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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF FENUGREEK SEED EXTRACT AGAINST ESCHERICHIA COLI AND PSEUDOMONAS

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ABSTRACT

The Fenugreek plant, besides having natural therapeutic values against various diseases, also provides high quality of food for livelihood. It is an ancient plant has been used throughout the world as medicine, food and spice. The present study was carried out with the extraction of dried seed of fenugreek using 70% methanol by Soxhlet apparatus. The Phytochemical screening was performed to identify the phyto constituents and revealed the presence of alkaloids, tannins, flavonoid and protein. It is working in different medicinal purposes in traditional systems. Fenugreek seeds contain phenolic compounds, which have antioxidant and antibacterial properties. The in vitro antibacterial activity was performed by well diffusion method. Extract of fenugreek revealed an elevated antimicrobial activity against *Escherichia coli* and *Pseudomonas* at ideal concentration of the crude extract. Present study suggests that the methanol extract of fenugreek an important scope to develop a novel broad spectrum of antimicrobial herbal formation.

KEY WORDS- Antimicrobial activity, fenugreek seed, phytochemical analysis, TPC, TLC.

INTRODUCTION

The fenugreek is an annual crop of Fabaceae family. It is a self pollinating dicot plant with trifoliate leaves and branched stems which, bears white flowers and produces golden yellow seeds (Acharya et al., 2010). Although fenugreek cultivation is mostly concentrated in Asia and the Mediterranean region, but now widely cultivated in Northern Africa, central Europe, North America and Australia (Fotopoulos, 2002). It is one of the oldest therapeutic plants, originating in Northern Africa and India. The leaf and seeds are used to prepare extracts or powders for medicinal use. Uses of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies. In modern Egypt, it is still used as a supplement in wheat and maize flour for bread-making (Morcos et al., 1981). In olden Rome, fenugreek was purportedly used to aid labor and delivery. In traditional Chinese medicine fenugreek seeds are used as a tonic as well as a treatment for weakness and edema of the legs (Yoshikawa et al., 1997). Fenugreek seeds are the most significant and valuable part of plant. Seed is 3-6 mm tall, 2-5 mm broad and 2 mm thick in geometry. Raw fenugreek seeds have maple flavor and bitter taste but by the process of roasting, their bitterness can be reduced and flavor can be enhanced (Altuntas et al., 2005).

In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant (Patil *et al.*, 1997). There are numerous other folkloric uses including the treatment of indigestion and baldness. The possible hypoglycemic and anti hyperlipidemic properties of oral fenugreek seed powder have been suggested by the results of preliminary animal and human trials. it is a promising protective medicinal herb for complementary therapy in cancer patients under chemotherapeutic interventions because fenugreek extract shows a protective effect by modifying the cyclophosphamide induced apoptosis and free radical-mediated lipid per oxidation in the urinary bladder of mice. Diosgenin is a crystalline steroid sapogenin originate in fenugreek and used as an initial material for the production of steroid hormones such as cortisone and progesterone. It has been found to be potentially important for cancer treatment (Aggarwal et al., 2006). The seeds contain L-tryptophan and lysine rich proteins, mucilaginous fiber and other rare chemical constituents such as Saponins, coumarin, nicotinic acid, sapogenin, phytic acid, scopoletin and trigonelline, it is rich source of calcium, iron and carotene and other vitamins (Sharma et al., 1996). Both leaves and seeds should be included in normal diet of family (Ody, 1993).

In this study, we tried antimicrobial activity extract of fenugreek seed against *Escherichia coli* and *Pseudomonas*. Both bacteria is gram negative bacteria and pathogenic. *Pseudomonas* is a mostly saprophytic in nature is found in soil, water and other moist environment. It has emerged as a main reason of health care associated and Opportunistic Infections. *E.coli* is a gram negative, motile, non Sporing bacillus, produces pink colonies on Mac-Conkey Agar. These species can be differentiated from other members of entero bacteria by biochemical reaction, being a member of enterobacteriaeace. *E. coli* is present as normal in the lower intestine of both humans and animals. However, some

strains can cause gastrointestinal illness ranging and may lead to potentially fatal complications, such as Hemolytic uremic syndrome (HUS) and Thrombotic thrombocytopenic purpura (TTP) in human beings (Hussein, 2007).

MATERIAL AND METHODS

Sample collection of Fenugreek Seed:

The *Trigonella Foenum-Graecum* Seed were collected from local market of Sehore (M.P.) in month of Feb 2018.Seed selection and collection were on the basis of ethno botanical survey, traditional use or literature survey. Various considerations involved in the proper selection of seed for the isolation of new chemical constituents, seed were selected on the basis of good activity according to traditional medicine for the treatment of some disease.

Preparation of seed material:

The seed materials were washed with distilled water. After washing we dry of seed and then after grind of seed in grinder machine. The powder was stored in the bottles for further analysis.

