Effect of processing methods on proximate composition and antioxidant activity of fenugreek (Trigonella foenum-graecum) seeds

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Abstract
Fenugreek (Trigonella foenum-graecum L.) is an old medicinal plant and has been commonly used as a traditional food and medicine. The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarine, sapogenins and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and thought to help lower sugar levels. Fenugreek seeds have also been reported to exhibit pharmacological properties such as anti-tumor, anti-viral and antioxidant activity. The aim of the present study was to investigate the effects of processing methods (roasted, soaked, germinated and pressure cooking) on the phenolic content and antioxidant activity of fenugreek seeds. The antimicrobial activity of fenugreek seeds was also analysed. Raw fenugreek seed flour contained higher amount of crude fiber (20.6%) followed by 15.8% in roasted seed flour, 10.2% in germinated and 6.21% in pressure cooked sample. Pressure cooking, germination and soaking enhanced the calcium and iron content of fenugreek seed flour. The protein content of raw fenugreek seed flour was 27.09% which was enhanced to 28.47% after germination. Germinated samples had highest DPPH % and FRAP content of 48.84% and 6.49mg ascorbic acid/g, respectively as compared to other treated samples. The aqueous extracts showed broadest antimicrobial activity by inhibiting most of the microbial strains involved. Among the microbial strains tested, Staphylococcus aureus and E. coli were the most susceptible strains whereas Salmonella enterica and Shigella flexneri were the most resistant microbes.

Key words: processing, antioxidant, fenugreek seeds, proximate, total phenolics.

INTRODUCTION
Fenugreek is a legume, originally from south Eastern Europe and western Asia, but grown now mainly in India and also in certain parts of Asia, northern Africa, Europe and the United States (Altuntas et al., 2005). It is known as methi in India and also as Fenugrec (France), Bockshorklee (Germany), Koroha (Japan), Hulba (Arab), Pazhitnik (Russia) and Ku-Tou (China). The seeds find extensive use in Indian cuisine to flavour many foods including curry powders, spice blends and tea. Its leaves are also used as a green leafy vegetable in the diet. Fenugreek seeds are aromatic but bitter with carminative, galactogogue, antibacterial and antiviral properties. The seeds contain a central hard yellow embryo surrounded by a corneous and comparatively large layer of white, semi-transparent endosperm. In Ayurveda, both fenugreek seeds and leaves are used to prepare extract or powder for medicinal use (Daniel, 2006).

The scientific name of fenugreek seeds is Trigonellafoenum-graecum. Fenugreek seeds are traditionally used for antioxidant properties. Many medicinal properties are attributed to fenugreek seeds and leaves. It is known to have several pharmacological attributes such as hypoglycemic, hypercholesterolemia, gastro protective, chemo-preventive, antioxidant, laxative and appetite stimulation (Sathyanarayana, 2011). Rheological properties and emulsions of galactomannans of fenugreek seed endosperm are also reported. The plant is known to contain alkaloids, flavonoids, salicylate, and nicotinic acid and used in treatment of many diseases. Studies show that the soluble fibre derived from fenugreek seeds has been identified as galactomannan similar to other soluble fibre of guar seeds, psyllium husk and other species. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that
antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenoids, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties are generally recognized as safe.

New antimicrobial agents are needed to treat diseases in humans and animals caused by drug resistant microorganisms. Interest in plant-derived drugs has been increasing, mainly due to the current widespread belief that they are safer and more dependable than costly synthetic drugs, many of which may have adverse side effects. The incidence and increasing frequency of microorganisms that are resistant to common and generally accepted effective first choice drugs is on the increase. A significant opportunity exists to identify new, natural plant derived antimicrobial agents for treatment of diseases or as food or cosmetic preservatives.

The present study investigates the effects of different processing methods (roasting, soaking, germination and pressure cooking) on the phenolic contents and antioxidant activities of fenugreek seeds. The anti-microbial activity of fenugreek seeds extracts has also been studied.

Materials and methods

Fenugreek seeds were procured from local market of Allahabad city; they were washed and dried under fanto remove excess moisture and stored at room temperature. All the chemicals were used for chemical analysis in extracts.

