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Quantitative determination of phytochemical constituents in seeds of common methi and Kasuri methi

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Abstract
Phytochemicals are chemical compounds produced by plants and play role in resistance against bacteria, fungi, and virus infections. Medicinal and spice crops are known to be rich in phytochemicals. Fenugreek is a multipurpose crop and is also considered Generally Recognised as Safe (GRAS) by the FDA. In order to estimate the phytochemicals abundance in different genotypes of fenugreek using seeds samples of methi (*Trigonella foenum-graecum* L.) (RMT-305, AFG-1, Pusa Early bunching) and Kasuri methi (*Trigonella corniculata* L.) were investigated for their potential phytochemicals abundance such as phenols, flavonoids, tannins, alkaloids and proteins by spectrophotometer method. Among genotypes, kasuri methi as found highest phytochemical contents such as phenol (71.80 mg/100 gm), flavonoids (90.28 mg/gm), tannins (244.53 gm), alkaloids (42.64/gm) and protein (55.10 gm) followed by RMT-305, AFG-1, and Pusa Early bunching. These findings showed that Kasuri methi had a significant quantity of phytochemicals. Further, such research outcomes useful for confirmation of effectiveness of phytochemicals on plant pathogens by using bioassay.

Keywords: Fenugreek, genotypes, phytochemicals, seeds, spectrophotometer

Introduction
From the ancient times plants have been used by human beings for medicinal purpose and it is the basis of traditional medicine. The component of plant that has health regulatory and disease preventive quality is the source of the herbal drug and it is called as phytochemicals. These herbal drugs are not only used as a medicinal entity but it can also be consumed as the supplementary diet (Dias *et al*., 2012) [8]. Now a days plant based products are used in various pharmaceutical companies, cosmetic products, nutraceutical, functional food as well as source of important trace element. About 80 percent population of whole world believe in these traditional medicines for their health care (Chan *et al*., 2003) [7].
The most important bioactive constituents of medicinal plants are the alkaloids, tannins, flavonoids, and phenolic compounds (Edeoga *et al*., 2005) [9]. These show considerable interest in the search for natural antioxidants and broad biological effects, including anti-inflammatory, anti-allergic and antibacterial (Pant *et al*., 2017) [21].
Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop which grows under arid and semi-arid regions of India. *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 percent of the total global fenugreek production takes place in India (Zahra and Jalal, 2018) [35]. Fenugreek is a multipurpose crop being used as a spice, vegetable and medicinal plant (Ahmed *et al*., 2016) [3]. The seeds of fenugreek are rich in minerals, vitamins and protein (Randhir *et al*., 2004) [23]. The seeds of fenugreek have aromatic properties which can be used as condiment during food preparation (Prajapati *et al*., 2017) [22].
Fenugreek seeds contain various phytochemicals and has been widely used in pharmaceutical industries due to its anti-diabetic, anticancer, antioxidant, antipyretic, antimicrobial, anthelmintic, anti-allergic and anti-inflammatory properties (Goyal *et al*., 2016) [12]. In order to understand the phytochemical abundance among common methi and kasuri methi the study has been undertaken.

Materials and Methods
Plant material
For the study, four genotypes of fenugreek seeds were used, including common methi (Rmt-305, AFG-1, and Pusa Early bunching) and Kasuri methi, both grown in the open field of

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Extraction preparation

Fenugreek seeds were collected and using a mechanical blender, the seed material was diced and blended with 80 percent ethanol. Whatman No. 1 filter paper was used to filter the solution. All of the extracts were stored at 4 °C in storage vials with the filtrate being collected in reagent bottles (Mona *et al*., 2014) [19].

