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Nutraceutical potential of Methi (*Trigonella foenum-graecum* L.) and Kasuri methi (*Trigonella corniculata* L.)

Vani Pasricha, Rajinder K Gupta

Abstract
The dried samples of methi leaves, stems (*Trigonella foenum-graecum* L.) and Kasuri methi *(Trigonella corniculata* L.) (Fabaceae family) were investigated for their potential for use as nutraceuticals. The former is commonly known as fenugreek while the latter is renowned as cultivated fenugreek. Thorough characterization of the both the samples demonstrated their capability as rich sources of energy, natural antioxidants, polyphenols and minerals. The methi leaves and kasuri methi showed to be good sources of protein. While both the samples are excellent sources of Calcium, Magnesium, Potassium, Phosphorus and moderate sources of Zinc, kasuri methi has a slightly richer amount of iron as compared to methi leaves. The samples revealed good amounts of alkaloids followed by moderate amounts of saponins. Total phenolics and flavonoids contents were obtained for methanolic and aqueous solvent extracts for each of the samples Antioxidant activity of the extracts was estimated using ABTS scavenging assay and FRAP assay. The methanolic extracts showed good antibacterial activity in *B. subtilis*, *E. coli and P. mirabilis.* GC-MS screening and flavour analysis showed the presence of several useful compounds indicating that the samples hold the potential to be stated under "nutraceuticals".

Keywords: Methi, Kasuri methi, Nutritional, Phytochemicals, Antioxidant, Antimicrobial, GC-MS, Flavor.

1. Introduction
Fruits and vegetables provide a small part of the daily caloric intake of our diet, along with certain substances that possess antioxidant capacity, for example, vitamin C (ascorbic acid), carotenoids polyphenols etc. Plants produce a diverse range of bioactive molecules, which allow them to be the sources of different pharmaceutical products [1]. Studies over the past decades have witnessed an increasing interest in the protective biochemical functions of these phytochemicals for the prevention of health damage to human beings [2]. In order to protect itself against free radicals, an organism has various antioxidant defense mechanisms, including endogenous, which include catalase, glutathione peroxidase/ reductase as well as exogenous including, vitamin C, E and beta carotene [3]. But these mechanisms solely are not sufficient in critical situations like oxidative stress, contamination, UV exposure etc. where there is a significantly increased production of free radicals [4]. Several recent epidemiological studies have shown a remarkable positive correlation between the consumption of fruit and vegetables and decreased incidences of diseases like coronary heart disease, aging, cancer and Alzheimer's disease [5]. Much before the discovery of vitamin C in 1932, experts recognized that something in citrus fruits could prevent scurvy, a disease that was responsible for killing about 2 million people between 1500 and 1800 [6]. Studies indicate that a diet rich in antioxidant phytochemicals, such as poly-phenolics, carotenoids, flavonoids and terpenoids protects against cellular damage due to its potency to scavenge oxygen-derived free radicals [7, 8, 9]. Flavonoids and phenolic compounds are widely distributed in plants and are reported to show various biological effects, including anti-inflammatory, anti-carcinogenic activities etc. Various researches have also suggested a role of flavonoids as potential iron chelators [10, 11]. The association between the anti-oxidative properties of food and health has been extensively investigated over the years. Natural antioxidants are also in high demand for application as nutraceuticals/functional foods and bio-pharmaceuticals because of consumer preferences [12].
Fenugreek (*Trigonella foenum-graecum* L.) commonly known as methi, is a self-pollinating, leguminous crop native to the Indian subcontinent and the Eastern Mediterranean region [13].

It is currently widely cultivated in central Asia, central Europe, northern Africa, North America and parts of Australia, with India being the leading fenugreek producer in the world [14]. It belongs to the family Leguminosae. The genus name Trigonella, has the Latin meaning 'little triangle', owing to the triangular shape of its flowers. The species '*foenum-graecum'* means 'Greek hay' [15]. It is also known as 'ox horn' or 'goat horn' because its two seed pods project in opposite directions from the nodes of the stem base and resemble an ox or goat horns [13]. A study on the quantification of phytochemicals in different extracts of seeds, leaves and stems of methi leaves indicates that green leafy vegetables are rich sources of phytochemicals [16].

