 2mg/ml and 1mg/ml for *Salmonella paratyphi.* A clear zone of inhibition of 15mm was obtained for concentration 8mg/ml, 14mm was obtained for concentration 4mg/ml and 2 mg/ml, 10mm was obtained for concentration 1mg/ml for *candida albican.* As the concentration of extract increased to 8mg/ml the inhibition activity of the extract also increased, maximum activity was showed at concentration 8mg/ml.Whereas Acetone extract did not show any activity for *Klebsiellapnemoniae*, *Staphylococcus aureus*, *Pseudomonasaeruginosa*. DMSO was taken as negative control which showed no activity. Table 3.1 shows the antimicrobial activity of acetone fenugreek seed extract.

Table 3.1: Antimicrobial Activity ofAcetone Extract of Fenugreek Seed.

Micro organisms	Zone of inhibition (mm) at different concentration				Standard Antibiotic
	8mg/ml	4mg/ml	2mg/ml	1mg/ml	Tetracyclin
Escherichia coli	24	22	22	20	22
Proteus vulgaris	24	22	21	21	21
Bacillus subtilis	15	14	9	8	19
Salmonella paratyphi	10	9	8	8	18
					Nystatin
Candida albican	15	14	14	10	17

According to Upadhyay*et al*., 2008 Acetone extract of fenugreek showed promising inhibitory effect for *B. cereus, L. acidophilus* and *Pneumococcus*[16].Nandagopal*et al*., 2012studies revealed that acetone extract of fenugreek has antibacterial activity against *Pseudomonas aerogenosa, E.coli, Salmonella paratyphi, Staphylococcus aureus*[15],Methanol extract of Fenugreek revealed antimicrobial activity against *Pseudomonas* spp. whereas acetone extract of spices exhibited highest activity against *Escherichia coli*[3]. From the present study and literature it can be concluded that acetone extract of fenugreek has promising activity against *E.coli.* The present studies shows acetone extract of fenugreek can be further researched for the antimicrobial drug for infectious diseases caused by *Proteus vulgaris*.

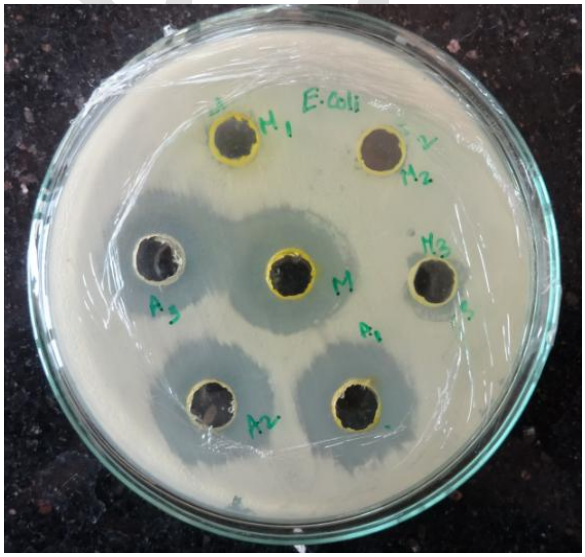

Fig 3.1: Antimicrobial activity of acetone fenugreek seed extract for*Escherichia coli*.

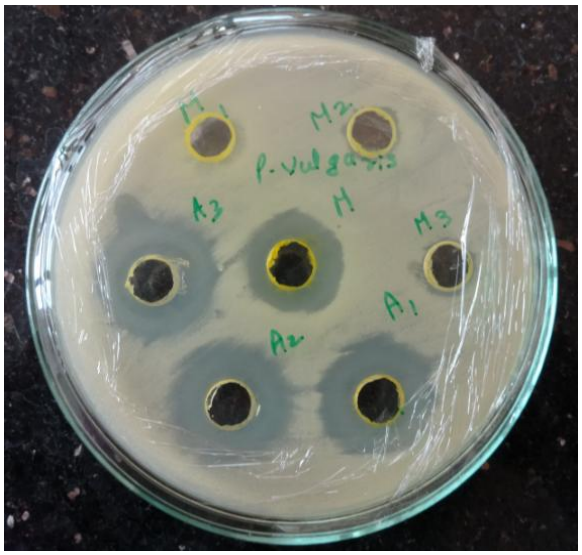

Fig 3.2: Antimicrobial activity of acetone fenugreek seed extract for*Proteus vulgaris.*

