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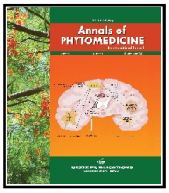

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Proximate and mineral analysis of *Trigonella foenum-graecum* L. (Fenugreek) seeds and leaves of variety HM444

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Abstract

The history of herbs and spices is as long as the history of mankind. These plants have been used by humans since the dawn of time. Spices and herbs have been effectively utilized in the indigenous systems of medicine and food in India. India has often been referred to as the medicinal garden of the world as nature has bestowed our country with an enormous wealth of medicinal plants. Annual herb, *Trigonella foenum-graecum* L. is locally known as "Methi". Fenugreek is well known for its gum, fiber, alkaloids, flavonoids, saponins, amino acids and volatile contents. The investigation aims to assess the proximate and mineral analysis of Fenugreek seeds and leaves of variety HM444 samples. The moisture content (%), ash (%), crude Fat (%), crude fiber (%), crude protein (%), total carbohydrates (%) and mineral content (ppb) were evaluated. Seeds had moisture content (8.96 ± 0.08%), ash (3.26 ± 0.01%), crude Fat (6.72 ± 0.01%), crude fiber (8.89 ± 0.02%), crude protein (27.99 ± 0.01%) and total carbohydrates (44.15 ± 0.09%) while leaves had moisture content (10.26 ± 0.16%), ash (8.72 ± 0.19%), crude fat (4.36 ± 0.31%), crude fiber (7.95 ± 0.01%), crude protein (36.66 ± 0.27%) and total carbohydrates (32.07 ± 0.07%). Seeds and leaves of Fenugreek of variety HM444 were found to be rich source of phytochemicals and minerals. Therefore, these had good nutritional and medicinal value.

1. Introduction

Medicinal plants contain phytochemicals that are found in various parts of plants, including leaves, fruits, seeds, flowers, stems, and roots (Goel *et al*., 2022; Kumari *et al*., 2022). Alkaloids, flavonoids, tannins, phenolics, saponin, steroids, glycosides, terpenes, and other phytochemicals are naturally occurring secondary metabolites generated from primary metabolites that give therapeutic benefits (Kabera *et al*., 2014; Devi *et al*., 2020; Aggarwal *et al*., 2022). These are an important component of Ayurvedic rasayans and Unani treatments (Sharma *et al*., 2008; Goel *et al*., 2022). Annual herb *T. foenum-graecum* commonly known as Methika (Sanskrit), Greek hay, Fenugreek (English), Kasuri methi, Methi, Sag methi (Hindi), Methi (Marathi) belongs to family Fabaceae. Fenugreek is both a spice and medicinal plant, used from times immemorial by human beings as spices and against various ailments and diseases. It is grown as a semi-arid crop all over the world (Brar *et al*., 2013, Moond *et al*., 2023). It is well known for its miraculous medicinal properties. Fenugreek works as an antidiabetic, anticarcinogenic, antioxidant, antibacterial agent, gastric stimulant, and anti-anorexia agent (Yadav *et al*., 2011).

Minerals are essential for both humans and plants for growth and development (Suman *et al*., 2022; Nehra *et al*., 2023). Both humans and plants need to consume enough of each of the important minerals, although the amount required for various microminerals and macrominerals vary depending on the species and individual organisms (Barroso *et al*., 2009). Plants are much more significant source of minerals for humans than animal products are, as they contain nearly all of the minerals thought to be necessary for human nutrition (Gupta and Gupta, 2014). But, each mineral is important for a variety of biochemical processes in plants. The amount of minerals in plants depends on various factors such as soil fertility, soil composition, root-soil interface, translocation of minerals and environmental conditions (Welch, 1999).

The present study was aimed to analyse proximate composition and mineral content in *T. foenum-graecum* (Fenugreek) seeds and leaves of variety HM (Hisar Mukta) 444. Both microminerals, *i.e.,* iron (Fe), copper (Fe), zinc (Zn), manganese (Mn), nickel (Ni), boron (B) and macrominerals, *i.e.,* sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) were analysed using ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

2. Materials and Methods

2.1 Sample collection

Seeds and leaves of Fenugreek (variety HM 444) were procured from the field of Chaudhary Charan Singh Haryana Agricultural University, Hisar.The collected sample was verified by Dr.Anita, Assistant Professor, Department of Botany and Plant physiology, CCS HAU, Hisar, India by using online platform (Tropicos & IPNI). The voucher specimens were verified by Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding,

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CCS HAU Hisar by voucher specimen number 20. The proposed study was conducted in Department of Chemistry, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

2.2 Sample preparation

The procured plant seeds and leaves were dried under shade, grinded into fine powder and stored in airtight containers for analysis.