Cultivated Fenugreek (*Trigonella corniculata* L.), also known as Kasuri methi, is a small annual herb with leaflets 1-4 cm long, 8-35 mm broad, oblong-wedge-shaped and tips being notched. Its flowers bloom in 8-20 flowered clusters, a 1.5-6 cm long peduncle. The flower-stalks are 3 mm long, sepal cup 3-4 mm long, sepals nearly equal, shorter than or as long as the tube. The flowers are 6-7 mm long, yellow and wings are shorter than the keel. Dried leaves of this plant are used in Indian cooking as kasuri methi. It is the most renowned Pakistani fenugreek with a remarkable aroma and has its origin of cultivation in the Kasur district of Punjab province of Pakistan and thus has its name 'Kasuri methi'. Methi from Kasur is very famous for its fragrance throughout India and is also a geographical indicator of Kasur (Pakistan) as Kasuri Methi [17].

Methi seeds and leaves are being consumed almost all around the world owing to its several uses. Major medicinal uses of methi include its anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial activities. The Methi is also used for various culinary purposes like use in syrups, use of mixed seed powder with flour for baking of breads, curries, dyes, young seedlings eaten as a vegetable, roasted methi grain as a coffee-substitute, particularly in Africa. It has also been used for controlling insects in grain storages and perfume industries. The biological and pharmacological properties of methi are accredited to the diversity of its constituents like poly-phenolic substances, volatile constituents, amino acids, etc [18]. Despite immense health favouring characteristics, the potentials of methi and kasuri methi have not yet been fully exploited in the fields of pharmaceuticals and nutraceuticals. The present study, therefore, aims at exploring the potential of Methi and Kasuri methi for their use as nutracetuticals.

2. Materials and methods

2.1. Sample collection and extraction

Fresh samples of Fenugreek (Methi) and Cultivated Fenugreek (Kasuri Methi) were taken from a local market of Dwarka, Delhi. The samples were first washed and air dried for 24 hours. The dried leaves and stems of methi were separated and ground separately. The samples were labelled as Methi leaves (ML) and Methi stems (MS), respectively. The cultivated fenugreek leaves and stems were ground collectively and labelled as Kasuri methi (KM). 50 g of the grounded samples (Methi leaves, Methi stems and Kasuri methi) were weighed on an electronic balance and extracted with 200 ml of each, Methanol and Water. The mixtures were incubated for 24 h and then filtered. The solvents were evaporated under vacuum and resulting extracts were stored at 4 ^{o}C and were labeled ME and WE respectively for the respective samples [19].

2.2. Nutritional analysis

The powdered samples of methi leaves and Kasuri methi were analysed for their nutritional composition. Moisture content (AOAC, 1999) [20], ash content [22], protein content using a kjeldahl method [21], crude fat content (AOAC method Ref. 2003.06) [21] were evaluated for the samples. Crude fiber content was estimated (Ref. 978.10 of AOAC, 2005) [21]. Mineral contents for the two samples by ICP-OES (Ref 956.52 AOAC, 2005) [21] were also determined. Total carbohydrates and energy content were calculated using formulae:

Total carbohydrates (% fresh weight) = 100 - moisture (%) - protein content (% fresh weight) - crude fat (% fresh weight) - ash (% fresh weight) and reported as total carbohydrates in % [19].

The calorific value in kilocalories (kcal) was calculated according to the system of Atwater, namely: kcal = (3.36 × % protein fresh weight) + (3.60 × % total carbohydrate fresh weight) + (8.37 × % fat) [19].

2.3. Phytochemical analysis

The Total Phenolics content of the samples of methi leaves, stems and kasuri methi were estimated by Folin-Ciocalteu reagent method [23]. The absorbance of the standard (Gallic acid) and the respective extracts was measured spectrophotometrically at 765 nm against DMSO blank. The results were expressed as Gallic acid equivalents (GAE, µg/mg of weight of extract).

The Total Flavonoids content was determined using Aluminium chloride colorimetric method [24]. The optical density for the standard (Catechin) and the sample extracts were measured at 765 nm against DMSO blank , the total flavonoids content was expressed in µg of Catechin equivalents per mg of weight of extracts (CE, µg/mg of weight of extract).

Concentration of crude alkaloids and saponins was also estimated using the methods already described by Harborne [25] and Obadoni & Ochuko [26] respectively. The results were calculated in %.

2.4. GC-MS Profiling

Other secondary metabolites in the samples of methi leaves and kasuri methi were identified by analyzing 1µl each, of mix solvent and Petroleum ether extracts of the samples by using GC/MS analysis. Helium was used as carrier gas. An Agilent 6890 GC with 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050) for all samples. Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST'05 library [19]. The transfer line temperature was set to 300 °C, solvent delay was 3 min, ion source and quadruple temperature were 230°C and 150 °C, respectively [27]. The detected compounds have been identified by NIST'05 mass spectrum library and more than 90% matching value were reported.