B. Phytochemical screening

Phytochemical analysis was done to determine the phyto-components present in the seed extract.In the present study, phytochemical screening of the acetone seed extract showed the presence of flavonoids, phenols, tannin, terpenoid, saponin and proteins but the absence of alkaloid. In Nandagopal*et al*., 2012 acetone extract of fenugreek extract showed the presence ofVolatile oils, Fattyacids and Triterpenoids[16]. Phytochemical screening of Trigonellafoenum-graecum seed showed different phytochemical from different solvent extract like Petroleum ether, Chloroform, Acetone, Methanol, Water. Acetone extract consisted of Alkaloids, Flavonoids and Amino Acids[9].

C.*Total phenol content.*

Total polyphenol content in fenugreek seed was estimated by folinciocalteau method. Phenols react with phosphomolybdic acid in FCReagent in alkaline medium and produce blue coloured complex (molybdenum blue)[12]. A graph of standard gallic acid curve was used for calculating the amount of polyphenol content in fenugreek seed with absorbance at 650nm. After plotting the graph and determining the concentration of polyphenol for different dilutions of extract used. Total polyphenol concentration in the extract was estimated to be 1.9 mg/ml of extract in terms of gallic acid equivalent. Acetone extract of fenugreek seed was used because acetone extracts high amount of polyphenols. This was demonstrated by Chavan et al ., 2013, where extracted polyphenol was extracted from different solvents like 80% methanol, 80% acetone and 80% ethanol and higher amount of polyphenols were found in 80% acetone extract[5]. Poly phenol content of fenugreek seed extract may contribute to the antimicrobial activity. ReenaRandhir et al., 2004 proposed the relation between phenolicsand their antimicrobial and antioxidant activity in dark germinated fenugreek sprouts[10].

D. Total Protein content.

The aqueous extract of *Trigonellafoenum-graecum* seed was used to determine the protein content in the extract. The protein concentration was measured in Benchtop spectrophotometer (BioPhotometer® D30) at 280nm with albumin as reference. The total protein concentration was found to be 175.5mg/ ml of fenugreek seed extract.Shakuntala*et al*., 2011 reported the estimation of protein in germinated endosperm of fenugreek seed to be 39.25%,protein was estimated by Kjeldahlmethod[11].Table 3.2 summarises the phytochemicals present in the acetone extract of fenugreek seed extract.

Table 3.2: Summary of Phytochemicals in Acetone Extract of Fenugreek Seed.

Tests	**Results**
Flavanoid	**+ve**
Phenol	**+ve**
Tannin	**+ve**
Terpanoid	**+ve**
Alkaloid	**-ve**
Saponin	**+ve**
Protein	**+ve**

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IV. CONCLUSION

Plants during their life time are attacked by many pathogenic microorganisms, and they protect themselves by producing secondary metabolites. These secondary metabolites can be used as an alternative for the chemical drugs and antibiotics to which pathogenic microorganisms are getting resistance.*Trigonellafoenumgraecum* is a multipurpose seed which is used for many ailments in traditional medicine. The present study focus is to find the inhibitory effect of this multipurpose seed.The acetone extracts of *Trigonellafoenumgraecum*seeds showed effective antimicrobial activity against *Escherichia coli* and *Proteus vulgaris*, maximum activity was shown for concentration 8mg/ml. The phytochemical analysis showedthe presence of flavonoids, phenols, tannin, terpenoid, saponin and proteins. The antimicrobial activity might be due to individual or combined effect of the above mentioned phytochemicals. Further studies are required to determine the exact mechanism by which the acetone extracts of *Trigonellafoenumgraecum*seeds shows the antimicrobial activity.

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