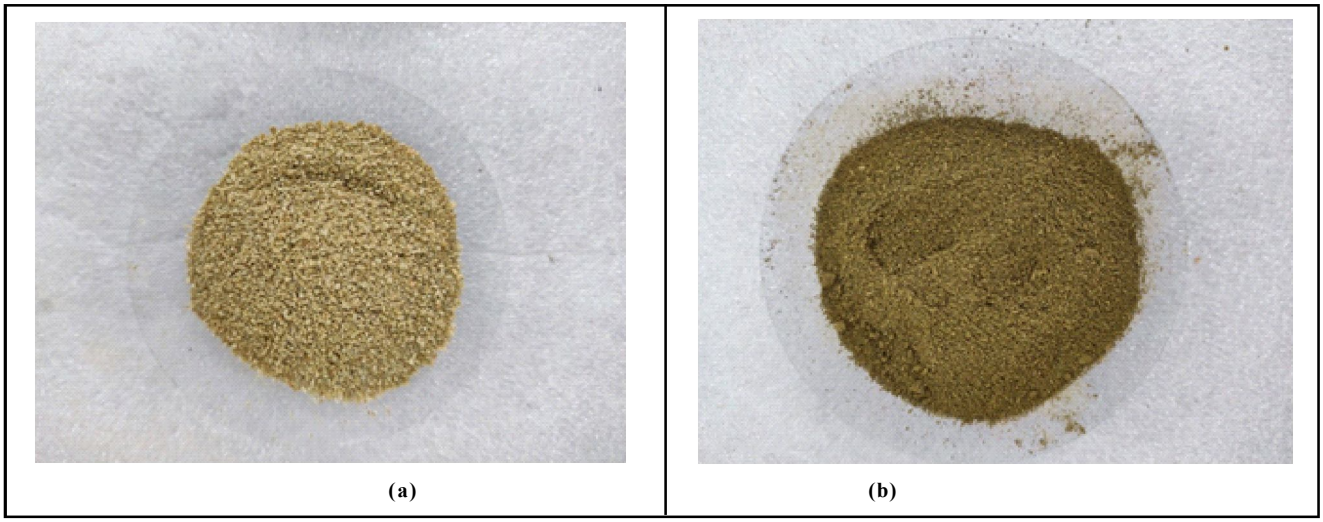

Figure 1: Seeds (a) and Leaves (b) of Fenugreek (HM 444) in dry powdered form.

2.3 Proximate analysis

The proximate analysis (moisture, ash, crude fat, crude fiber, crude protein and total carbohydrates) were determined in triplicates as per standard techniques of Association of Official Analytical Chemists (AOAC).

2.3.1 Estimation of moisture content

Moisture content in Fenugreek seeds and leaves was determined by the Association of Official Analytical Chemists (AOAC, 1995) method. Three replicates of Fenugreek seeds and leaves powder (2 g) was taken and were air dried first under shade, then in an oven, first at 85-90°C, then at 100-105°C. Dry sample weights were recorded at regular intervals until a constant weight was obtained. The moisture content (%) was calculated by using the formula:

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh leaves} - \text{Weight of dried leaves}}{\text{Weight of fresh leaves}} \times 100$$

2.3.2 Estimation of ash content

Three replications of 2 g of Fenugreek powdered samples of seeds and leaves were weighed, placed into a crucible that had already been heated, and then placed in a muffle furnace for 2 h at 750°C.The samples were then allowed to cool in desiccator while their weight was recorded. Ash content (%) was calculated as follows:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.3.3 Estimation of crude fat

A completely dried empty thimble was weighed and 2 g oven-dried sample of Fenugreek seeds and leaves was taken in a thimble. Soxhlet extraction was performed with petroleum ether for 8 h. After extraction, thimble was taken out and oven-dried after drying at room temperature. The dried thimble was weighed once again. The crude fat content (%) was evaluated:

$$\text{Crude fat content (\%)} = \frac{\begin{array}{c}(\text{Weight of empty thimble} + \text{sample}) - \\ (\text{Weight of dried thimble after exraction})\end{array}}{\text{Weight of sample}} \times 100$$

2.3.4 Estimation of crude fiber

Crude fiber was evaluated by Maynard (1970) method with some suitable modifications.