Extraction of fenugreek Powder by Soxhlet apparatus:

A quantity of 170 gm of the dried powder of *Trigonella Foenum-Graecum* with hydro alcoholic (Merck) were used Soxhlet apparatus. Placed of powder in Soxhlet apparatus for 8 to 10 days to get the extract with methanol and distilled water. After that, the extract was evaporated on water Bath at 80°C for 6 hours to obtained crude for antioxidant assay and phyto chemical analysis (**Khosla** *et al.*, **1995**).

Phytochemical Analysis of Fenugreek seed extract:

The extracts were tested for the presence for bioactive compounds by using standard methods some important standard methods are present here on the bases of bioactive compounds of fenugreek seed Powder (**Trease and Evans, 1989**).

Test for Flavonoid (Shinoda test):

A pinch of dried extract was dissolved in ethanol, mixed thoroughly and filtered. To the filtrate, magnesium metal pieces and concentrated hydrochloric acid were added and heated gently. Appearance of magenta colour indicates the presence of Flavonoid.

Test for Phenols (Ferric chloride test):

In 1ml of extract, add 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Saponins (Foam test):

5ml of distilled water mixed with extract in a test tube, then after shaken vigorously. The formation of stable foam is taken as an indication for the presence of Saponins.

Test for Alkaloids (Wagner's test):

2 ml of 1% HCl mixed with crude extract. And after that we heated of test tube. Then after Mayer's and Wagner's reagent are added to the mixture. Turbidity of the resulting precipitate is taken as evidence for the presence of alkaloids.

Test for Carbohydrates (Molisch test):

In 1ml of extract, add 2 drops of alcoholic alpha-naphthol solution in a test tube. Then add 1 ml of H_2SO_4 by the side of test tube Formation of the violet ring at the junction indicates the presence of carbohydrate.

Test for Tannins:

A pinch of dried extract was dissolved in ethanol, mixed thoroughly and filtered. The filtrate was treated with the following reagents.

1. Lead acetate solution- formation of white precipitate shows the presence of tannins.

2. Ferric chloride solution- formation of deep blue colour shows the presence of tannins.

3. Aqueous gelatin solution- formation of white precipitate shows the presence of tannins.

Test for Protein:

Biuret test- The extract was dissolved in 1 ml of ethanol, filtered and the filtrate was added with 40% v/v sodium hydroxide and copper sulphate solution. Formation of violet colour indicates the presence of proteins.

Ninhydrin test- The extract was dissolved in 1 ml of ethanol, filtered and the filtrate was treated with ninhydrin reagent. Formation of purple colour indicates the presence of proteins.

Test for Steroids (Liebermann's Burchard test):

The extract was dissolved in 2 ml of chloroform and 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid were added. Formation of green colour indicates the presence of phyto sterols.

Test for 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.

Test for Anthraquinones:

To test the presence of anthraquinones in fenugreek seed extract. We keep1 ml of extract with 10% HCl for boil in water bath Then it is filtered and allowed to cool. Equal volume of $CHCl_3$ is added to the filtrate and few drops of 10% ammonia are added to the mixture and heat. Rose pink colour is found which indicates the presence of anthraquinones.

Qualitative analysis by thin layer chromatography (TLC):

TLC slide was prepared with silica gel G. then after we keep of slide on heating mantle for drying at 100°C for 30 minute. Chloroform, methanol and toluene (5:1:1) was used as Solvent system. And observe at under UV light. After development, Number of spots was noted and Rf values were calculated using the formula.

Distance travelled by solute

Determination of total phenolic content (TPC)

The amount of total phenolic content was performed by the Folin–Ciocalteu method as described by (Kim *et al.*, 2003). Mix 9 ml distilled water in a test tube with 1 ml extract and add One ml Folin- Ciocalteu phenol reagent was added to the mixtures and shaken well. After 5 min, 14 ml of 20 %

 Na_2CO_3 solution is added. Solution was diluted to 25 ml with distilled water and mixed well. Then after kept of solution at normal room temperature for 15 min for incubation and take OD at 550 nm in spectrophotometer (Table 1).

		r r r r r r r r		
S.No	Sample / Standard	Distilled Water	Na ₂ Co ₃	Folin reagent
1	20µ1	980 µ1	2 ml	2 ml
2	40 µ1	960 µ1	2 ml	2 ml
3	60 µl	940 µl	2 ml	2 ml
4	80 µ1	920 µl	2 ml	2 ml
5	100 µ1	900 µ1	2 ml	2 ml
6	Blank	1 ml	2 ml	2 ml

Antimicrobial property of fenugreek seed Extract by Well diffusion method:

The antimicrobial activity was performed using well agar diffusion method (Kavanagh, 1972). For this purpose we fenugreek seed extract with different concentrations (25%, 50%, 75% and 100%). Two ml of a standardized bacterial cells suspension were thoroughly mixed with 200 ml of sterile nutrient agar media (NAM), which was maintained at 37 °C and then the medium was poured into sterilize Petri dishes, and left to solidify at room temperature. And saturated with the extract at different concentrations (25%, 50%, 75% and 100%) and placed centrally on the surface medium. Control sample were only saturated with sterilize distilled water. The plates were then incubated at 37°C for 48 hours and the inhibition zones were measured each 24 hours as was described by (Barry *et al.*, 1970; Cruikshank *et al.*, 1975).

