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COMPARATIVE ANALYSIS OF BIOACTIVE COMPONENTS AND ANTIFUNGAL POTENTIAL OF *TRIGONELLA FOENUM-GRAECUM* SEEDS

Riti Thapar Kapoor
Plant Physiology Laboratory
Amity Institute of Biotechnology, Amity University, Noida - 201 313, India
E.mail: rkapoor@amity.edu

ABSTRACT

The seeds of *Trigonella foenum-graecum* L. contain various biochemical components such as saponin, terpenoids, phenolic compounds, glycosides, flavonoids and steroids etc. The different concentrations of seed extracts of *Trigonella foenum-graecum* L. (varieties PEB and Prabha) were screened for their antifungal activity against six fungal species such as *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Fusarium solani, Fusarium oxysporum* and *Fusarium moniliforme*. The results revealed that seed extracts of *Trigonella foenum-graecum* L. showed significant antifungal activity against fungal species by dry mycelial weight method and poisoned food technique and it may be due to the presence of active biochemical components. The antifungal activity of fenugreek seeds followed the order: Fenugreek variety PEB > variety Prabha.

Keywords: Antifungal activity, biochemical components, *Trigonella foenum-graecum*

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop which grows under arid and semi-arid regions of India during rabi season. *Trigonella foenum-graecum* L. commonly known as Methi or Fenugreek and it belongs to the family Fabaceae. India is leading in fenugreek seed production and around 90% of the total global fenugreek production takes place in our country (Acharya *et al*., 2008). *Trigonella foenum-graecum* L. is a multipurpose crop being used as a spice, vegetable and medicinal plant (Wani and Kumar, 2018). The leaves and shoots of fenugreek plant are rich in protein, minerals and vitamins (Arya, 2000; Kashyap *et al*., 2018). The fresh and dried leaves of fenugreek have aromatic properties which can be used as condiment during food preparation (Prajapati *et al*., 2017). Fenugreek seeds contain a substantial amount of fiber phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid, choline, vitamins such as A, ascorbic acid, thiamine, riboflavin, nicotinic acid and niacin (Meghwal and Goswami, 2012).

Despite its exceptionaFenugreek seeds are bitter in taste due to the presence of trigonelline alkaloids and they also contain volatile and fixed oil in less amount (Sowmya and Rajyalakshmi, 1999). Fenugreek plant has been widely used in pharmaceutical industries due to itsanti-diabetic, anticancer, antioxidant, antipyretic, antimicrobial, anthelmintic, anti-allergic and anti-inflammatory properties (Krishnaswamy, 2008).Fenugreek leaves and seeds contain various phytochemicals and can be used for the treatment of cardiovascular diseases, gastric inflammation and cancer etc. (Kumari *et al*., 2016). Fenugreekine, a steroidal sapogenin peptide ester is present in fenugreek which is used to control blood sugar level in human-beings in both type I and II diabetes (Idries, 2014).

The phytopathogenic fungi cause approximately 20% reduction in the crop productivity (Agrios, 2000). More than nineteen thousand fungi are known to cause diseases in crop plants worldwide (Jain *et al*. 2019). They may remain dormant but alive on both living and dead plant tissues for long duration and become active when conditions become favourable for their proliferation. The fungal spores can be easily dispersed by wind, water, soil and insects and can easily infest crop plants (Lazarovits *et al*. 2014). *Fusarium oxysporum* is a cosmopolitan fungus and it is known for causing wilt and rot diseases in many economically important crops (Tripathi *et al*. 2009). The application of synthetic fungicides to control fungal infections on crop plants may cause health hazards to human-beings and animals directly or indirectly and may also increase environmental pollution (Gnanamangai and Ponmurugan, 2012). Most of the fungicides are expensive and non-biodegradable and they tend to persist for many years in environment (Brady, 1984). Due to this, fungi may develop resistance against synthetic chemicals. There is an urgent need to use plant extracts as environmentally safe alternative option for control of fungal diseases (Lee *et al*., 2007; Abdel-Monaim *et al*., 2011). The literature reveals that no study has been done till date to study the comparative analysis of the biochemical components present in two different varieties i.e. PEB and Prabha of fenugreek plant with their antifungal potential. Hence, present investigation was conducted to compare the bioactive compounds present in fenugreek seeds which may act as an important source for development of novel antifungal drugs.