2 g of moisture and fat free powdered sample of Fenugreek seeds and leaves were weighed and placed in a one litre spoutless beaker. 200 ml of 1.25% (w/v) sulphuric acid was poured in it. The beaker was then put on a hot plate for refluxing, which took 30 min (counted from the start of boiling), with constant shaking every 5 min. Prepared extract was filtered through a muslin cloth using suction after 30 min. The material was transferred to the same beaker and the contents were once more refluxed for 30 min with 200 ml of 1.25% NaOH solution after being cleaned of acid by washing the residues with hot water. With the help of vacuum pump, it was filtered again through muslin cloth and the residue was washed with hot water to make it free from alkali and placed into a crucible in a heated air oven at 80-110°C till constant weight achieved. Weight was recorded and finally residue was ignited in muffle furnace for 2-3 h at 550-660°C, then cooled, and the weight of the crude fiber which represents the weight lost as a result of ignition was estimated.

$$\text{Crude fiber content (\%)} = \frac{\text{Weight of crude fiber}}{\text{Weight of sample}} \times 100$$

2.3.5 Estimation of crude protein content

Nitrogen was estimated by Kjeldahl's method described in the AOAC (Association of Official Analytical Chemists) (Horwitz *et al*., 1970).

Percentage of nitrogen is multiplied by the factor 6.25 to obtain crude protein content. 1 g sample of Fenugreek seeds and leaves was transferred to 250 ml digestion tube. 8 g of catalytic mixture and 12 ml conc. H_2SO_4 was added to the digestion tubes, shake it gently to wet the sample. Digestion of sample was done until clear blue or green colour was observed (about 60 min), followed by cooling for 10 to 20 min after that dilution of digested sample with 80 ml of distilled water and 50 ml of 40 % NaOH was done. 25-30 ml of 4% boric acid solution was taken in receiver flask. Distillation was performed automatically. The NH_3 gas liberated from distillation was trapped in receiver flask containing 4 % boric acid solution with indicator. The distillate thus collected was titrated against standard 0.1 N HCl. Similarly, a blank was run and % of nitrogen content in the sample was calculated:

$$\% \text{ nitrogen} = \frac{(T - B) \times N \times 14.007}{\text{Weight of sample}} \times 100$$

T = titration volume for sample (ml)

B = titration volume for blank (ml)

N = normality of titrant

% protein = N × F

F = conversion factor for nitrogen to protein (6.25)

2.3.6 Total carbohydrates

Total carbohydrates content was calculated by difference as follows:

Total carbohydrates content (%) = 100 – [Moisture (%) + Ash (%) + Crude fat (%) + Crude fiber (%) + Crude protein (%)]

2.4 Minerals analysis

Minerals content was estimated by using the ICP-MS technique (Kýlýç Altun *et al*., 2017).

Following microwave-assisted acid digestion, mineral contents of Fenugreek seeds and leaves were examined using inductively coupled plasma mass spectrometry (ICP-MS). All glassware was first cleaned for a day with a 10% (v/v) HNO_3 solution, and then it was rinsed with ultrapure water. In PTFE vessels using a microwave system, 0.2 g of each sample were digested with 5.0 ml of HNO_3 acid and 2 ml of H_2O_2. Similarly, blank digest was carried out. In microwave, the following digestion conditions were used: 120°C for 15 min, then at a constant temperature for 10 min; 170°C for 20 min; and then, after cooling to 22°C for 20 min, diluting to 50 ml with deionized ultrapure water. Thus, using an ICP-MS with a concentric nebulizer, a quartz torch with a quartz injector tube, and a cyclonic spray chamber; this solution was employed for the micronutrient analysis. Micronutrient levels were assessed in samples of fenugreek seeds and leaves.

2.5 Statistical analysis

For statistical analysis, triplicates of each sample were performed and the obtained values were represented as mean ± standard error (S.E.). Online atatistical analysis (OPSTAT) was used for one-way and two-way analysis of variance (ANOVA) to evaluate any significant differences among the mean values of data.

3. Results and Discussion

3.1 Proximate analysis

The proximate composition data of Fenugreek seeds and leaves of variety HM 444 is reported in Table 1.