RESULTS AND DISCUSSION

Phytochemical Analysis of fenugreek seed extract:

Some tests were positive but few tests were negative on the bases of phytochemical methods. Result is below here.

Flavonoid test:

Appearance of magenta colour after few minutes indicates the presence of flavonoid. This test was positive.

Phenols test:

Bluish black colour coloration indicates the presence of phenols. Test was positive.

Saponins test:

The formation of stable foam is taken as an indication for the presence of saponins. This test was positive.

Alkaloids test:

Turbidity of the resulting precipitate is taken as evidence for the presence of alkaloids. Test was positive.

Carbohydrate test:

Formation of the violet ring at the junction indicates the presence of carbohydrate. Test was positive.

Tannins test:

Formation of white precipitate shows the presence of tannins. Test was positive.

Protein test:

Biuret test- Formation of violet colour indicates the presence of proteins. Test was positive.

Ninhydrin test -Formation of purple colour indicates the presence of proteins. Test was positive.

Steroids test:

Formation of green colour indicates the presence of phyto sterols. Test was negative.

Glycosides test:

Red ppt indicates the presence of glycosides. Test was negative.

Anthraquinones test:

Rose pink colour is found which indicates the presence of anthraquinones. Test was negative



FIGURE: 1 Phytochemical Analysis of Fenugreek seed extract

Qualitative analysis by thin layer chromatography (TLC):

The extract of fenugreek seed was definite by TLC. Chromatogram of culture extract mark when observed in UV

light showed the different Rf value. Qualitative analysis had done by TLC (thin layer chromatography) using chloroform, methanol and toluene (5:1:1) solvent system. Rf value of seed extract is 2.50 and 3.10 (Table 2).

TABLE 2: Rf value of Seed extract				
S. No.	Sample	Rf Value		
1	P-1 with water	2.50		
2	P-2 with methanol	3.10		

Determination of total phenolic content (TPC):

Showed significant difference in the total phenolic content of fenugreek seed. Fenugreek seed were found to have the highest TPC values. Tannic acid and ascorbic acid was used as a standard to estimate the concentration of unknown (Table 3 and 4). There have been significant effects on the antioxidant activities of fenugreek seeds (Fig. 2 and 3) based on the solvent (Turkmen *et al.*, 2006).

TABLE 3: Total antioxident of ascorbic acid					
Concentration	Ascorbic Acid Standard	Extract\ Sample			
50	2176.404	2203.37			
100	2297.303	2185.393			
150	2068.876	2252.808			
200	2147.865	2225.842			
250	2201.46	1924.719			

TABLE 4: Total antioxident of tanic acid

Concentration	Absorbance
20	0.15
40	0.27
60	0.36
80	0.54
100	0.74



FIGURE: 2 Total antioxident of seed extract with standard ascorbic acid

Antimicrobial property of fenugreek seed Extract:

Fenugreek seed extract showed higher antibacterial property against *E.coli* and *Pseudomonas* where the inhibition zones were very well (Fig. 4). When the concentration of seed

extract was different but treated the extract at various concentrations such as 25%, 50%, 75% and 100%, the antibacterial activity increased (Table 5).



FIGURE 3: Total antioxident of seed extract with Standard Tannic Acid

TABLE 5: Zone of inhibition of Fenugreek seed extract at different concentration.

Concentration	E.coli	Pseudomonas
25%	09 mm	11 mm
50%	11 mm	11 mm
75%	13 mm	13 mm
100%	15 mm	16 mm



FIGURE 4: Antimicrobial property of fenugreek seed Extract against Pseudomonas and E.coli.

CONCLUSION

From the present study, it could be concluded that the solvent play an essential function in the extraction of the plant constituents. As methanol and ethanol are very polar among the solvents used therefore, they include high yield of phenolic compounds as compared to the other solvents. A methanolic extract of fenugreek seeds was show highest antioxidant activity .The results obtained from these methods provide some important factors responsible for the antioxidant potential of fenugreek seeds. And second is seed extract shows strong inhibitory effect against Pseudomonas and E.coli. Present study proved that fenugreek seed extract has antibacterial activity against pathogenic bacteria for Pseudomonas and E. coli.

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