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Phytochemical analysis and anti-microbial activity of *Trigonella foenumgracum* (Methi seeds)

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Abstract

Trigonella foenum-graecum Linn. (*fabaceae*), a spice seed used to flavour, color and texture of food and it is employed in various medicinal purposes in traditional systems. *Trigonella foenum-graecum* commonly known as fenugreek is a plant extensively used as source of antidiabetic compounds from its seeds. It has been acutely lower postprandial glucose levels. Number of laboratory research gives the information about the biological actions of fenugreek. The aim of the study was to screen the medicinal and antibacterial activities of distilled water, methanol, acetone; ethanol extract of the spice. The invitro antibacterial activity was performed by agar well diffusion method. Methanol, acetone, ethanol and distilled water extract of Fenugreek revealed an elevated antimicrobial activity against *Bacillus Subtilis* and *Candida parapsilosis* at lower concentration of the crude extract. The results obtained in the present study suggest that the methanol extract of *Trigonella foenum* L. revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formation. In the phytochemical analysis, there is absence of anthraquinones by extracting with distilled water and glycosides by methanol.

Keywords: *Trigonella foenum gracum*, fenugreek, phytochemical analysis, antimicrobial activity

Introduction

Natural products have been a major source of new drugs (Vuorelaa P, 2004) [22]. Plants possess medicinal and drug activities. Medicinal plants are used by 80% of the world population and in developing countries (Hasim H, 2010) [6]. Current study on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses (Arora DS, 2007) [2]. They can be extracted and used for chronic and infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics (Periyasamy A, 2010) [13]. The active drugs which play role are secondary metabolites. The antimicrobial activities of plant extract which produces different components including aldehyde and phenolic compounds (Lai PK, 2004) [9]. Fenugreek is used traditionally as demulcent, laxative, lactation stimulant and exhibits hypocholesterolemic, hypolipidemic and hypoglycemic activity in healthy and diabetic animals and humans. The defatted seeds of fenugreek reduce gastrointestinal absorption of glucose and cholesterol and bile acid secretion.

The herb fenugreek (*Trigonella foenum-graecum* L., *Fabaceae* family) is used both in cooking and for the treatment of diabetes in many parts of the world especially in China, Egypt, India and middle eastern countries. (Kirtikar KR, 2000, Saxena A, 2004, Wang E, 2008) [8, 18, 23]. In India, it is widely used in Bangladesh. Active compounds of fenugreek included soluble fiber (Neeraja A, 1996, Raghuram TC, 1994, Hannan JM, 2007) [12, 15, 5], trigonelle (Moorthy R, 2010) [11], diosgenin (Uemura T, 2010) [21] and 4- hydroxyisoleucine (Singh AB, 2010, Sauvaire Y, 1998) [20, 17]. Hypoglycemic activities have mainly been attributed to dietary fiber (Neeraja A, 1996, Raghuram TC, 1994) [12, 15] and Saponin (Lu F, 2008) [10]. Fenugreek is a widely used herbal medicine for diabetes, but its efficacy for glycemic control remains unclear.

Fenugreek (*Trigonella foenum-graecum*) being rich in phytochemicals has traditionally been used as a food, forage and medicinal plant (Puri D, 1998) [14]. It contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreek, nicotinic acid, saponin, phytyc acid, scopoletin and trigonelle which has therapeutic effects. (Billaud C, 2001) [3] The component called fenugreekine a steroidal saponin peptide ester has hypoglycemic properties (Jellin JM, 1999) [7]. Thus it is best use is to control blood sugar in both insulin dependent (type 1) and noninsulin dependent (type 2) diabetes (Anuradha CV, 2001, Sharma RD, 1996, Bordia A, 1997) [1, 19, 4].

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Materials and Methods

Plant material was obtained from Osmania university botanical garden. Then identified and authenticated by Department of Botany, Osmania University. Specimens were submitted to the herbarium. Reagents and chemicals such as wagner's reagent, chloroform, 2% H₂SO₄, concentrated sulphuric acid, 10% lead acetate, Benedict's reagent, 0.1% ferric chloride, Fehling's solution, dilute NaOH, 2% HCL, 10% ammonia, 10% HCL, distilled water, ethyl alcohol are used in this study.