Materials and Methods

The experiment was conducted in the Plant Physiology Laboratory, Amity Institute of Biotechnology, Amity University, Noida, India.

Study material

The healthy seeds of *Trigonella foenum-graecum* L. (varieties: PEB and Prabha) were washed with tap water only for few seconds to avoid loss of water soluble components, followed by quick rinsing in distilled water and drying with clean absorbent paper. The seeds of fenugreek were kept in a single layer on plastic tray under the shade for air drying for 72 hours. Then seeds were powdered in a grinder and stored in sterilized polythene bags to avoid contamination.

Preparation of methanolic seed extracts

Ten grams of dried fenugreek seed powder was mixed with 100 ml of methanol and kept on rotary shaker for 24 hours at 190-220 rpm. After 24 hours, mixture was filtered and supernatant was collected and it was evaporated to 1/4th of its original volume. The methanolic seed extract obtained was used for the analysis of biochemical components present in the fenugreek seeds.

Characterization of biochemical components

Different biochemical components present in the methanolic seed extracts of *Trigonella foenum-graecum* L. were analyzed by using the standard procedures described by Harborne (1998). The test for various biochemical components are given below:

Test for tannin

Approximately 0.5 grams of the dried seed powder of *Trigonellafoenum-graecum* L. was boiled in 20 ml of distilled water and then filtered. A few drops of 0.1% ferric chloride solution was added. A brownish green or blue-black colour of the test solution indicated the presence of tannin in the seeds.

Test for saponin

Two grams of the powdered seed sample was boiled with 20 ml of distilled water in a water bath and filtered. The filtrate (10 ml) was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The foamy leather formation showed the presence of saponin in the test solution.

Test for proteins

The proteins present in the fenugreek seed powder were analyzed by Biuret test. The test solution turned into violet colour showed the presence of proteins.

Keller-Kiliani test for glycosides

Few drops of glacial acetic acid and 2-3 drops of ferric chloride solution were added to 2 ml of seed powder extract along with 1 ml of concentrated sulfuric acid. Appearance of brown ring at the interface confirmed the presence of glycosides in the given sample.

Test for phenolic compounds

The seed powder of *Trigonella foenum-graecum* L.(500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds in the test sample.

Test for flavonoids

One gram of the powered dried seeds of fenugreek were boiled with 10 ml of distilled water for 5 minutes and filtered. Few drops of 20% NaOH solution were added to 1 ml of cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution showed the presence of flavonoids in the fenugreek seeds.

Test for terpenoids

The presence of terpenoids in the fenugreek seeds was analyzed by Salkowski test. The seed extract (5 ml) was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was also added from the sides of the test tube to form a layer. A reddish- brown colour of test solution showed the presence of terpenoids in the fenugreek seeds.

Test for steroids

The acetic anhydride (2 ml) was added to 0.5 ml offenu greek seed extracts. Then 2 ml of concentrated $H_2SO_4$was added from the side of the test tube. The change in colour of test solution from violet to blue-green colour indicated the presence of steroids in the fenugreek seeds.

Bioassay for anti-fungal activity of *Trigonella foenum-graecum* L. seeds

Determination of mycelial growth inhibition by dry mycelial weight technique

The seed powder of two different varieties of fenugreek was added to Richard's solution to achieve 5, 15 and 25% concentration of the seed extracts in the liquid medium. The extract amended media (50 ml) was taken in a 100 ml of Erlenmeyer conical flask and sterilized at 121°C for 20 minutes. Richard's solution without any aqueous extract of fenugreek seeds served as control. The flasks were inoculated with 5 mm diameter mycelia disc of different fungi taken from one week old culture respectively and incubated for 8 days at 23 ± 2^0C temperature under 12 h light and 12 h dark conditions. After incubation, the content of the each flask was poured into a pre-weighed Whatman No. 1 filter paper. The filter paper with the mycelial mat was dried in an oven at 60^0C. The dry weight of the mycelia was determined by subtracting the weight of the filter paper from total weight of the filter paper with mycelia (Kumar and Prasad, 1992). The percent inhibition of mycelial growth was calculated by using the following formula:

Inhibition (%) = C – T / C X 100 where C = mycelial weight in control and T = mycelial weight in treatment.

