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Phytochemical analysis and antioxidant activity of *Trigonella foenum-graecum* seeds

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Abstract
Trigonella foenum graecum (Fenugreek) (Family: Leguminosae) is used as a traditional medicine in treating various diseases due to its antiinflammatory, antidiabetic and antilipidemic properties. In our study, aqueous extract of seeds were subjected for *in vitro* antioxidant activity by 1- diphenylpicryl-hydrazyl radical (DPPH), and ABTS radical cation assay. Total phenol and total flavonoid content were estimated. It was observed that free radicals were scavenged by the extract in a dose dependent manner. The content of total phenolics (expressed as mg of gallic acid equivalents/gm extract) and total flavonoids (expressed as mg of quercetin equivalent/gm extract) were determined along with antioxidant enzymes. The aqueous extract of fenugreek exhibited potent DPPH and ABTS radical scavenging activity with IC_{50} values of 62.67 µg/mL and 71.44 µg/mL, respectively. Based on the results it can be concluded that aqueous seed extract of fenugreek seeds may have potential antioxidant effects against several oxidants generated in the body.

Keywords: Fenugreek, antioxidant, trigonella, polyphenols, oxidative stress

Introduction
Plants are used as a source of medicine in the past centuries. Nowadays, scientists have recognized their value as a source of new or complimentary medicinal products. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal products to supplement the diet, mainly to improve the quality of life and preventing the diseases. Fenugreek (*Trigonella foenum-graecum* L.) has been attributed to possess multipotent antioxidant, antimicrobial, anticancer, gastroprotective and antidiabetic properties reported by Zameer *et al*. (2017) [1], Basch *et al*. (2003) [2]. Generally, antioxidants have been identified as major health beneficial compounds reported from varieties of medicinal plants and are sources for alternative medicines. Szymanska *et al*. (2016) [3] found the role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents reported by. The protective effect of plant products are due to the presence of several components such as enzymes, proteins, vitamins, flavonoids, carotenoids, and other phenolic compounds. Generally, the antioxidant capacity of phenols in plant extracts is effective at low concentrations, and in humans, it is associated with the prevention of cardiovascular disease and cancer reported by Duthie *et al*. (2000) [4], Li *et al*. (2014) [5], Balmus *et al*. (2016) [6]. Thus, studies for the determination of the antioxidant activity of the aqueous extract of fenugreek seeds could contribute to establishing the value as a source of new antioxidant compounds noticed by Miliauskas *et al*. (2004) [7], Gouthamchandra *et al*., (2010) [8].
Free radicals or reactive oxygen species are formed in our body as a result of biological oxidation. Thomson (1995) [9] reported that over production of free radicals such as hydroxyl radical, super oxide anion radical, hydrogen peroxide can cause damage to the body and contribute to oxidative stress. Lee *et al*. (2000) [10] found that oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases including cancer, coronary artery disease, hypertension, diabetes etc and compounds that can scavenge free radicals have great potential in ameliorating these disease processes reported by Kris-Etherton *et al*. (2002) [11], Behara *et al*. (2006) [12], Di Malteo and Esposito (2003) [13]. Most of the reactive oxygen species are scavenged by endogenous defense systems such as catalase, super oxide dismutase and peroxides-glutathione system as noticed by Rice-evans and Bourdon (1993) [14]. Hazra *et al*. (2008) [15] focused on natural antioxidants and numerous crude extracts and pure natural compounds have been recognized to have beneficial effects against free radicals in biological systems as antioxidants.
Fenugreek is a widely used in folk and Ayurvedic systems of medicine. Fenugreek belonging to the family Leguminosa, an aromatic, 30-60 cm tall, annual herb, cultivated throughout the

country. Flavonoids of fenugreek extract have been observed to possess antioxidant activity reported by Moskaug *et al.* (2005) [16], Myhrstad *et al.* (2002) [17], Ozcan *et al.* (2005) [18]. The objective of the present study was to determine the antioxidant activity of fenugreek seeds extract using standard methods.