3.1.1 Moisture content

The statistics of moisture content in Fenugreek seeds and leaves of variety HM 444 was given in Table 1. Moisture content in seeds and leaves was 8.86% and 10.26%, respectively. Statistical analysis of data revealed that the difference between mean value of seeds and leaves was more than CD (%) which indicates that moisture content differs significantly among the plant parts used.

3.1.2 Ash content

The ash content was found to be higher in leaves (8.72 %) as compared to seed (3.26%). Statistical analysis of data revealed that the difference between mean value of seeds and leaves was more than CD (%) which implicates that ash content varies significantly among the plant part investigateds.

3.1.3 Crude fat

The crude fat content was found to be higher in seeds (6.72 %) as compared to leaves (4.36 %). Statistical analysis of data revealed that the difference between mean value of seeds and leaves was greater than CD (%) which implicates that crude fat content varies significantly among the plant parts used.

3.1.4 Crude fiber

The crude fiber content was found to be higher in seeds (8.89 %) as compared to leaves 7.95 %. Statistical analysis of data revealed that the difference between mean value of seeds and leaves was more than CD (%) which implicates that crude fiber content (%) varies significantly among the plant parts used.

Table 1: Proximate composition in Fenugreek seeds and leaves (variety HM444) dry powder

S.No.	Plant parts	Moisture content (%)	Ash (%)	Crude fat (%)	Crude fiber (%)	Crude protein (%)	Total carbohydrates (%)
1.	Seed	8.96 ± 0.08	3.26 ± 0.01	6.72 ± 0.01	8.89 ± 0.02	27.99 ± 0.01	44.15 ± 0.09
2.	Leaves	10.26 ± 0.16	8.72 ± 0.19	4.36 ± 0.31	7.95 ± 0.01	36.66 ± 0.27	32.07 ± 0.07
SE (m)		0.13	0.14	0.22	0.01	0.20	0.08
CD at 5%		0.52	0.55	0.89	0.06	0.79	0.32
CV %		2.33	3.93	6.89	0.28	1.05	0.36

3.1.5 Crude protein

The crude protein was found to be higher in leaves (36.66%) than seeds (27.99 %). Statistical analysis of data revealed that the difference between mean value of seeds and leaves was more than CD (%) which implicates that crude protein (%) varies significantly among the plant parts used.

3.1.6 Total carbohydrates

Total carbohydrates were higher in seeds (44.15 %) as compared to leaves (32.07 %). Statistical analysis of data revealed that the difference between mean value of leaves and seeds was more than CD (%) which implicates total carbohydrates varies significantly between the plant parts used.

Table 2: Trends in proximate composition

Parameters	Trends
Moisture content	Leaves > Seeds
Ash content	Leaves > Seeds
Crude fat content	Seeds > Leaves
Crude fiber content	Seeds > Leaves
Crude protein content	Leaves > Seeds
Total carbohydrates	Seeds > Leaves

The trends in proximate composition were reported in Table 2. According to Table 1 data, the moisture content, ash content and crude protein content were higher in leaves as compared to seeds whereas crude fat, crude fiber and total carbohydrates were higher in seeds as compared to leaves. Figure 2 represents comparison of proximate composition in Fenugreek seeds and leaves (variety HM444) dry powder.