Quantification of phytochemicals

Determination of total phenol content

According to the method described by Vidyasagar *et al*., (2009) [33] the total phenols were estimated using the Folin-Ciocalteau reagent (FCR) method. One gram of seeds was crushed with 10 ml of 80% (v/v) ethanol and was meticulously filtered through layers of muslin cloth and then kept in bottles at 4 °C. In a hot water bath, one millilitre of the aliquot evaporated. The residue was combined with 0.5 ml of FCR and dissolved in 1 ml of distilled water. After fully mixing, 2 ml of sodium carbonate (20%) solution was added. The tubes were then immersed in boiling water for one minute, allowed to cool, and the absorbance at 650 nm was measured using a visible spectrophotometer (Visiscan 167 model) using reagent blank. The concentration of phenols in the test sample was determined from the standard curve using catechol as the standard, and it was expressed as mg phenols/100 g.

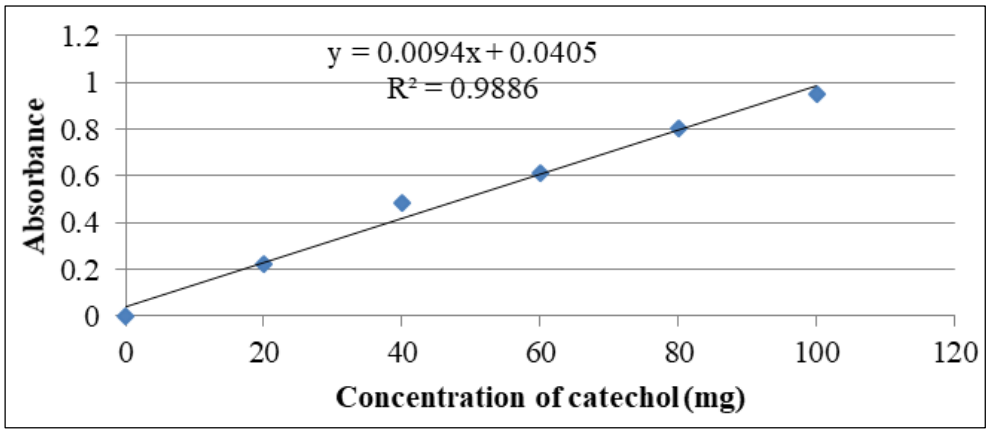

Fig 1: Calibration curve of catechol

Determination of total flavonoid content

Total flavonoid content was determined using the aluminium chloride colorimetric method. Briefly, 0.5 ml solutions of ethanol extract separately mixed with 1.5 ml of ethanol, 0.1 ml of (10%) aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm with a visible spectrophotometer. Total flavonoid content was calculated as quercetin from a calibration curve. Quercetin was used as standard and the flavonoid content is expressed in terms of milligrams of quercetin equivalents (QE) 100 per gram of extract (Ghasemzadeh *et al*., 2010) [11].

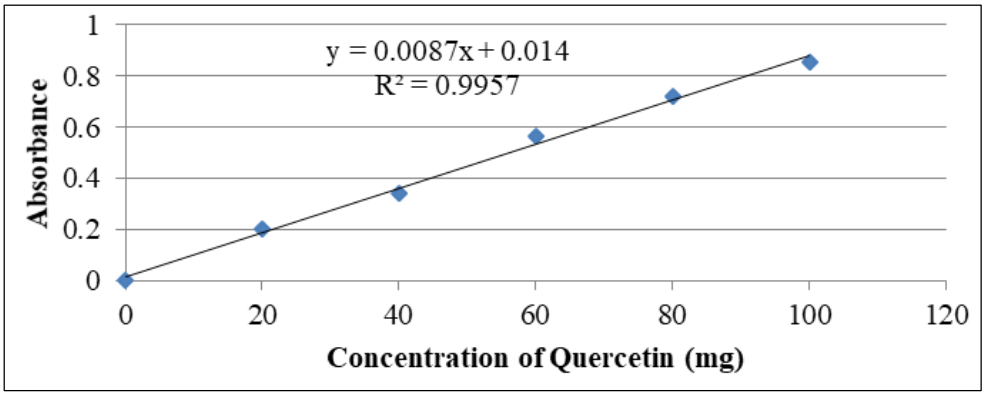

Fig 2: Calibration curve of Quercetin

Determination of total tannin content

The total tannins were determined by Folin - Ciocalteu method. About 0.5 ml of the sample extract was added to a test tube (15 ml) containing 5 ml of distilled water and 2.5 ml of Folin -Ciocalteu phenol reagent, 5 ml of 35 percent sodium carbonate (Na_2CO_3) solution and dilute to 2 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Absorbance for test and standard solutions were measured against the blank at 700 nm spectrophotometer. The tannin content was expressed in terms of mg of tannic acid /100 g of extract (Afify *et al*., 2012) [2].