2.5. Determination of antioxidant activity

The total antioxidant capacity of the extract was determined using ABTS radical scavenging activity and FRAP assay.

2.5.1. The ABTS radicals are generated through a chemical oxidation reaction with potassium persulfate. The ABTS (2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) method used was previously described by Re *et al* [28]. The $ABTS^+$ radical cation was produced by mixing ABTS and potassium persulfate, the mixture was then kept in the dark at room temperature for 16 h. For the analysis, the reagent was diluted in ethanol until the absorption at 734 nm was 0.7± 0.02. A 20 µl of extract was mixed with 980 µl of ABTS reagent. The absorption was measured in a Hewlett-Packard spectrophotometer after 6 min of the addition of the sample. Each determination was performed in triplicates. The percentage of radical scavenging activity was calculated using the formula by using Ascorbic acid as a control:

Scavenging activity (%) = (1-Absorbance sample/ Absorbance control) x 100

2.5.2. The FRAP (Ferric reducing antioxidant power) assay was carried out following the methodology of Benzie and Strain [29]. The FRAP reagent consisted of TPTZ in 40 mM HCl, $FeCl_3$ and sodium acetate buffer (pH 3.6). FRAP reagent was freshly prepared. A 100 µl of extract solution containing 0.1 mg extracts was mixed with 900 µl of FRAP reagent. The mixture was incubated at 37 °C for 4 min, the absorbance at 593 nm was then determined against blank. BHT was used as calibration standard. FRAP values were calculated as µg of BHT equivalents/g extract from three determinations and were averaged.

2.6. Antimicrobial activity

Antibacterial activities of the extracts were evaluated by agar well diffusion method against three Gram-positive bacteria and two Gram negative bacterial test pathogens [30]. Extracts were reconstituted to a final concentration of 350 mg/ml. Nutrient agar was inoculated by spreading 100 µl of the bacterial inoculums. Wells (6 mm diameter) were punched in the agar and 100 µl of extracts were loaded into the wells. The plates were then incubated at 37 ºC for 24 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and reported on the scale of millimeters.

DMSO was used as control in one of the wells.

2.7. Flavour and Fragrance Profiling

Thermal desorption system was used for profiling of volatile and semi-volatile compounds which are responsible for flavour and fragrance in the sample which uses heat and a flow of inert carrier gas to extract volatile or semi volatile organic compounds from a sorbent or other sample matrix. It is used for sample matrices that cannot be directly introduced to the GC analyzer for example, dilute vapour samples, solids, foods, drinks etc. Analysis was done using GC HP5MS column and the conditions for GC were same as mentioned in 2.4 except for the oven programming which was set from 60 ˚C (0 min) at 3 ˚C/min to 240 ˚C (6 min) at 5 ˚C/min to 280 ˚C (15 min). The compounds were eluted from GC in total ion chromatograph (TIC), which was searched against two databases: Agilent NIST'05 library developed by The National Institute of Standards and Technology, Flavor 2 developed by Agilent.

Table 1: Proximate Analysis of methi leaves

Parameter	Result
Energy	310.98 kcal
Carbohydrates	46.30%
Protein	28.45%
Fat	5.82%
Ash	13.36%
Moisture	6.07%
Dietary fiber	2.60%

Table 2: Proximate Analysis of kasuri methi

Parameter	Result
Energy	319.41 kcal
Carbohydrates	56.76%
Protein	24.06%
Fat	4.12%
Ash	9.84%
Moisture	5.22%
Dietary fiber	2.4%

Table 3: Mineral content estimation for methi leaves

Analyte	Concentration (µg/g)	Analyte	Concentration (µg/g)
Cu	9.6	Na	24098
P	3545	K	17644
Mg	4501	Fe	293
Ca	10988	Mn	36.4
As	*ND	Zn	49.6
Be	ND	Li	ND
Cd	ND	Mo	6.8
Sb	ND	Ni	1.4
Cr	2.4	V	ND
Co	ND	Pb	ND
Se	ND	Sn	ND
Sr	149	Ti	ND
T l	ND		

