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Evaluation of bioactive compounds in the extract of *Trigonella foenum-graecum* seeds and leaves

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Abstract
Trigonella foenum-graecum Linn. seeds and leaves 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 a source of antidiabetic compounds from its seeds. Fenugreek seeds and leaves contain phenolic compounds, which have antioxidant and antibacterial properties. The results demonstrated that fenugreek seeds showed the highest TPC contents while leaves showed maximum TFC content. The primary phytochemical analysis confirmed the presence of all essential bioactive compounds like phenol, flavonoid and alkaloids.

Keywords: *Trigonella foenum-graecum*, TPC, TFC, TLC

Introduction
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. 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 (Kumari *et al*., 2016) [2]. It contain lysine and L-trytophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreeke, nicotinic acid, sapogeninsphytic acid, scopoletin and trigonelle which has therapeutic effects (Srinivasan *et al*., 2006) [6]. The component called fenugreekine a steroidal sapogenin peptide ester has hypoglycemic properties. Thus it is best use is to control blood sugar in both insulin dependent (type 1) and noninsulin dependent (type 2) diabetes (Idries, 2014) [7].
Fenugreek has been referred to as a medicinal herb both in IndianAyurvedic and traditional Chinese medicines. Medicinal uses vary from wound-healing to bust enhancement and from promotion of lactation in weaning mothers, to its use as a sex stimulant or aphrodisiac. Fenugreek plant extract was successful in complete removal of head lice from infected patients within a week (Shaikh *et al*., 2013) [1].

Material and Method
Sample Collection
Sample (fenugreek seeds and leaves) was collected from bittan market (vegetable market) in Bhopal. After that fenugreek (methi) leaves were dried in shade, then they were powdered in a blender. Then extract was prepared of both the leaves and seeds.

Extraction
In this process solid ingredients are placed in a stoppered bottle or container with the whole of the solvent and allowed to stand for a period of at least 3-4 days with frequent agitation, until soluble matter is dissolved. The mixture is then strained (through sieves or net), the marc pressed and the combined liquids clarified (cleaned by filtration) or by decantation after standing.

Phytochemical Tests
Phytochemical analysis of the extract was done with the reference of Harborne, (1984) [8].

1. Carbohydrates (Molisch test)
   In 1ml of extract, add 2 drops of alcoholic alpha-naphthol solution in a test tube. Then add1ml of H2SO4 by the side of test tube. Formation of the violet ring at the junction indicates the presence of carbohydrate.
2. Alkaloids (Wagner's test)
   In 1 ml of extract, add few drops of Wagner reagent (Iodine in KI). Formation of a brown or reddish precipitate indicates the presence of alkaloid.
3. Saponins (Foam test)
   In 1ml of extract, add 1ml of water and shake it. If foam produced persist for 10 min, it indicates the presence of water.
4. 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.
5. Flavonoids (Lead acetate test)
   In 1ml of extract, add few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.
6. Steroids (Salkowski's test)
   In 1ml of extract, add 1ml of Salkowski's reagent. Formation of golden yellow, red, green colour indicates the presence of steroids.

Total Flavanoid Content (Tfc)
The total flavonoid content (TFC) of the extract was

determined using the aluminium chloride assay through Spectrophotometer (Samatha *et al.*, 2012) [9]. Take 0.5ml extract in test tube then add 1ml distilled water and add 0. 5ml, 5% sodium nitrite ($NaNO_2$) and allow to stand for 6min. Later add 0.5ml of 10% $AlCl_3$ and incubate for 6min. After that add 2ml of 4% NaOH and make up the volume to 5ml with distill water. After 15 minute of incubation take OD at 510 nm.

Total Phenol Content (TPC)

The total phenolic content (TPC) of the crude extracts of plant were determined using the method of Singleton *et al.*, (1999) with slight modifications. To 0.5 ml of test sample, 1.5 ml (1:10 v/v diluted with distilled water) Folin Ciocalteau reagent was added and allowed to stand for 5 min. After 5 min, 2.0ml of 7.5% of sodium carbonate was added. These mixtures were incubated for 90 min. in the dark with intermittent shaking. After incubation, development of the blue colour was observed. Finally absorbance of blue colour in different samples was measured at 725 nm using spectrophotometer. The phenolic content was calculated as Tannic acid equivalents TA/g on the basis of standard curve of Tannic acid. The results were expressed as tannic acid equivalents TA/g of the plant material. All the determinations were carried out in triplicates.