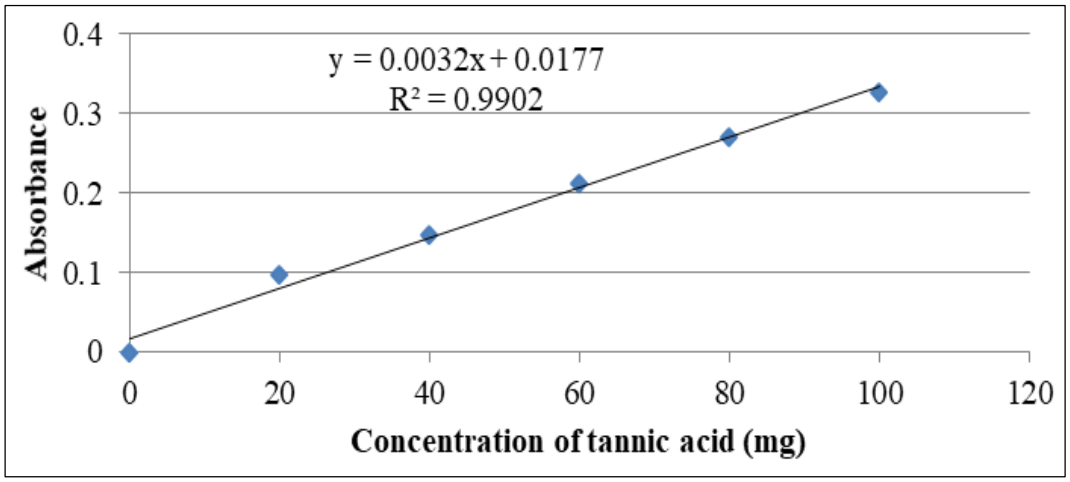

Fig 3: Calibration curve of tannic acid

Determination of total alkaloid content

The plant extract of 0.5ml was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. Later, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 15 ml test tube and diluted to the volume with chloroform. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm spectrophotometer. The total alkaloid content was expressed as mg of (atropine) AE/100 g of extract (Fazel *et al*., 2010) [10].

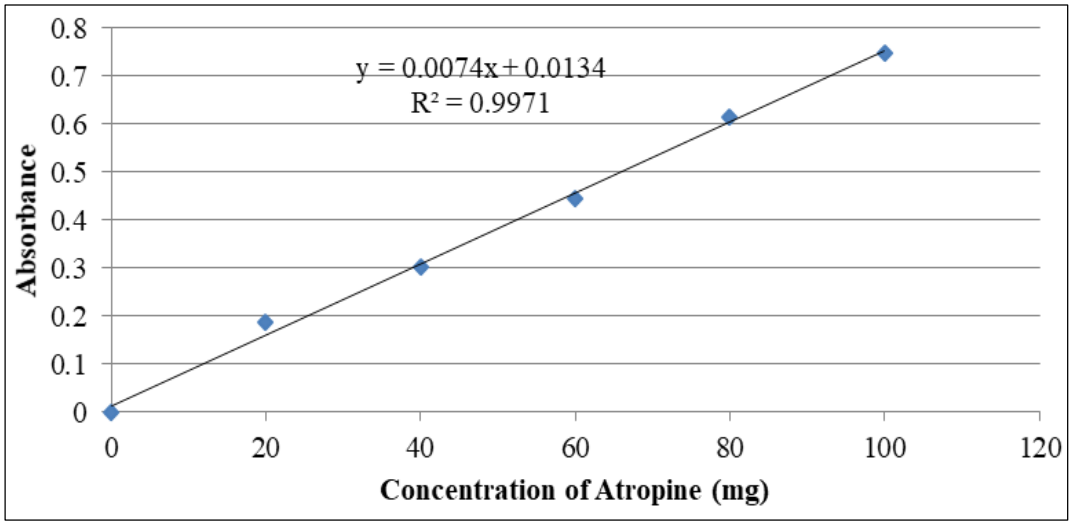

Fig 4: Calibration curve of atropine

Estimation of protein content

The Lowry *et al*. (1951) method was used to quantitatively determine the total protein content in all samples.