Materials and Methods

Plant material and extraction procedure

The fenugreek seeds sample was collected from the Vegetable Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The fenugreek seeds were identified (Ref. No.: NBRI/CIF/541/2017) by CSIR-National Botanical Research, Lucknow, Uttar Pradesh, India. Dry fenugreek seeds were cleaned and ground into coarse powder. Distilled water was used for extraction method. The extract was filtered. The residue was re-extracted twice under the same condition to ensure complete extraction. The extracts were evaporated to dryness under reduced pressure at 60°C by a rotary evaporator, lyophilized and stored at -20 °C for further study. All chemicals were used are analytical reagent grade.

Phytochemical analysis

Determination of total phenol

Estimation of total phenol content was estimated as per the method explained by Mena *et al.* (2011) [19]. Gallic acid was used to draw standard calibration curve (Figure 1). Total phenol content was calculated and expressed as milligram per liter gallic acid equivalent of per gram of dry weight of extract (mg Eqv GA/gm EW).

Assay of total flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric method described by Zhishen *et al.* (1999) [20]. Quercetin was used as a standard flavonoid to produce the calibration curve (Figure 2). The flavonoid was expressed as mg of equivalent of quercetin per gram of extract weight (mg ER /g EW).

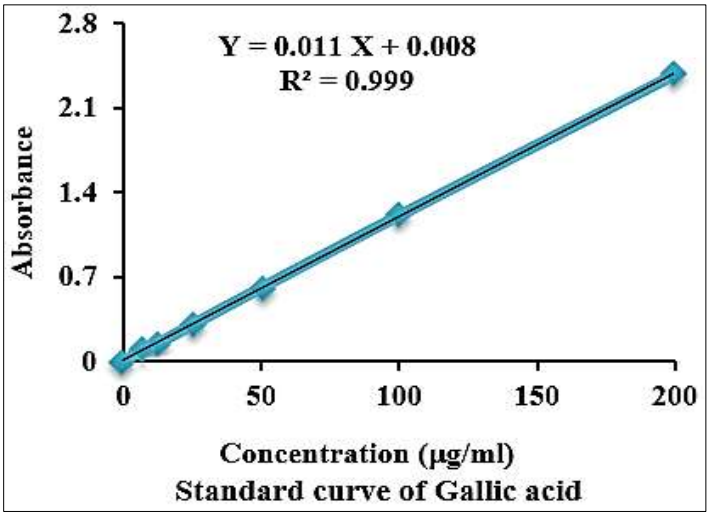

Fig 1: Total phenolic content of fenugreek

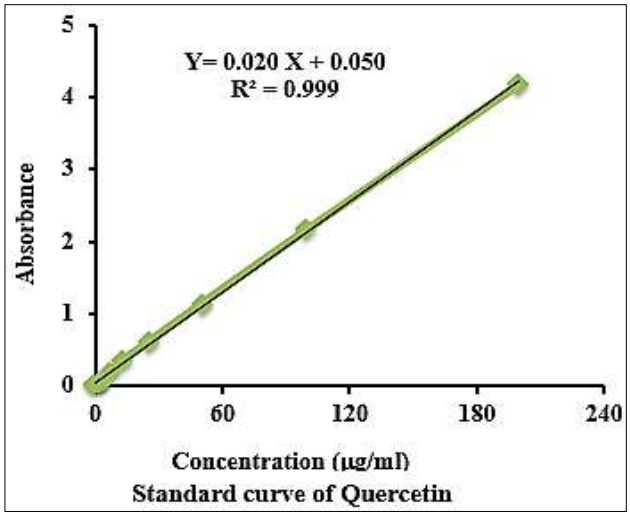

Fig 2: Total flavonoid content in fenugreek

Determination of antioxidant properties

DPPH free radical scavenging system

Antiradical activity of seed extract was evaluated by measuring scavenging activity on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by using method described by Shekhar and Anju (2014) [21]. Absorbance was measured at 517 nm by using spectrophotometer. The DPPH scavenging activity was calculated by using following equation:

$$\text{DPPH scavenging activity (\%)} = [\frac{(Ab-As)}{Ab}] \times 100$$

Where, A_b is the absorbance of the control (DPPH solution without test sample), and A_s is the absorbance of the test sample (DPPH solution plus seed extract).