The seeds were processed in the terms of dry heating at 40-50°C in a cabinet tray drier. Soaking of Fenugreek seeds (50g) was done in distilled water at the ratio of 1:5 (w/v) at room temperature for 12 hrs. The water was intermittently changed every 6 hrs. After 12 hrs, the excess water was discarded. Germination was done by soaking the seeds (50g) overnight in water at the ratio of 1:5 (w/v). The excess water was drained and seeds were kept in the dark for germination (tied in muslin cloth) at 27±2°C temperature for 24 hrs. The germinated seeds were dried in an oven at 40°C for 6 hrs. Another 50 g of Fenugreek seed sample was roasted in an open pan at 130±5°C for 7 min. It was continuously stirred with ladle for proper and uniform roasting until it became slight brown and left a peculiar aroma. Pressure cooking of seeds (50g) was done in a pressure cooker for 20 min at a water ratio of 1:5 (w/v).

All the processed samples were dried at 40°C for 6 hrs.

Proximate Analysis:

All the samples were analyzed for proximate composition using AOAC (2005) methods. All the chemicals used were of analytical grade obtained from Mercrk or Sigma.

Antioxidant Analysis:

Preparation of solvent extract: Raw and processed fenugreek seed powder (100 g) was extracted with 500 ml of 70% methanol (w/v) using a shaker. The sample was shaken occasionally for 24 h. The extracts were centrifuged at 5,000 rpm for 20 min and the supernatants obtained were concentrated with a rotary vacuum evaporator (RV-10, IKA) at 45°C. The resultant extracts were stored in amber vials at 4°C until assayed.

Estimation of DPPH free radical scavenging method

The free radical scavenging activity of the fenugreek seed extracts was measured as decrease in absorbance of ethanolic DPPH solution at 517 nm in the presence of the extract (Krings and Berger, 2001).

Ferric reducing power of plasma

The ferric reducing power of the seed extracts was determined by using potassium ferricyanide- ferric chloride method (Oyaizu, 1986). Different dilutions of extracts accounting to 1 ml were added to 2.5 ml 0.2 M phosphate buffer (pH=6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 minutes, after which 2.5 ml trichloroacetic acid (10%) was added. 2.5 ml of the mixture was taken and mixed with 2.5 ml water and 0.5 ml 1% ferric chloride. The absorbance at 700 nm was measured after allowing the solution to stand for 30 minutes.

Total Phenolic Content:

Total phenolic content was determined using Folin-Ciocalteau method (Velioglu et al., 1998). Absorbance was measured using spectrophotometer at 725 nm after keeping for 1.5 hours at room temperature. Results were expressed as gallic acid equivalent in mg/100 g dry weight (DW).

Total Tannin Content:

The tannin content was determined by the method of Ranganna (2005). Powdered sample (0.5 g) was boiled with water (75ml) for 30 minutes and centrifuged at 2000 rpm for 20 minutes and the supernatant was collected. Folin Denis reagent and sodium carbonate is added to the sample extract, solution is diluted to 100ml with water proper shaking and absorbance is taken at 700 nm after 30 minutes.

Antibacterial activity

Test organisms

Four pathogenic bacterial strains were selected for this study to assess antimicrobial activities of spices against those strains. The strains were Escherichia coli MTCC 1687, Staphylococcus aureus MTCC 7443, Salmonella enteric MTCC 3219, Shigella flexneriMTCC 1457 were obtained from MTCC, IMTECH, Chandigarh, India. All bacterial cultures were maintained on tryptic soy agar (HiMedia) and sub-cultured regularly. Standard inoculum was prepared by sub-culturing 4-5 freshly grown isolated colonies of each strain in Tryptic soy broth (TSB) and incubated at 35-37 °C for 24 hours. Inoculum was standardized with sterile TSB to give final cell load of 105-106 CFU/ml.

Preparation of aqueous decoction:

The seeds were washed thoroughly with sterile double distilled water to make these leaves completely free from any possible contamination. Aqueous decoction of seeds was prepared by boiling 20 grams of seeds in 100 ml sterile distilled water over moderate flame for 20 minutes. The aqueous extract was cooled, filtered through Whatman No. 1 filter paper and then kept in sterile screw capped glass vials at 4°C. These crude extracts were reconfirmed as free of any

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Preparation of solvent extract: 20 g sample of raw and processed fenugreek seeds were soaked in ethanol (80%) for 48 hours at 24°C with stirring (Liu and Nakano, 1996). The extracts were centrifuged and filtered through Whatman No. 1 filter paper and evaporated using vacuum rotary evaporator or water bath to near dryness and stored in glass vials in dark at 4°C and extracts were used to determine the antioxidants. These crude extracts were diluted with 10% dimethyl sulphoxide to obtain required concentration.  