Determination of mycelial growth inhibition by Poisoned food technique

Czapekdox agar (CDA) and Malt extract salt agar (MESA) media with 5, 15 and 25% concentrations of fenugreek seeds were prepared and sterilized at 121^0C for 20 minutes. The media (15 ml) was separately poured into petriplates and allowed to cool and solidify. After complete solidification of the medium, 5mm disc of seven days old culture of *Fusarium* and *Alternaria* species were inoculated into CDA and *Aspergillus*species were inoculated into MESA at the centre of petri dishes. The petri plates were incubated at 23±2^0C for seven days. The petri dishes contained media without seed extract but same amount of distilled water served as control. After incubation, the colony diameter was measured in mm (Singh and Tripathi, 1999). The percentage inhibition of mycelial growth was calculated by using the formula:

Inhibition (%) = C - T / C X 100, where C = Average increase in mycelial growth in control plate and T = Average increase in mycelial growth in treatment.

Statistical analysis

Treatments were arranged as randomized block design with three replications. Data were statistically analyzed using analysis of variance (ANOVA) by using SPSS software (Version 16 SPSS, US). The treatment mean was analyzed by using Duncan's multiple range test (DMRT) at $P < 0.05$.

Results and Discussion

In the present investigation, biochemical characterization of seeds of *Trigonella foenum-graecum* L. (varieties: PEB and Prabha) was done for the identification of biologically active components. The methanolic extracts of fenugreek seeds contain several phytochemical components such assaponin, terpenoids, phenolic compounds, glycosides, flavonoids and steroids etc. which may play a significant role in antifungal activity of fenugreek plant (Table-1).

Table 1: Priliminary characterization of biochemical components present in the seeds of *Trigonella foenum-graecum* L.

S.No.	Biochemical components	Methanolic extract of seed powder of *Trigonella foenum-graecum* L.	
		T. foenum-gracecum (var. PEB)	*T. foenum-gracecum* (var. Prabha)
1.	Tannin	+	+
2.	Saponin	+++	++
3.	Protein	+++	+
4.	Glycosides	+	+
5.	Phenolic compounds	+++	++
6.	Flavonoids	++	++
7.	Terpenoids	+	+
8.	Steroids	+	+

(+) sign indicates the presence of biochemical components in the seeds of *Trigonella foenum-graecum* L.Sign: (+++): highly present,(++): moderately present and(+): low content.

The growth of fungal mycelia with different concentrations of fenugreek seed extracts was recorded by two different methods such as dry mycelial weight method and poisoned food technique. The results revealed that dry mycelial weight of the tested fungi grown in Richard's medium varied with different concentrations of both of the varieties of fenugreek seed extracts. The inhibition in fungal mycelial growth with fenugreek seed extracts was in the order: 25% > 15% > 5%.

The inhibition in mycelial growth of *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Fusarium solani, Fusarium oxysporum* and *Fusarium moniliforme* was48, 53, 41, 81, 94 and 97%respectively as observed by dry mycelial weight method with 25% concentration of fenugreek (var. PEB) seed extracts (Table-2).The seed extracts of fenugreek (var. Prabha) also exhibited significant decrease in mycelial growth 41, 49, 38, 77, 91 and 92% but lesser as compared to fenugreek variety PEB.