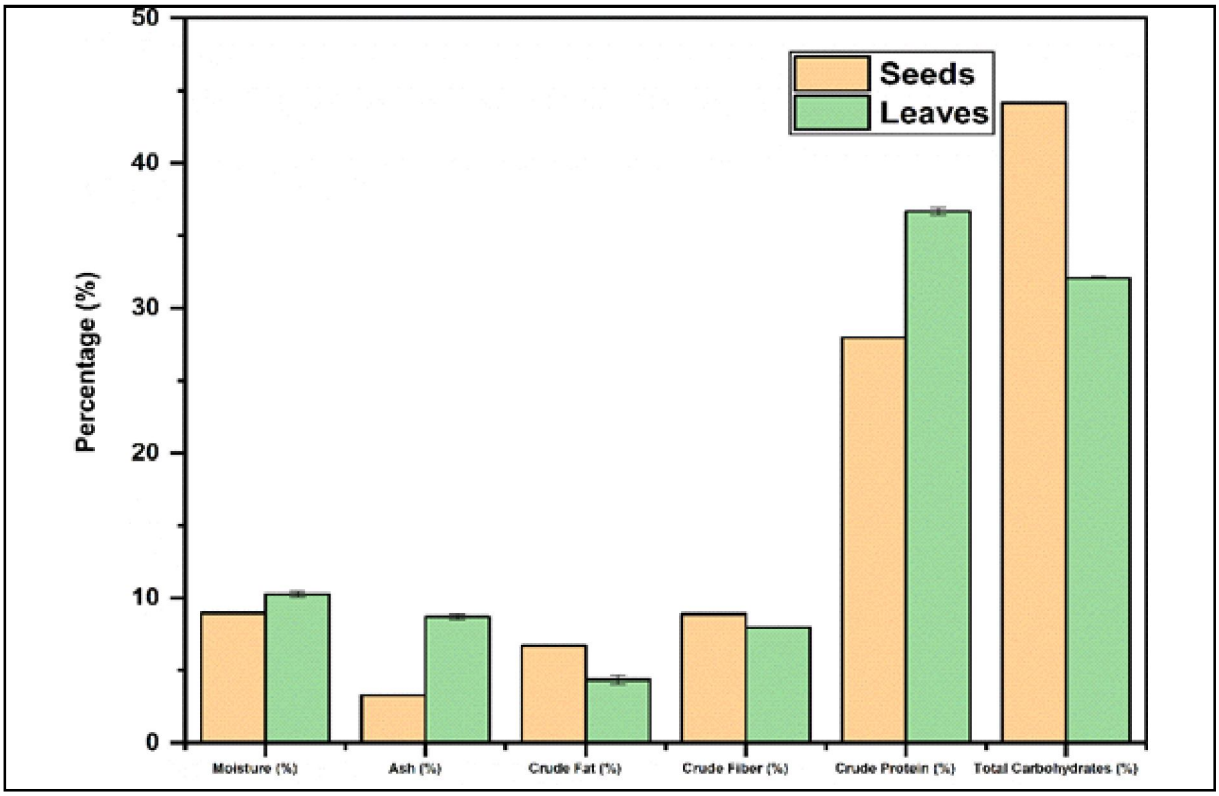

Figure 2: Comparison of proximate composition in Fenugreek seeds and leaves (variety HM444) dry powder.

The difference of proximate composition might be attributed to the conditions in which medicinal plant was harvested along with environmental factors.

3.2 Minerals analysis

3.2.1 Microminerals

The data of microminerals in Fenugreek seeds and leaves of variety HM 444 is reported in Table 3.

Iron (Fe) content

The data of Fe content in Fenugreek seeds and leaves of variety HM 444 was given in Table 2.

The Fe content in Fenugreek seeds and leaves was 42.56 ppb and 54.93 ppb, respectively. Fe content was higher in leaves as compared to seeds.

Copper (Fe) content

The Cu content in Fenugreek seeds and leaves was 1.13 ppb and 1.41 ppb, respectively. Cu content was found to be higher in leaves as compared to seeds.

Zinc (Zn) content

The Zn content in Fenugreek seeds and leaves was 114.04 ppb and 1265.92 ppb, respectively**.** Leaves had higher amount of Zn than seeds.

Manganese (Mn) content

The Mn content in Fenugreek seeds and leaves was 0.73 ppb and 3.65 ppb, respectively. Mn content was found to be higher in leaves as compared to seeds.

Nickel (Ni) content

The Ni content in Fenugreek seeds and leaves was 2.26 ppb and 1.55 ppb, respectively. Ni content as found to be higher in seeds as compared to leaves.

Boron (B) content

The B content in Fenugreek seeds and leaves was 45.50 ppb and 17.45 ppb, respectively. Leaves had higher amount of boron as compared to seeds.

Statistical analysis of data revealed that the difference between mean value of seeds and leaves was more than CD (%) which implicates that microminerals varies significantly among the plant parts used.

Table 3: Microminerals in Fenugreek seeds and leaves (variety HM444)

S.No.	Plant parts	Microminerals (ppb)					
		Fe	Cu	Zn	Mn	Ni	B
1.	Seed	42.56 ± 0.65	1.13 ± 0.01	114.04 ± 0.91	0.73 ± 0.01	2.26 ± 0.07	45.50 ± 0.67
2.	Leaves	54.93 ± 0.96	1.41 ± 0.06	1265.92 ± 3.85	3.65 ± 0.02	1.55 ± 0.05	17.45 ± 1.55
SE (m)		0.82	0.47	2.8	0.01	0.06	1.35
CD at 5%		3.29	0.19	11.28	0.06	0.25	5.44
CV %		2.90	10.65	0.70	1.13	5.61	0.07