*ND: Not detected

3. Results and Discussion

3.1. Nutritional profiling

Nutritional studies of the samples have demonstrated their functionality as nutraceuticals (Table 1 & 2). Both the samples were found to be very rich sources of energy. The calorific value of methi leaves was estimated to be 310.98 kcal, and for kasuri methi, 319.41 kcal. The ash contents of fenugreek leaves and cultivated fenugreek were found to be 13.36% and 9.84%, respectively, and the respective moisture contents were 6.07% and 5.22% which which have an influence on the shelf stability of the samples. Fat contents were estimated to be 5.82% and 4.12% for methi leaves and kasuri methi, respectively. The total protein contents in fenugreek leaves and cultivated fenugreek were estimated to be 28.45% and 24.06%, respectively. Crude fiber was obtained in appreciable amounts in both the samples and the respective values were found to be 2.6% and 2.4%, respectively. Mineral determination by ICP-OES revealed the presence of excellent amounts of essential minerals like calcium, iron, phosphorus, potassium and magnesium which assure a direct correlation of both the samples and good health. Iron is useful in treating anemia, tuberculosis etc [31]. A high content of phosphorous can play an important role in

bone formation and other essential metabolic activities of the body. Calcium is known to play a crucial role in maintaining the rigidity of the skeleton. It is also known to have a part in blood clotting, and other metabolic processes [32]. Zinc supplementation in diabetes mellitus proved to have antioxidant effect [33]. The amounts of minerals obtained in the samples are represented in Tables (3 & 4).

Table 4: Mineral content estimation for kasuri methi

Analyte	Concentration (µg/g)	Analyte	Concentration (µg/g)
Cu	9.8	Na	15890
P	3933	K	12260
Mg	4282	Fe	318
Ca	11364	Mn	27.2
As	ND	Zn	32.7
Be	ND	Li	ND
Cd	ND	Mo	1.1
Sb	ND	Ni	1.1
Cr	2.4	V	ND
Co	ND	Pb	ND
Se	ND	Sn	ND
Sr	96	Ti	ND
T l	ND		

* ND: Not detected

3.2. Phytochemical analysis

Plant extracts containing different classes of polyphenols are very attractive in the food industry. Phytochemicals, primarily phenolics are considered to be the chief bioactive compounds for providing health benefits. Flavonoids have also been recognized to show antioxidant activity [34]. The total flavonoids content of the reconstituted extract was estimated by the aluminium chloride method and the results were expressed in µg of Catechin equivalents (CE) per mg of dry weight of the extract based on the calibration curve of the standard. The present study was used to investigate the total phenolics and the total flavonoids contents of the methi leaves, stems and kasuri methi samples.

The presence of crude alkaloids and saponins in moderate amount in the food samples divulge their therapeutic significance. A diet containing alkaloids may prove helpful for healing wounds, ulcers, hemorrhoids and burns. Saponins, on the other hand, may be anti-nutritional factors and may reduce the uptake of certain nutrients, including cholesterol and glucose at the gut through intra lumenal physicochemical interaction (Okwu & Okwu) [35]. They should therefore be present in lesser amounts in food samples. The phytochemical analysis of methanolic and aqueous extracts of methi leaves, methi stems and kasuri methi are mentioned in the respective tables (Tables 5, 6 & 7).

Table 5: Phytochemical analysis of methi leaves

Extract	Total phenolic content (µg GAE/ mg sample).	Total flavonoid content (µg CE/mg sample)	Crude Alkaloids (%)*	Saponins*
Methanolic extract (ML ME)	8.74	6.42	8.16	2.37
Aqueous extract (ML WE)	22.47	3.72	*Calculated for powdered samples	

Table 6: Phytochemical analysis of methi stems

Extract	Total phenolic content (µg GAE/ mg sample).	Total flavonoid content (µg CE/mg sample)	Crude Alkaloids (%)*	Saponins*
Methanolic extract (MS ME)	40.19	7.51	3.68	0.58
Aqueous extract (MS WE)	18.36	3.40	*Calculated for powdered samples	

Table 7: Phytochemical analysis of kasuri methi

Extract	Total phenolic content (µg GAE/ mg sample).	Total flavonoid content (µg CE/mg sample)	Crude Alkaloids (%)*	Saponins*
Methanolic extract (KM ME)	17.30	7.40	10.86	2.79
Aqueous extract (KM WE)	39.83	5.53	*Calculated for powdered samples	

3.3. Characterization of GC-MS analysis

GC-MS results revealed the presence of common fatty acids and other phytochemicals (Tables 8 - 11) which are essential for the regulation of several significant functions like immune response, blood pressure, lipid levels and inflammation response to injuries. Secondary metabolite estimation of the PE and mixed solvents' extracts of methi leaves and kasuri methi revealed the presence of several useful compounds such as phytols followed by sterols such as campesterol, stigmasterol and others. One essential role of plant sterols in a regular diet is in the way these sterols balance the cholesterol in the body. Stigmasterol possesses potent anti-diabetic and thyroid inhibiting properties and campesterol is a valuable element in controlling cholesterol and lowering the risk of heart diseases. The samples also indicated the presence of phytol, which is a precursor for Vitamin E and vitamin K. Other useful compounds such as nonanoic acid, which is a food grade flavor ingredient and glycerine which is used as a moisturizer to prevent dry, rough, scaly skin and minor skin irritations, were also observed in the GC-MS profile of the samples. The presence of these compounds indicate the goodness of methi for skin. Cultivated fenugreek revealed the presence of lupeol, which is reported to be anti-cancerous and anti-inflammatory [36] and vitamin E which is used for treating and preventing heart diseases, diabetes and lung cancers.