Thin Layer Chromatography (TLC)

Prepare silica gel in the ratio 1: 2 with distill water. Pour few drops of silica gel on the glass slide on the one end and spread it evenly on the whole slide by tilting the slide slightly at the angle of 45º. After drying plates was activated at 100ºc for 30 min.

Different solvent in various ration was used for TLC.

Ethyl acetate, water and ethanol in the ratio of 6:8:6

Ethyl acetate, chloroform and ethanol in the ratio of 3:3:6.

Chloroform, ethyl acetate and acetic acid in the ratio of 8:4:4.

Chloroform, ethanol and glacial acetic acid in the ratio of 3:6:3.

Result

The preliminary phytochemical screening of the fenugreek seed and leaves extracts using hydroalcohol solvents was reported (Table 1). By this analysis we can conclude that fenugreek seeds contain more phytochemicals than fenugreek leaves. It consists of phenol, alkaloids, flavonoids and carbohydrates. Mahmooda and Yahya, 2017 [3] reported that the aqueous extract was found to contain a rich amounts of alkaloids, flavonoids, carbohydrates, phenolic compounds and tannins, terpenoids, saponins, amino acids, protein, with a less amount of glycosides, steroids, and absence of Oil, fat. *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 (Kumari *et al*., 2016) [2].

Table 1: Phytochemical Screening of Seeds and leaves of *Trigonella foenum-graecum*

Test	Seed Extract	Leaf Extract
Carbohydrate (Molish Test)	+	+
Alkaloids (Wagner`S Test)	Partially +	-
Saponin (Foam Test)	+	+
Phenol (Ferric Chloride Test)	+	+
Flavonoids (lead acetate test)	+	+
Steroids (salkowski test)	-	-

Where, + indicate presence and – indicate absence

Total Flavonoid Content

The data on flavonoid content (TFC) in hydroalcohol crude extract of fenugreek seeds and leaves is presented in Figure 2. TFC in seeds was 0.15mg QE per g extract and 0.51mg QE per g extract in leaves. In the present study, leaves shows maximum flavonoid content in the hydroalcoholic extract. Quercetin used as standard in present experiment figure 1. Similarly, S N Saxena *et al*., 2016 reported the effect of cryogenic grinding on phenolic compounds and antioxidant properties of fenugreek (*Trigonella foenum-graecum L*.) seed extract.

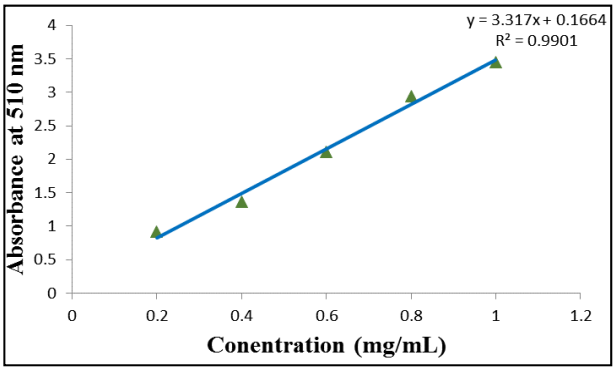

Fig 1: Standard graph of Quercetin for TFC

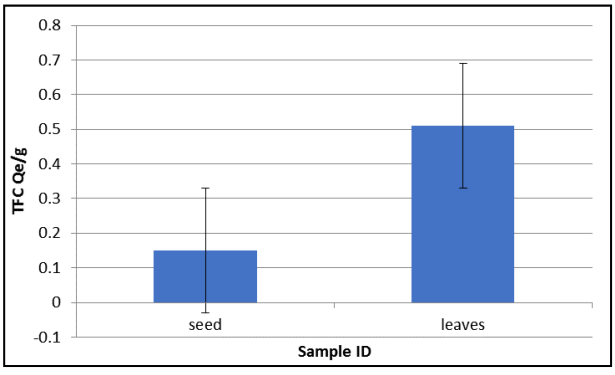

Fig 2: Total flavonoid content in seeds and leaves of fenugreek

Total Phenol Content

The variation found in the total phenolic content of fenugreek seeds and leaves was shown in Figure 4. The content of phenolics was significantly not different in the both explant; it ranges from 2.74 to 2.90 mg TA/g extract. Fenugreek seeds were found to have the highest TPC values 2.90 mg. Tannic acid was used as a standard to estimate the concentration of unknown (Figure 3).