Disc diffusion bioassay  
The disc diffusion test was performed as described by Jorgensen et al. (1999). A 0.5 ml standardised inoculum suspension of each bacterial strain was spread on TSA plates with a sterile bent glass rod spreader. Sterile 6-mm Whatman no.1 filter paper discs were aseptically placed on plates. Extracts of standard concentrations (10 mg dry weight) were aseptically poured onto the discs along with plates. Extracts of standard concentrations (10 mg dry weight/ml) of the extracts were added to broth immediately after inoculating with fresh 0.2 ml culture of the strain, keeping final volume at 5 ml. The cultures were incubated on a rotary shaking incubator at 37°C for 48 hours. The lowest concentration of the extracts showing no visible growth was considered as the MIC.  

Result and discussion  
Raw fenugreek seed had a moisture content of 6.92%, crude fibre (7.51%), ash (3.65%). Raw fenugreek seed showed higher amount of iron content (11.19mg/100g) than processed seeds (Table 1). The total phenolic content 173.38mg GAE/g and DPPH radical scavenging ability (13.72%) was significantly lower than processed fenugreek seed samples. Panday and Awasthi (2013) have also reported lower antioxidant activity in raw fenugreek seed as compared to processed seed samples.  

Table 1: Proximate Composition (%), mineral content (mg) and antioxidant activity of raw and processed Fenugreek Seeds  

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw</th>
<th>Roasted</th>
<th>Soaked</th>
<th>Germinated</th>
<th>Pressure cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture%</td>
<td>6.92±0.83b</td>
<td>4.25±0.23c</td>
<td>8.81±1.2c</td>
<td>6.39±0.55b</td>
<td>8.24±0.17c</td>
</tr>
<tr>
<td>Fat%</td>
<td>4.23±0.75c</td>
<td>4.05±0.56b</td>
<td>3.66±0.28a</td>
<td>3.48±0.35a</td>
<td>3.95±0.57b</td>
</tr>
<tr>
<td>Crude fibre%</td>
<td>7.51±0.53b</td>
<td>7.86±0.92c</td>
<td>7.22±0.52b</td>
<td>9.26±0.59d</td>
<td>6.21±0.58c</td>
</tr>
<tr>
<td>Ash%</td>
<td>3.65±0.46b</td>
<td>4.06±0.81c</td>
<td>4.17±0.96c</td>
<td>1.04±0.83a</td>
<td>3.04±0.74b</td>
</tr>
<tr>
<td>Iron mg/100g</td>
<td>11.19±0.92d</td>
<td>5.17±0.15c</td>
<td>9.75±0.62c</td>
<td>6.53±0.58b</td>
<td>9.45±0.52c</td>
</tr>
<tr>
<td>Calcium mg/100g</td>
<td>84.17±1.28b</td>
<td>87.21±2.54c</td>
<td>76.10±1.01a</td>
<td>90.2±1.57c</td>
<td>73.2±1.91a</td>
</tr>
<tr>
<td>Phosphorus mg/100g</td>
<td>515.7±2.17a</td>
<td>596.2±1.81c</td>
<td>604.27±2.68d</td>
<td>612.17±2.54d</td>
<td>549.35±1.72b</td>
</tr>
<tr>
<td>Phosphorus mg/100g</td>
<td>515.7±2.17a</td>
<td>596.2±1.81c</td>
<td>604.27±2.68d</td>
<td>612.17±2.54d</td>
<td>549.35±1.72b</td>
</tr>
<tr>
<td>Tannin content (mg GAE/g)</td>
<td>173.38±12.29b</td>
<td>172.25±13.94b</td>
<td>157.92±5.70a</td>
<td>184.31±7.02c</td>
<td>178.16±3.7c</td>
</tr>
<tr>
<td>Tannin content (mg Tannic Acid/g)</td>
<td>3.94±0.83b</td>
<td>2.6±0.41a</td>
<td>4.33±0.34c</td>
<td>5.5±0.19d</td>
<td>5.34±0.58d</td>
</tr>
<tr>
<td>DPPH %</td>
<td>13.72±0.88a</td>
<td>24.81±0.74b</td>
<td>35.85±0.92c</td>
<td>48.84±1.12d</td>
<td>27.74±1.35b</td>
</tr>
<tr>
<td>FRAP (mg Ascorbic Acid/gm)</td>
<td>4.89±0.21b</td>
<td>3.44±0.25a</td>
<td>5.24±0.17c</td>
<td>6.49±1.33d</td>
<td>5.16±0.76c</td>
</tr>
</tbody>
</table>