Table 4: Trends in microminerals

Microminerals	Trends
Iron (Fe) content	Leaves > Seeds
Copper (Cu) content	Leaves > Seeds
Zinc (Zn) content	Leaves > Seeds
Manganese (Mn) content	Leaves > Seeds
Nickel (Ni) content	Seeds > Leaves
Boron (B) content	Seeds > Leaves

The trends in microminerals were reported in Table 4. According to Table 3 data, the Iron (Fe) content, Copper (Cu) content, Zinc (Zn) content and Manganese (Mn) content were higher in leaves as compared to seeds whereas Nickel (Ni) content and Boron (B) content were higher in seeds as compared to leaves. Figure 3 represents comparison of microminerals in Fenugreek seeds and leaves (variety HM444).

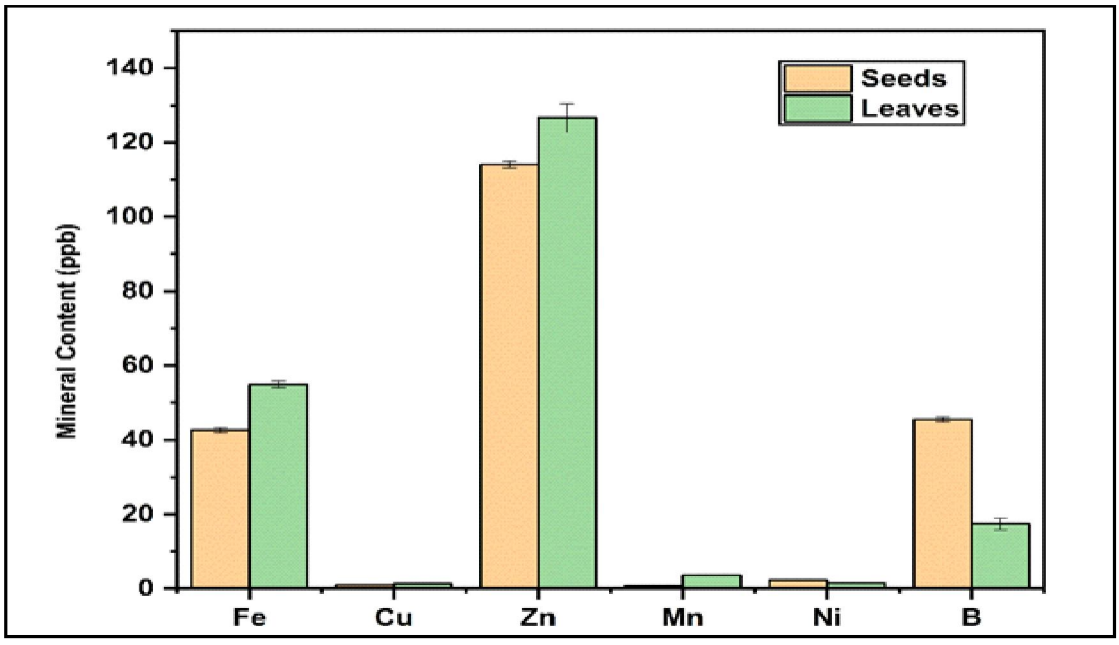

Figure 3: Comparison of microminerals in Fenugreek seeds and leaves (variety HM444).

3.2.2 Macrominerals

The data of macrominerals in Fenugreek seeds and leaves of variety HM 444 is reported in Table 5.

Sodium (Na) content

The data of Na content in Fenugreek seeds and leaves of variety HM 444 was given in Table 3.

The Na content in Fenugreek seeds and leaves was 4695.63 ppb and 3217.79 ppb, respectively. Na content was higher in seeds as compared to leaves.

Potassium (K) content

The K content in Fenugreek seeds and leaves was 4444.61 ppb and 2287.10 ppb, respectively. K content was higher in seeds as compared to leaves.

Magnesium (Mg) content

The Mg content in Fenugreek seeds and leaves was 996.49 ppb and 1160.50 ppb, respectively. Leaves had higher amount of Mg than seeds.

Calcium (Ca) content

The Ca content in Fenugreek seeds and leaves was 208.14 ppb and 303.73 ppb, respectively. Leaves had higher amount of Ca contentthan seeds.

Statistical analysis of data revealed that the difference between mean value of seeds and leaves was more than CD (%) which implicates that macrominerals varies significantly among the plant parts used.