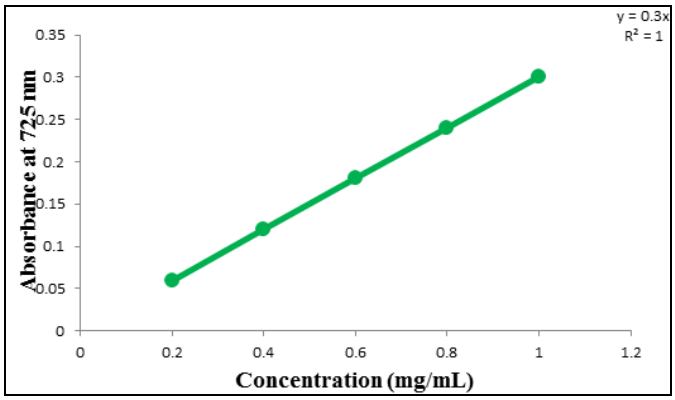

Fig 3: Standard graph of tannic acid

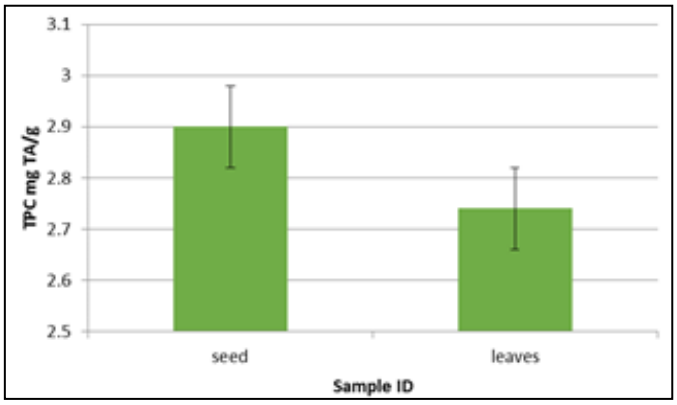

Fig 4: Total phenol content in fenugreek seed and leaves

Thin layer chromatography

A qualitative TLC method that gives dense and compact spots with significant values for simultaneous determination of phytochemicals in fenugreek seeds and leaves was developed Figure 5. The effect of extraction solvent was investigated by introducing a single factor test. As the flavonoids and saponins are polar compounds, the polar solvents like ethanol, methanol, water, and their combination can be employed for better extraction of these substances than nonpolar ones (Omi *et al*., 2014). Separated spots on TLC plates indicate presence of different compounds. The corresponding Rf values identical to seeds of fenugreek at 254 nm are 0.67 and 0.83 corresponding to the spot. Whenever the TLC profile for leaves extracts with the Rf values of 0.38, 0.50 and 0.78 developed chromatogram is shown in Figure 5. The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines.

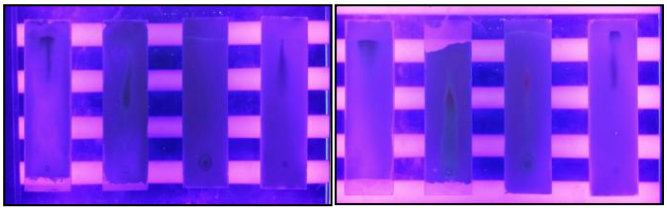

Fig 5(A-B): Thin layer chromatogram of fenugreek seeds and leaf extract

Conclusion

The preliminary phytochemical screening of *Trigonella foenum gracum* gives good results in the presence of flavonoids, alkaloids, tanins, phenolic compounds, terpenoids, saponins, carbohydrates and proteins. There is absence of anthraquinones by extracting with hydroalcohol. In the present study the hydroalcoholic extracts of the selected parts of fenugreek have been evaluated for potential antioxidant activities, phytochemicals and separation of active content. The amount of phenolic content in the extract of fenugreek may be the reason for its potent antioxidant activity whereas other spices have significant phenolic and flavonoids contents which assure their antioxidant properties.

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