The values are mean ± SD (n=3)  
Values with similar superscripts in a row do not differ significantly (p<0.05)  

Roasting  
Roasting of fenugreek seeds caused a decrease in moisture content (4.25%) and fat content (4.05%) whereas the crude fibre (7.86%) and the ash content (4.06%) increased significantly (table 1).The decrease in fat content on roasting may be attributed to loss of volatile oils on dry heating of fenugreek seeds (Mathur and Chaudhary, 2009). Roasting showed increase in calcium (87.21mg/100g) and phosphorus content (596.21mg/100g) of the fenugreek seeds. The increase of mineral may be related to breakdown of antinutritional compounds such as phytates and oxalates which bind these mineral and reduce their availability (Reddy et al., 1978).During roasting there is also breakdown of the bound between phytates and P. The tannin content decreased from 3.94 to 2.6mg tannic acid/g. The DPPH% increased significantly to 24.18% on roasting of fenugreek seeds. An increase in antioxidant activity of fenugreek seed on roasting has also been reported by Panday and Awasthi (2013).  

Soaking  
On soaking, a significant decrease in fat content (3.66%) was observed (Table 1). A similar trend in decrease in fat content in fenugreek seeds after soaking has been reported by Hooda and Jood (2003). An increase in moisture content (8.81%) was observed in fenugreek seeds where a significant decrease was observed in iron content.
(9.75mg/100g) and Calcium content (76.10mg/100g). The phosphorus content showed significant increase on soaking fenugreek seed (604.27 mg /100g). Comparative lower contents of mineral when soaked in water might be due to leaching of some amount in to soaking water (Nolan and Duffin 1987). The phenolic content was minimum in soaked fenugreek seeds (157.92 mg GAE/g) as compared to other processed samples. The DPPH % and FRAP content increased significantly to 35.85% and 5.24mg ascorbic acid/g, respectively on soaking fenugreek seeds. An increase in DPPH % on soaking of fenugreek seeds has also been reported by Panday and Awasthi (2013).

Germination
Germination of fenugreek seeds showed a significant decrease in fat content (3.48%) as compared to raw seeds (4.23%). El-Aal (1986) reported decrease in total fat content along with decrease in free fatty acids, monoglycerides and polar lipids upon germination. The crude fibre increased significantly to 9.26% (Table 1). Whereas the ash content decreased significantly to 1.04% on germination of fenugreek seeds. Increase in crude fibre content upon germination, might be attributed to the synthesis of structural carbohydrates, such as cellulose and hemicelluloses during germination (http://en.wikipedia.org/wiki/Sprouting,2012). Among the minerals, the iron content decreased whereas calcium and phosphorus increased significantly. Decrease in iron content in germinated fenugreek seed flour might be due to leaching of iron in to soaking medium (Duhan et al. 2002). A decrease in iron content during germination of fenugreek seeds was reported by El-S himi et al. (1984). The total phenolic content was observed to increase significantly (184.31mg GAE/g) as compared to other processed samples (Table 1). Randhir et al. (2004) reported higher antioxidant activity during early germination, which correlates to higher phenolic content suggesting that initially phinolics are antioxidants in nature. Shakuntala et al. (2011) also reported that sprout of germinated fenugreek seeds were rich in polyphenols(97.55mg/100g). Cevallos-Casals and Cisneros-Zevalos(2010) reported that germinated edible seed species are an excellent source of dietary phenolic content. They also observed that these phenolic compounds are responsible for the antioxidant property of the sample. DPPH % and FRAP content also showed to increased significantly to 48.84% and 6.49mg ascorbic acid/g, respectively. Naidu et al. (2011) also reported 50-70% free radical scavenging activity in different concentrations of fenugreek extracts.