Table 5: Macrominerals in Fenugreek seeds and leaves (variety HM444)

S.No.	Plant parts	Macrominerals (ppb)			
		Na	K	Mg	Ca
1.	Seed	4695.63 ± 6.68	4444.61 ± 2.34	996.49 ± 0.61	208.14 ± 0.81
2.	Leaves	3217.79 ± 6.67	2287.10 ± 1.32	1160.50 ± 1.19	303.73 ± 1.09
SE (m)		6.67	1.76	0.96	0.96
CD at 5%		3.08	7.12	3.88	3.88
CV %		26.92	0.09	0.15	0.65

Table 6: Trends in macrominerals

Macrominerals	Trends
Sodium (Na) content	Seeds > Leaves
Potassium (K) content	Seeds > Leaves
Magnesium (Mg) content	Leaves > Seeds
Calcium (Ca) content	Leaves > Seeds

The trends in macrominerals were reported in Table 6. According to Table 5 data, magnesium (Mg) content and calcium (Ca) content were higher in leaves as compared to seeds whereas sodium (Na) content and potassium (K) content were higher in seeds as compared to leaves. Figure 4 represents comparison of macrominerals in Fenugreek seeds and leaves (variety HM444).

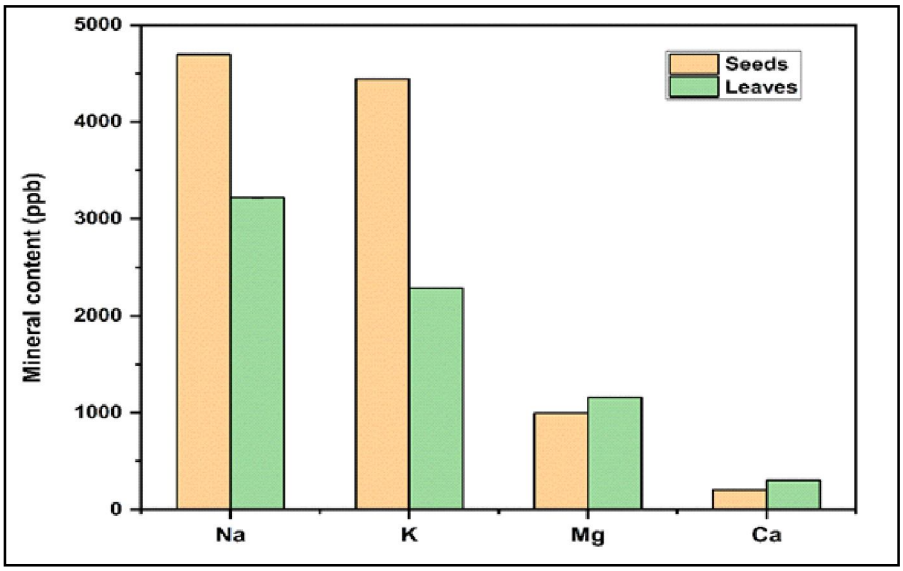

Figure 4: Comparison of macrominerals in Fenugreek seeds and leaves (variety HM444).

4. Discussion

The mineral content of medicinal plant species showed considerable variation among different micronutrients. However, for some parts of plant, higher concentration was recorded which may be due to prevailing environmental, soil conditions and season when the plant was collected for analysis (Bhatla *et al*., 2018). A variety of minerals are needed by the human body to maintain optimal health. As a plant accumulates minerals necessary for growth from the environment, a variety of minerals vital to human nutrition are accumulated in various parts of the plant. For numerous enzyme systems to operate normally, zinc is a necessary metal. Zn deficiency, particularly in youngsters, can result in an appetite loss, slow growth, weakness, and even delayed sexual development. Dietary iron intake must be adequate to reduce the prevalence of anaemia (Subramanian *et al*., 2012).Therefore, these elements play a very important role in the formation of the active chemical constituents present in plant and these are also responsible for their medicinal properties.

5. Conclusion

Different parts of Fenugreek (variety HM 444), namely; seeds and leaves are analysed in order to get some useful informationto be used in the preparation of therapeutic and nutraceutical foods. The present study revealed that the Fenugreek seeds and leaves of variety HM 444 are good source of minerals that are crucial for biochemical processes. From findings it can be concluded that fenugreek seeds and leaves are economicsource of nutrients that can be utilised as a food supplement for humans which includes fats, fiber, protein and carbohydrates.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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