Reagents

1. Reagent A: 2 percent sodium carbonate in 0.1 N NaOH (sodium hydroxide)
2. Reagent B: 1 percent solution of copper sulphate in distilled water
3. Reagent C: 2 percent solution of Na-K tartarate
4. Reagent D: Is freshly prepared prior to use by mixing B and C in a 1:1 ratio
5. Reagent E: Prepared by adding 1 ml of reagent D to 50 ml of reagent A
6. Reagent F: 1 N Folin-ciocalteau reagent.

Procedure

To one ml of suitably diluted protein sample 5 ml of reagent E was added, mixed well and was kept at room temperature for 10 minute. Then, 0.5 ml of reagent F was added and the content was mixed immediately on the vortex mixture. After 30 min, absorbance was read at 750 nm. The equivalent amount of protein was calculated from a standard curve prepared by using bovine serum albumin (2 mg BSA/ml). The protein content is expressed as mg/g.

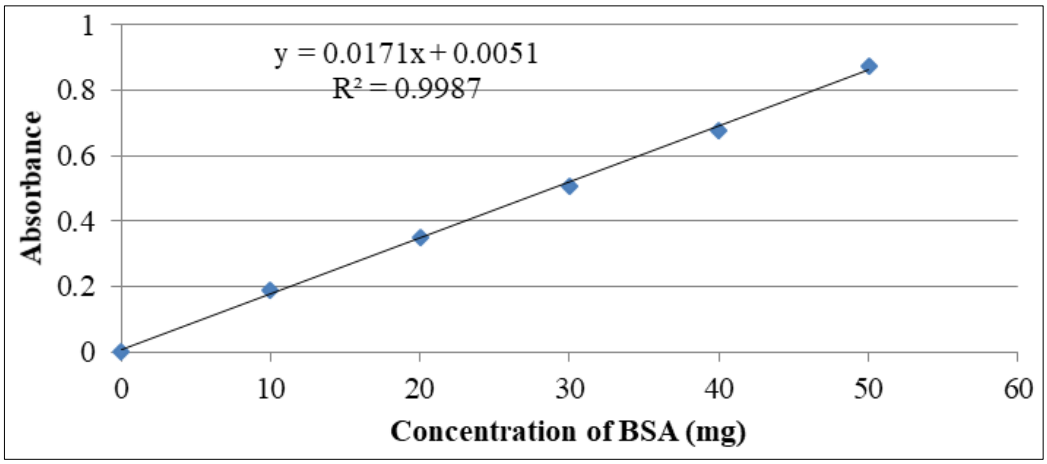

Fig 5: Calibration curve of BSA

Results and Discussion

Total phenols

Phenolic compounds are the main constituents of many plant species and play an important role in the regulation of plant growth and development. These compounds have a variety of functions in plants like defense mechanism against microbial attack through phytoalexins, lignification, and control of enzyme, auxin-activity and cell-wall synthesis. During the present studies, the total phenolic contents were examined at different genotypes of fenugreek seeds. Phenol content in seeds varied from 40.84 mg/100 g to 71.80 mg/100 g with a mean of 53.95 mg/100 g. Among selected genotypes, the maximum phenol content found in Kasuri methi (71.80 mg/100 g) followed by RMT-305 (59.35 mg/100 g) and AFG-1 (43.80 mg/100 g) whereas lowest level was observed in Pusa Early bunching (40.84 mg/100 g) these are depicted in table 1 and figure 6. The results indicate that seeds containing high phenolic may provide a source of dietary anti-oxidants (Jignesh *et al.*, 2015 [14]; Al-Maamari *et al.*, 2016[4]; Sahu *et al.*, 2022) [28].