The significant reduction in mycelial growth was observed bypoisoned food technique with different concentrations of fenugreek seed extracts. Maximum inhibition in mycelial growth of *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Fusarium solani, Fusarium oxysporum* and *Fusarium moniliforme* was 52, 56, 49, 82, 95 and 92% respectively with 25% fenugreek (var. PEB) seed extracts as observed by poisoned food technique (Table-3). The significant decrease in mycelial growth of all the fungal species was reported with both of the varieties of fenugreek seed extracts but maximum reduction was observed with fenugreek variety PEB as compared to variety Prabha. The exploitation of naturally available chemicals obtained from plants retards the growth of undesirable microbes and it would be eco-friendly method for crop protection(Mohana and Raveesha, 2007).The biologically active plant derived fungicides are expected to play a key role in the management of plant diseases and development of commercial fungicides for crop protection (Varma and Dubey, 1999;Gottlieb *et al*. 2002).The findings of the present study is an important step towards the characterization of the antifungal properties of fenugreek seeds. Further researches are required to study its commercial application at large scale in crop protection strategies.

Table 2: Comparative analysis of mycelial growth inhibition in different fungal species by aqueous seed extracts of *Trigonella foenum-graecum* L.

Fungal species	Mycelial inhibition (%) with different concentrations of fenugreek seed extracts					
	Dry mycelial weight method					
	Fenugreek (variety PEB)			Fenugreek (variety Prabha)		
	5%	15%	25%	5%	15%	25%
Aspergillus niger	14 ± 0.02	27 ± 0.14	48 ± 0.19	12 ± 0.04	22 ± 0.12	41 ± 0.13
Aspergillus flavus	19 ± 0.05	33 ± 0.28	53 ± 0.27	15 ± 0.08	29 ± 0.20	49 ± 0.21
Alternaria alternata	12 ± 0.06	23 ± 0.09	41 ± 0.36	10 ± 0.02	19 ± 0.07	38 ± 0.25
Fusarium solani	27 ± 0.19	52 ± 0.63	81 ± 0.67	24 ± 0.14	48 ± 0.51	77 ± 0.56
Fusarium oxysporum	32 ± 0.41	69 ± 0.57	94 ± 0.83	29 ± 0.36	67 ± 0.42	91 ± 0.81
Fusarium moniliforme	34 ± 0.28	73 ± 0.89	97 ± 0.92	29 ± 0.21	68 ± 0.61	92 ± 0.75

Data are mean ± standard error of three independent experiments with three replicates. Values followed by different letters show a significant difference at $P < 0.05$ significant level between treatments according to the ANOVA and Duncan's multiple range test.

Table 3: Comparative analysis of mycelial growth inhibition in different fungal species by aqueous seed extracts of *Trigonella foenum-graecum* L.

Fungal species	Mycelial inhibition (%) with different concentrations of fenugreek seed extracts					
	Poisoned food technique					
	Fenugreek (variety PEB)			Fenugreek (variety Prabha)		
	5%	15%	25%	5%	15%	25%
Aspergillus niger	18 ± 0.03	25 ± 0.12	52 ± 0.14	13 ± 0.03	23 ± 0.18	44 ± 0.14
Aspergillus flavus	25 ± 0.07	36 ± 0.16	56 ± 0.21	19 ± 0.07	31 ± 0.16	45 ± 0.26
Alternaria alternata	17 ± 0.03	28 ± 0.08	49 ± 0.34	14 ± 0.03	23 ± 0.08	41 ± 0.34
Fusarium solani	29 ± 0.14	54 ± 0.04	82 ± 0.42	22 ± 0.14	45 ± 0.14	78 ± 0.42
Fusarium oxysporum	38 ± 0.42	75 ± 0.37	95 ± 0.75	27 ± 0.42	66 ± 0.37	85 ± 0.71
Fusarium moniliforme	37 ± 0.25	81 ± 0.52	92 ± 0.72	33 ± 0.19	67 ± 0.48	88 ± 0.62

Data are mean ± standard error of three independent experiments with three replicates. Values followed by different letters show a significant difference at $P < 0.05$ significant level between treatments according to the ANOVA and Duncan's multiple range test.

Conclusion

The significant amount of phytochemicals were present in the seeds of *Trigonella foenum-graecum* L. The broad spectrum antifungal activity was detected in seed extracts of both varieties of fenugreek against wide range of fungal species. Therefore, *Trigonella foenum-graecum* L. seeds can be cost effective and eco-friendly solution for the treatment of field and storage fungi and it can be used for prevention of biodeterioration of grains during storage and spoilage of processed food products.

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