Table 8: Secondary metabolites in the GC-MS analysis of methi leaves (Mixed solvents extract)

Compound name	Cas#	RT	% Area
Propanoic acid, 2-methyl-	000079-31-2	4.231	0.84
Oxime-, methoxy-phenyl-4-Ethylbenzoic acid,	1000222-86-6	5.779	1.04
2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	014237-73-1	19.113	0.30
Hexadecanoic acid, methyl ester	000112-39-0	19.921	1.05
n-Hexadecanoic acid	000057-10-3	20.291	9.10
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	000112-63-0	21.570	0.67
Octadecanoic acid)-	000057-11-4	22.164	2.84
9,12,15-Octadecatrienoic acid, ethyl ester	001191-41-9	24.867	2.01
E,Z-1,3,12-Nonadecatriene	1000131-11-3	26.482	2.20
Nonanoic acid	055268-58-1	26.560	3.95
Phytol	000150-86-7	21.738	1.35
Glycerine	000056-81-5	6.967	0.86

Table 9: Secondary metabolites in GC-MS analysis of methi leaves (PE extract)

Compound name	Cas#	RT	% Area
n-Hexadecanoic acid	000057-10-3	20.258	6.86
Phytol	000150-86-7	21.738	1.99
9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	21.906	5.00
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	000506-44-5	21.974	16.53
2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	014237-73-1	19.113	0.73
9,12,15-Octadecatrienoic acid, (Z, Z,Z)-	000463-40-1	24.867	2.41

Table 10: Secondary metabolites in GC-MS analysis of kasuri methi (Mixed solvents extract)

Compound name	Cas#	RT	% Area
3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	014901-07-6	15.076	0.10
Hexadecane	000544-76-3	16.366	0.06
Octadecane	000593-45-3	18.620	0.09
2-Pentadecanone, 6,10,14-trimethyl	000502-69-2	19.125	0.20
Hexadecanoic acid, methyl ester	000112-39-0	19.932	0.86
n-Hexadecanoic acid	000057-10-3	20.415	1.05
Hexadecanoic acid, ethyl ester	000628-97-7	20.594	0.30
Eicosane	000112-95-8	20.639	2.84
10,13-Octadecadienoic acid, methyl Ester	056554-62-2	21.581	0.62
Phytol	000150-86-7	21.940	14.26
Hexadecanoic acid, butyl ester	000111-06-8	22.400	0.30
Nonadecane	000629-92-5	22.512	0.63
Vitamin E	000059-02-9	31.921	2.92
Campesterol	000474-62-4	33.750	1.38
Stigmasterol	000083-48-7	34.411	1.15
Octadecane	000593-45-3	35.095	0.89
Lupeol	000545-47-1	37.987	0.92

Table 11: Secondary metabolites in GC-MS analysis of kasuri methi (PE extract)

Compound name	Cas#	RT	% Area
2-Pentadecanone, 6,10,14-trimethyl	000502-69-2	19.125	0.21
Hexadecanoic acid, methyl ester	000112-39-0	19.932	0.89
n-Hexadecanoic acid	000057-10-3	20.415	1.03
Eicosane	000112-95-8	20.639	0.14
Hexadecanoic acid, ethyl ester	000628-97-7	20.594	0.25
10,13-Octadecadienoic acid, methyl ester	056554-62-2	21.581	0.65
Phytol	000150-86-7	21.940	14.89
Hexadecanoic acid, butyl ester	000111-06-8	22.400	0.32
Nonadecane	000629-92-5	22.512	0.66
1-Heneicosyl formate	077899-03-7	24.946	1.41
Heptacosane	000593-49-7	25.002	1.53
Vitamin E	000059-02-9	31.921	2.97
Campesterol	000474-62-4	33.750	1.44
Stigmasterol	000083-48-7	34.411	1.24
Octadecane	000593-45-3	35.095	0.93
Lupeol	000545-47-1	37.987	0.96