Total flavonoids

Flavonoid has been reported to have an inherent ability to modify the body's reaction to allergen and they show anti-allergic, anti-inflammatory as well as anthelmintic activity (Siddhartha *et al.*, 2009) [30]. The content of flavonoids was expressed in terms of quercetin equivalent mg of QE/100 g of extract. In our study the results revealed that highest flavonoid content was found in Kasuri methi (90.28 mg/100 g) followed by RMT-305 (81.54 mg/100 g) and AFG-1 (81.26 mg/100 g) whereas lowest level was observed in Pusa Early bunching (72.92 mg/100 g) these are depicted in table 1 and figure 6. The results were in similar with results of Sagwan *et al.* (2010) [27], Singh *et al.* (2010) [31].

Total tannins

Plant tannins are a broad, diversified category of polyphenolic chemicals that are present in many different plant species. Tannins serve as a protective element in the bark of a plant's roots, stems, or other exterior layers. They have a high polyphenol content, which gives them an astringent quality. This characteristic grants the capacity to solidly combine with proteins, carbohydrates, and other macromolecules (Hattenschwiler *et al.*, 2000) [13]. The values obtained for the concentration of tannin contents are expressed as mg of TA/100 g of extract. Among selected genotypes, Kasuri methi found the highest tannin content (244.53 mg/100 g) followed by RMT-305 (220.77 mg/100 g) and AFG-1 (219.99 mg/100 g) whereas lowest level was observed in Pusa Early bunching (197.34 mg/100 g) these are depicted in table1 and figure 6. The results were in similar with results of Al-Maamari *et al.* (2016) [4] and Sahu *et al.* (2022) [28].

Total alkaloids

The presence of alkaloids might be associated with medicinal uses and thus the plant is reported as a potential source of some useful drugs. The alkaloid contents were expressed in terms of atropine (AE) equivalent as mg of AE/100 g. Among selected genotypes, the maximum alkaloid level found in Kasuri methi (42.64 mg/100 g) followed by RMT-305 (30.41 mg/100 g) and Pusa Early bunching (22.29 mg/100 g) whereas lowest level was observed in AFG-1 (20.60 mg/100 g) these are depicted in table 1 and figure 6. The results were in similar with results of Singh *et al.* (2010) [31], Abdouli *et al.* (2014) [1], Al-Maamari *et al.* (2016) [4], Burham (2017) [6].

Total protein

Proteins are biochemical compounds consisting of one or more polypeptides typically folded into a globular or fibrous form, facilitating a biological function. Proteins are the chief actors within the cell, said to be carrying out the duties specified by the information encoded in genes. Protein contents have been estimated in several medicinal plant species.

Proteins are the primary components of living things. The presence of higher protein levels in the plant points towards their possible increase in food value or that a protein base bioactive compound could also be isolated in the future.

Among selected fenugreek genotypes, the maximum protein level found Kasuri methi (55.10 mg/g) followed by RMT-305 (47.85 mg/g) and Pusa Early bunching (25.12 mg/g) whereas lowest level was observed in AFG-1 (20.20 mg/g) these are depicted in table 1 and figure 7. The current study results were agreement with results of Rao and Sharma (1987) [24], Naidu *et al.* (2010) [20], Kalidass *et al.* (2012) [15], Rasheed *et al.* (2015) [25], Bhagyawant *et al.* (2015) [5], Rawani (2022) [26] and Sahu *et al.* (2022) [28].