Micro Organisms

To determine the viability of organisms, the organisms are cultured on nutrient agar. The organisms which are used for antimicrobial activity are obtained from Primer Biotech Research Centre, Hyderabad. The organisms which are used for antimicrobial activity are *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Trichophyton rubrum*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida parapsilosis*, *Candida albicans*, *Aspergillus niger*. They are maintained as glycerol stocks at 4 °C to check their viability from time to time.

Preparation of Solutions

- Fehling's solution:** Take a beaker and mix it with equal volume of copper sulphate, sodium potassium tartarate and sodium hydroxide.
- Wagner's reagent:** Take 2gm of iodide and 6gm of potassium iodide in 100ml of water and mix it well.

Preparation of fenugreek seed extract with ethyl alcohol:

The finely grinded seed powder was taken. From the total extract 10gm of seed powder was taken and 50ml of ethyl alcohol was added to that extract stirred it constantly for 30min and the solution was kept in room temperature for 24 hrs and then filtered. The filtered solution is again filtered with whatmann filter paper no.3 and then it was stored at 4°C for further use.

Tests

- Phytochemical screening:** In every phytochemical analysis, ethyl alcohol is mainly used as standard to identify various residues in the extract.
- Procedure for alkaloids:** To identify the presence of alkaloids in the extract 2ml of extract is taken and to that 2ml of wagner's reagent is added. A brownish precipitation formation is observed. Thus it indicates the presence of alkaloids.
- Cardiac glycosides:** To test the presence of glycosides, 2ml of extract is dissolved with 2ml of chloroform then carefully add concentrated sulphuric acid to form a layer. Deep reddish brown colour at the interface of steroid ring indicates the presence of cardiac glycosides.
- Flavonoids:** To know the presence of Flavonoids in the seeds, 2ml of extract is added to 2ml of 10% lead acetate. Yellowish green colour indicates the presence of flavonoids.
- Saponins:** For this, 2ml of extract is dissolved with 2ml of benedict's reagent. Blue black ppt indicates the presence of saponins.
- Tanins:** To know the presence of tanins, 2ml of extract is treated with 0.1% of Ferric chloride. Brownish green layer indicates the presence of tannins.

- Terpenoids: (salkowski test):** To identify the presence of terpenoids, 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. A reddish brown colour is observed which indicates the presence of terpenoids.
- Anthraquinones:** To test the presence of anthraquinones in fenugreek seed extract, 1ml of extract is boiled with 10%HCL for few minutes in boiling water bath. Then it is filtered and allowed to cool. Equal volume of CHCL₃ is added to the filtrate and few drops of 10% Ammonia is added to the mixture and heat. A rose pin colour is found which indicates the presence of anthraquinones.
- Reducing sugars:** The extract was shaken with distilled water and filtered. The filtrate is boiled with Fehling's solution A and B for few minutes an orange red ppt indicates the presence of reducing sugars.
- Glycosides:** To identify this, extract is hydrolysed with HCL solution and neutralized with NaOH solution. Few drops of Fehling's solution A and B are added, Red ppt indicates the presence of glycosides.
- Phlobatanins:** The test the presence of Phlobatanins, the extract is dissolved in distilled water and filtered. The filtrate is boiled with 2% HCL solution. Red precipitate shows the presence of phlobatanins.

Evaluation of antimicrobial activity

Preparation of fenugreek seed extract

Seeds were taken and grounded into fine powder. The powder (100gm) was extracted with methanol and rotavaporized at 40-50 °C for 3-4 hrs. Crude extract will be obtained. This extract was processed for further experiment to examine the results of antimicrobial activity.

Equipment preparation

To process the experiment, 28g of nutrient agar is dissolved in 1000 ml distilled water. Along with the agar medium, petri dishes, forceps, spreader, cotton balls and 25ml conical flasks, whatmann no 1 filter paper are kept in autoclave. The sterilized agar was then transferred to petri dishes and allowed to solidify. The anti-microbial activities were formed using whatmann no 1 paper.

The fine round paper discs were obtained and were sterilized. The whole process executed under aseptic conditions under laminar air flow.