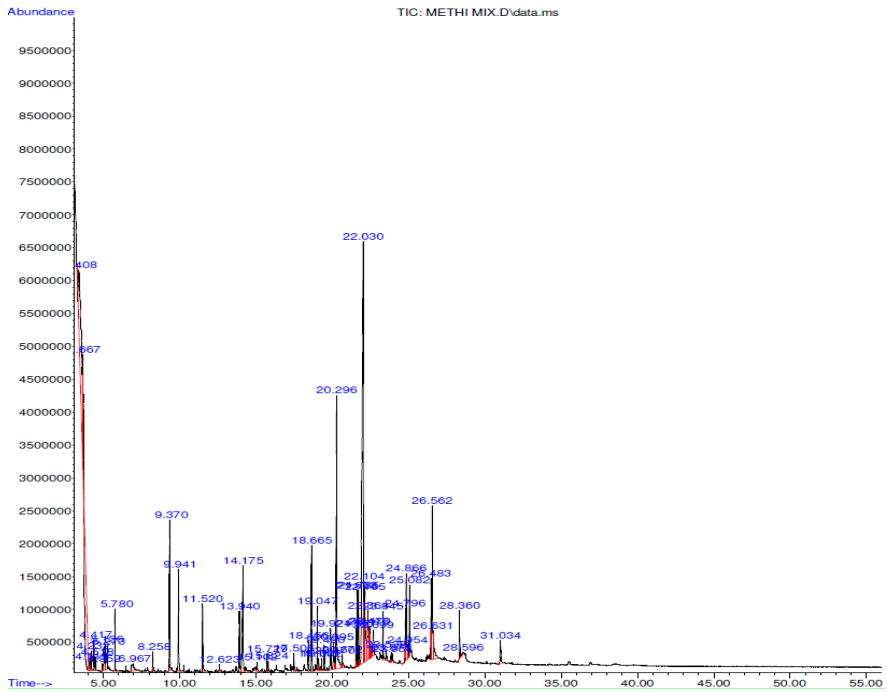

Fig 1: Chromatogram for GC-MS analysis of methi leaves Mixed solvents extract

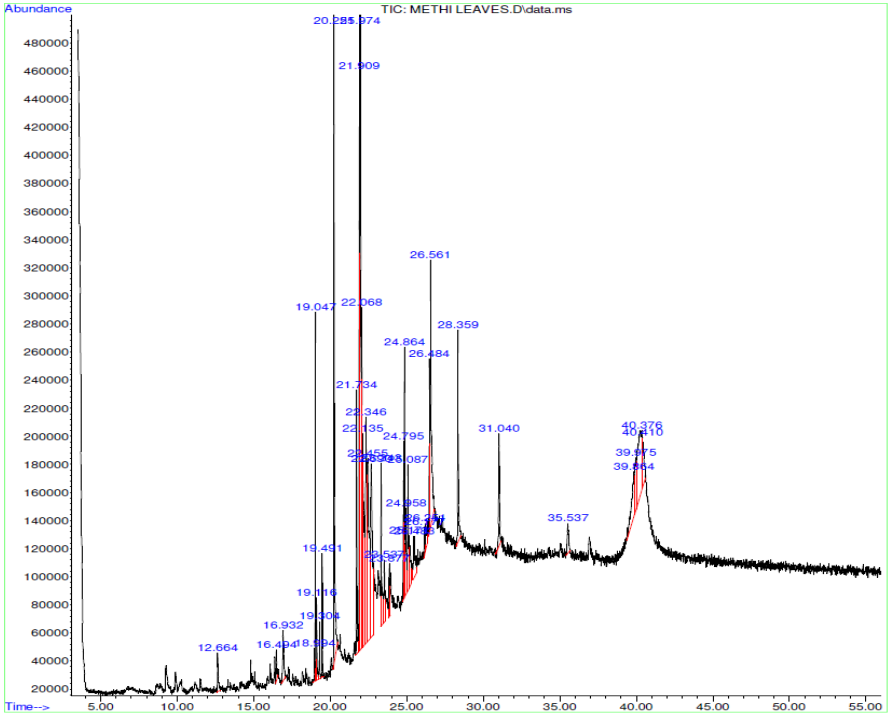

Fig 2: Chromatogram for GC-MS analysis of methi leaves PE extract

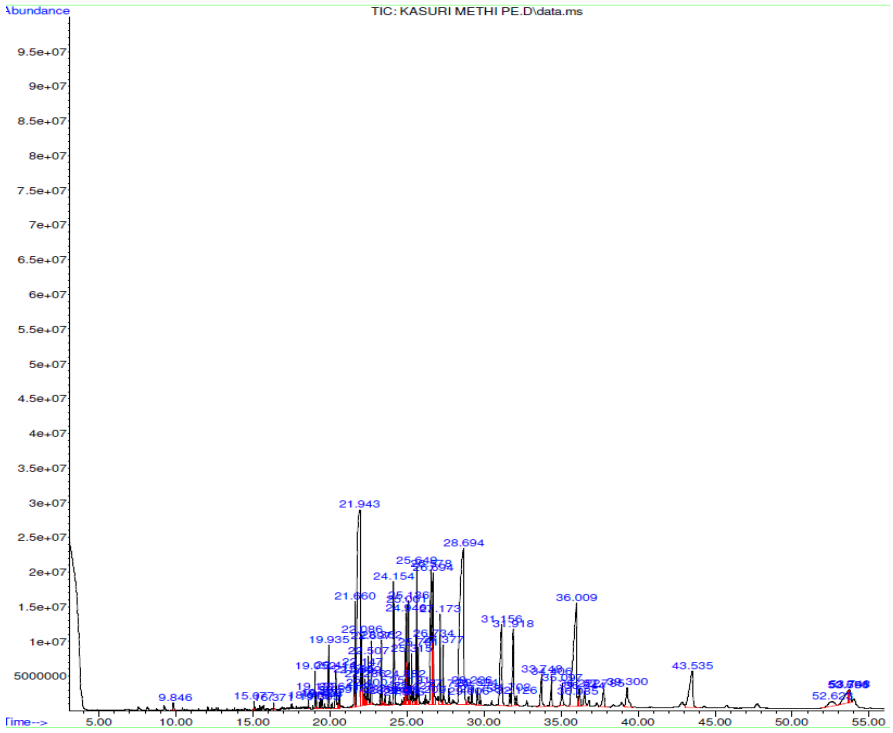

Fig 3: Chromatogram for GC-MS analysis of kasuri methi Mixed solvents extract

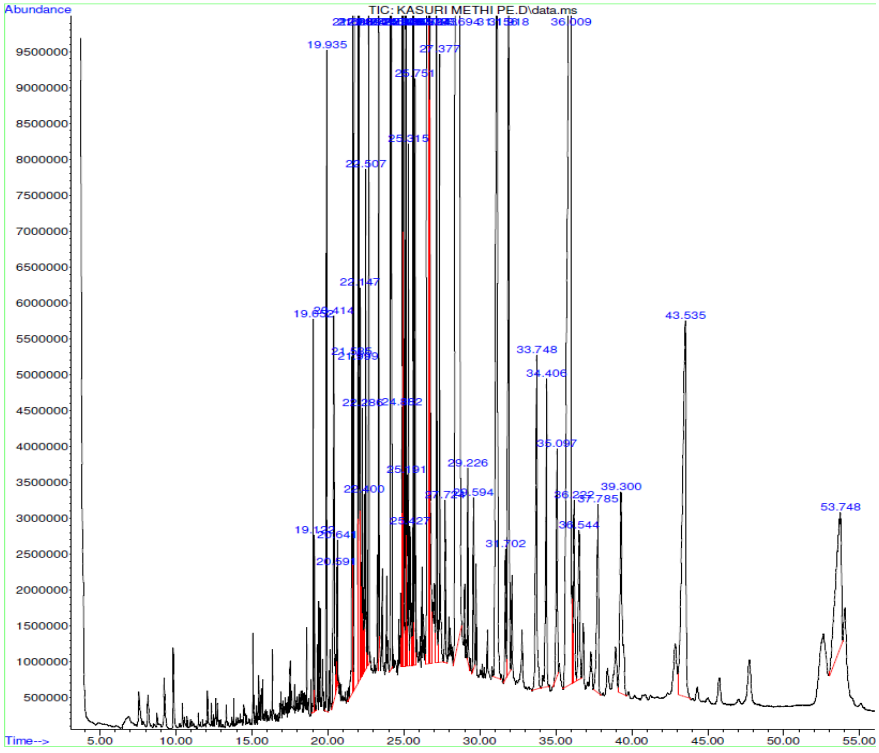

Fig 4: Chromatogram for GC-MS analysis of kasuri methi PE extract

3.4. Determination of antioxidant activity

3.4.1. ABTS Scavenging assay

The total antioxidant activity of the samples was evaluated in accordance with the decolorization of ABTS to its radical cation. ABTS+ as percentage inhibition using the % radical scavenging formula. IC_{50} values for the extracts in mg/ml as estimated are represented in the respective tables (12, 13). Methanolic extracts for all three samples have shown the maximum scavenging activity proving the samples to be excellent antioxidants.