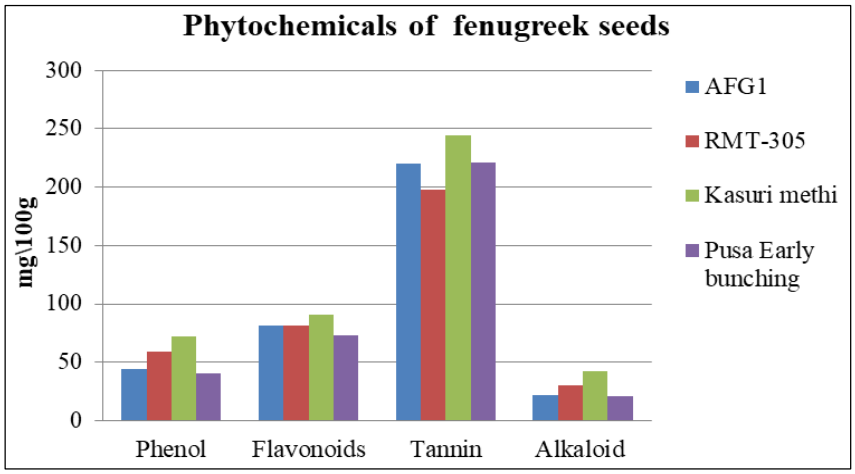

Fig 6: Determination of phytochemicals in seeds of fenugreek genotypes

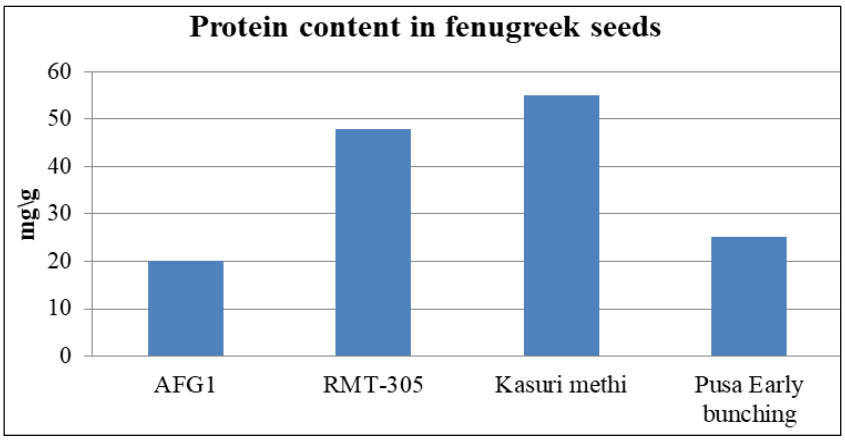

Fig 7: Determination of total protein content in seeds of fenugreek genotypes

Table 1: Total phenols, flavonoids, tannins, alkaloids and protein contents of ethanol extracts of the seeds of selected fenugreek genotypes

SL. No.	Genotypes	Phenol (mg/100 g)	Flavonoid (mg/100 g)	Tannin (mg/100 g)	Protein (mg/g)	Alkaloid (mg/100 g)
1.	AFG1	43.80	81.26	219.99	20.20	22.29
2.	RMT-305	59.35	81.54	197.34	47.85	30.41
3.	Kasuri methi	71.80	90.28	244.53	55.10	42.64
4.	Pusa Early bunching	40.84	72.92	220.77	25.12	20.60
	Mean	53.95	81.50	220.66	37.07	28.98
	Range	40.84	72.92	197.34	20.20	20.60
		71.80	90.28	244.53	55.10	42.64
	C.V.	1.30	0.80	0.80	0.99	2.00
	S.Em±	0.35	0.32	0.88	0.18	0.29
	CD@ 1%	1.08	1.00	2.73	0.56	0.89

Conclusion

The present study revealed that genotype of Kasuri methi and RMT-305 contains high content of phenols, flavonoids, tannins, alkaloids and protein contribute to the antioxidant activity and is considered to be great potential source of antimicrobial principles. Natural substances which are part of daily diet nutritional supplement with antimicrobial property constitute a new source of herbal drugs. The antimicrobial potential of this plant is a remarkable relevance as fenugreek seeds are edible and generally consumed and no extra processing is needed for its administration. Further isolation and exploration on the isolated chemical constituents on antimicrobial activity may lead to chemical entities for clinical application.

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