Pressure cooking
Pressure cooking of fenugreek seeds caused a significant increase in moisture content (8.2%). The fat content and crude fibre content showed a significant decrease to 3.95% and 6.21%, respectively. The iron and calcium content decreased whereas the phosphorus content increased significantly, on pressure cooking of fenugreek seeds. Increase in P might be due to decrease in phytates, tannins and other anti-nutritional factors that bind the minerals (El- Mahdy and El-Sebaiy, 1982). Total phenolic content and tannin content were significantly higher than raw samples (Table 1). Similarly the DPPH% and FRAP values were also significantly higher for pressure cooked sample than raw and roasted fenugreek seed samples. Nutritional composition, antinutritional and antioxidant activity compared well with the fenugreek seeds composition reported by Hoo da and Jood (2003), Sharma(1986) and Gopalan et al. (2004).

The antimicrobial activity of fenugreek seed extracts
The disc diffusion assay showed that the fenugreek seed extracts have different degrees of bacterial growth inhibition, depending on the strains (Table 2). The aqueous extract showed broadest antimicrobial activity by inhibiting most of the microbial strains involved. Ethanolic extracts indicated higher anti-microbial activity showing greater diameter of inhibition zones. Hexane extracts did not show any zone of inhibition and Shigella flexneri was found to be the most sensitive strain. It is clear that different extracts ordecoctions of fenugreek seeds differ in their antimicrobicactivities, which may depend on solubilityof the active constituents.

Table 2: Antibacterial activity of fenugreek seed extracts, indicated by diameter of inhibition zone (DIZ, mm, for 10 mg dry weight/disc)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Staphylococcus aureus</th>
<th>Salmonella enterica</th>
<th>Shigella flexneri</th>
<th>E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>18±3</td>
<td>16±1</td>
<td>14±1</td>
<td>18±1</td>
</tr>
<tr>
<td>Acetone</td>
<td>38±1</td>
<td>40±2</td>
<td>43±1</td>
<td>42±1</td>
</tr>
<tr>
<td>Hexane</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>34±1</td>
<td>37±1</td>
<td>37±2</td>
<td>39±1</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>18±1</td>
<td>0</td>
<td>18±2</td>
<td>18±1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>35±2</td>
<td>41±2</td>
<td>28±4</td>
<td>33±1</td>
</tr>
</tbody>
</table>

The values are mean ± SD (n=3)

Shigella flexneri were the most resistant microbes. Saini and Singh (2015) studied the antimicrobial activity of alcoholic and aqueous extracts of asafetida, carom seeds, nutmeg and mace against various pathogens and found Staphylococcus aureus and E. coli as the most susceptible strains. Difference in antimicrobial activity of herbs and spices against different bacteria might be due to presence of

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different active phyto-compounds. Among the antimicrobial compounds, phenolic compounds, terpenoids, and alkaloids are considered very important for their antimicrobial and antioxidant effects (Hoult and Paya, 1996; Rios and Recio, 2005). Nowadays microbes are increasingly developing resistance against the drugs in use. To combat against these drug resistant microbes, a large library of novel compounds is required. Natural products from plants may give us a solution to this alarming problem.

**CONCLUSION**

From the present study, it can be concluded that nutritional quality and antioxidant activity of fenugreek seeds may be enhanced or improved through various processing methods such as soaking, germination, pressure cooking and roasting. The above results also signify the fact that natural products like spices can be seen as alternatives to chemical preservatives used in various food industries so as to minimize their side effects and simultaneously improving the shelf life of the food products. The fenugreek seed extracts can also be exploited for their anti-microbial activity against various pathogens viz. *Salmonella, Shigella, Staphylococcus* and *E.coli*. Processed fenugreek seed can be utilized in functional foods, therapeutic agents as well as antimicrobial agent in food. The inhibitory factor responsible for the antimicrobial activity can further be identified and used as an alternative to currently used drugs against the pathogenic microbes under study.

**ACKNOWLEDGEMENT**

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**References**


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**Table 3: Minimum Inhibitory Concentration of fenugreek seed extracts (MIC, for mg dry weight/ml)**

<table>
<thead>
<tr>
<th>Extracts</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Salmonella enterica</em></th>
<th><em>Shigella flexneri</em></th>
<th><em>E.coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>10</td>
<td>30</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>Acetone</td>
<td>15</td>
<td>20</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Hexane</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Ethanol</td>
<td>7.5</td>
<td>20</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>7.5</td>
</tr>
</tbody>
</table>

“*” shows no MIC value.


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