The total experiment was undergone on aseptic conditions. Nutrient agar medium (25ml) was taken in a sterile petridish and broth cultures of the test isolate (0.1ml) containing 1.0X10⁵ CFU/ml of organisms were used. Those culture extracts which have grown under aseptic conditions were dissolved in ethyl alcohol and used. To examine the extracts different concentrations are used i.e., 10, 20, 40 and 50 mg/ml. Ampillicin (10µg/ml) was used as standard antibacterial agent and Griseofulvin was used as standard antifungal agent (*Sah P 2008*).

Results and Discussion

Results of phytochemical analysis: By this analysis we can conclude that fenugreek seeds consists of Tanins, anthraquinones, flavonoids, alkaloids, terpenoids, saponins, cardiac glycosides, reducing sugars, phlobatanins, steroids, aminoacids, phenolic and proteins.

Table 1: results of Phyto Chemical Analysis of *Trigonella foenumgracum* (Methi seeds)

Sl. No	Phytochemicals	Distilled Water	Methanol	Acetone	Ethanol
1	Tanins	Positive	Positive	Positive	Positive
2	Anthraquinones	Negative	Positive	Positive	Positive
3	Flavonoides	Positive	Positive	Positive	Positive
4	Alkaloides	Positive	Positive	Positive	Positive
5	Terpenoids	Positive	Positive	Positive	Positive
6	Saponins	Positive	Positive	Positive	Positive
7	Cardiac glycosides	Positive	Positive	Positive	Positive
8	Glycosides	Positive	Negative	Positive	Positive
9	Reducing Sugars	Positive	Positive	Positive	Positive
10	Phlobatanins	Positive	Positive	Positive	Positive
11	Steroids	Positive	Positive	Positive	Positive
12	Phenolic	Positive	Positive	Positive	Positive
13	Aminoacids	Positive	Positive	Positive	Positive
14	Proteins	Positive	Positive	Positive	Positive
15	Quinones	Positive	Positive	Positive	Positive

Results of antimicrobial activity

Gram positive and Gram negative bacterial and fungal strains are used to examine the anti-microbial activity of *Trigonella* seed crude extract. Antimicrobial activity is identified by measuring the zone of inhibition. The antimicrobial activity was processed by agar well diffusion method by using

different concentrations 2.5,5.0,7.0,10µg/ml.

Ampicillin (anti-bacterial), Itraconazole or Griseofulvin (antifungal) as the standard drug at a concentration of 200µg/ml. LB agar was used as the culture media and Potassium dextrose was used as antifungal activity.

Table 2: Antimicrobial activity of *Trigonella foenum gracum* seed extract

Organism/conc of extract	2.5µg/ml	5.0µg/ml	7.5µg/ml	10µg/ml
<i>E. Coli</i>	1.0	1.2	1.2	1.0
<i>Staphylococcus aureu</i>	1.1	1.0	1.1	1.1
<i>Bacillus subtilis</i>	1.3	1.1	1.2	1.0
<i>Salmonella typhi</i>	1.0	1.1	1.1	1.0
<i>Aspergillus niger</i>	1.1	1.2	1.2	1.1
<i>Candida parapsilosis</i>	1.3	1.1	1.2	1.1
<i>Trichophyton rubrum</i>	1.0	1.1	1.1	1.0
<i>Candida albicans</i>	1.1	1.2	1.1	1.0

The seed extract of *Trigonella* showed high activity against *Bacillus subtilis*, *Candida parapsilosis* at low concentration (2.5µg/ml). The zone of inhibition measured in cm.

Conclusion

The preliminary phytochemical screening of *Trigonella foenum gracum* gives good results in the presence of flavonoids, alkaloids, anthraquinones, tanins, phenolic compounds, terpenoids, saponins, carbohydrates and proteins. There is absence of anthraquinones by extracting with distilled water and glycosides with methanol extraction. The antimicrobial activity screening also shows good results at *Bacillus subtilis* and *candida parapsilosis* at lower concentrations. This phytochemical and antimicrobial bioprocesses concludes that *Trigonella foenum gracum* (fenugreek) seeds have good medicinal and therapeutic compounds. By observing the recent research studies it can also be used to reduce the level of glucose levels and can be used as one of the best anti-diabetic component.

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