ABTS radicals scavenging assay

Antiradical activity of extract based on ability to scavenge ABTS (2, 2'- azinobis-3-ethylbenzthiazoline-6-sulfonic acid) radical cation as mentioned by Bernini *et al.* (2018) [22]. Distilled water was used as a blank. Absorbance was calculated by using following formula. The result was compared with control (only ABTS solution).

$$\text{ABTS radical scavenging activity (\%)} = [\frac{(Ab-As)}{Ab}] \times 100$$

Where, A_b is the absorbance of the control (ABTS and methanol solution without sample), and A_s is the absorbance of the test sample {represents the mixture of ATBS solution (*i.e.*, working solution) plus seed extract} was expressed as µmol.

Result and Discussion

The quantitative analysis of the aqueous extract of fenugreek seeds showed the presence of high amount of total phenols i.e., 73.54 milligram gallic acid equivalent per gram extract and flavonoids 70.30 milligram quercetin equivalent per gram extract. The compounds derived from secondary metabolism, specifically phenolic compounds, play a fundamental role against oxidative stress reported by Pang *et al.* (2018) [23]. These compounds are known to act as antioxidants not only for their ability to donate hydrogen or electrons but also because they are stable radical intermediates as reported by Niciforovic *et al.* (2010) [24]. Phenolic compounds also have protective effects on humans when the plants are consumed as food.

The aqueous extract of fenugreek seeds was also evaluated for their *in vitro* antioxidant activity using two different methods such as, 1- diphenylpicryl- hydrazyl radical (DPPH), and ABTS radical cation assay. It was observed that free radicals were scavenged by the extract in a concentration dependent

manner. The extract of fenugreek seeds exhibited potent DPPH and ABTS radical scavenging activity with IC_{50} values 62 mg/ml, and 71 mg/ml respectively. All the experimental values were compared with standards. The antioxidant activity of the extracts was also evaluated by their ability to inhibit the $ABTS^{*+}$ radical obtained from ABTS. This activity increases in a dose-dependent manner with the concentrations of the different extracts. The IC_{50} value of the extract is close to that of the standard.

The DPPH radical has been used widely to test the potential of the compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. Shimada *et al*. (1992) [25] found that DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by extract either by transfer of hydrogen or of an electron**.** The result of DPPH scavenging activity assay in this study indicates that the seeds were active against free radical scavenging. This suggests that the seed extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The ability of this seed extract to scavenge DPPH could also reflect its ability to inhibit the formation of ABTS+. The scavenging activity of ABTS+ radical by the seed extract was found to be appreciable; this implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentration mentioned by Wang *et al*. (1998) [26].

Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, gastritis, renal failure and neurodegenerative diseases as a result of deficient natural antioxidant defense mechanism reported by Parr and Bolwell (2008) [27], Kumpulainen *et al*. (1999) [28]. Fenugreek seed with antioxidant activities have been reported to possess free radical scavenging activity as found by Das and Pereira (1990) [29]. Antioxidants are the compounds which help to delay or inhibit the oxidation of lipids and other molecules through the inhibition of either initiation or propagation of oxidative chain reactions reported by Jaleel *et al*. (2007) [30]. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented by Pourmorad *et al*. (2006) [31]. Medicinal plants can protect against harmful effects of ionizing radiation. Natural plant extracts or pure compounds are safe ingredients, which do not have any serious toxic effects. The therapeutic benefit of medicinal plants is usually attributed to their antioxidant properties and oxidative stress is a prominent feature of these diseases as mentioned by Feher *et al*. (1998) [32], Aboutwerat *et al*. (2003) [33].

Conclusion

The present study provides the useful information about antioxidant properties and polyphenolic contents of fenugreek seeds, which is consumed in India as a source of potential antioxidants. This may be helpful in preventing or slowing the progress of diseases involved as a result of oxidative stress.

Acknowledgments

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