Table 12: ABTS scavenging activity for methi extracts

Sample	IC_{50} value(mg/ml)
ML ME	0.85
ML WE	5.5
MS ME	0.27
MS WE	7.5

Table 13: ABTS scavenging activity for kasuri methi extracts

Sample	IC_{50} value (mg/ml)
KM ME	0.9
KM WE	7

3.4.2. Ferric reducing antioxidant power (FRAP)

The principle of this method is the reduction of ferric tripyridyl-s-triazine complex to ferrous colored form in the presence of antioxidants. The procedure was described by Benzie and Strain. Antioxidants in the samples reduce ferric tripyridyl-s-triazine complex to form a blue colored complex which results in an increase in the absorbance at 593 nm. The calibration curves revealed highly positive linear relation between mean FRAP values and the concentration of BHT standard. The BHT equivalents for the respective samples are mentioned below (Tables 14, 15).

Table 14: BHT equivalents (µg BE/mg sample) for methi leaves and stems

Sample	BE equivalents (µg BE/ mg sample)
ML ME	65.179
MLWE	19.67
MS ME	20.53
MS WE	30.564

Table 15: BHT equivalents (µg BE/mg sample) for kasuri methi

Sample	BE equivalents (µg BE/ mg sample)
KM ME	23.917
KM WE	2.277

3.5. Antimicrobial activity

Three gram positive (*Staphylococcus aureus, Staphylococcus epidermidis and Bacillus subtilis*) and two gram negative (*E. coli*, *Proteus mirabilis*) bacterial test pathogens were used to evaluate the antibacterial activity of fenugreek leaves and cultivated fenugreek. Agar well diffusion method was used to assess the activity against the bacterial test pathogens. Results were analyzed by measuring the zones of inhibition. Inhibiting concentrations used for both samples was 350 mg/ml. The methanolic extracts showed significant zones of inhibition (in mm) for the bacterial test pathogens viz. *E. coli, P. mirabilis and B. subtilis.*

Table 16: Antibacterial activity against various bacteria for fenugreek leaves

Test pathogens	Diameter of zone of inhibition (mm)			
Gram positive	**MLME**	**MLWE**	**KM ME**	**KM WE**
E. coli	13	--	12	--
S. aureus	--	--	--	--
S. epidermidis	--	--	--	--
Gram negative				
B. subtilis	16	--	12	11
P. mirabilis	16	--	16	--

* -- indicates: No Zone of inhibition

3.6. Flavor and Fragrance analysis

Flavor and fragrance analysis of fenugreek and cultivated fenugreek revealed the presence of several industrially useful important aroma compounds, some of which are described as follows:

Aldehydes such as tetradecanal were observed in the flavor analysis of fenugreek leaves, which is myristyl aldehyde and is used as a flavoring agent, also, 1,19- eicosadiene is an essential volatile oil which imparts aroma to the food products along with antibacterial activity [37]. n-hexanoic acid also known as Palmitic acid provides fragrance to food products and its usage in fragrance concentrates is permitted upto 1.00%. Similarly, for kasuri methi which is known for its flavor in the Indian cuisine, compounds such as 2-methoxy, 4-vinylphenol were detected which is a flavor and fragrance agent for food products. 3-Amino-4, 5-dimethyl-2(5H)-furanon is another powerful flavor compound found in several foods and spices. It contributes to the burnt sweet note of aged sake, cane sugar, and coffee, to the spicy/curry and condiments as well as to the nutty sweet flavor of botrytized wines and flor-sherry wines [38]. Tables 17 and 18 represent the flavor and fragrance profiles of the samples. Figures 5 & 6 represent the chromatographs for the flavor analysis of both the samples.

Table 17: Flavor analysis of methi leaves

Compound name	Cas#	RT	% Area
Tetradecanal	000124-25-4	14.551	0.17
2-Pentadecanone, 6,10,14-trimethyl	000502-69-2	17.773	0.78
n-Hexadecanoic acid	000057-10-3	18.994	1.38
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	000506-44-5	20.694	2.88
1,19-Eicosadiene	014811-95-1	23.960	1.64
13-Tetradecen-1-ol acetate	056221-91-1	26.970	5.18
beta-Sitosterol	000083-46-5	33.647	1.92

Table 18: Flavor analysis of kasuri methi

Compound name	Cas#	RT	% Area
3-Amino-4,5-dimethyl-2(5H)-furanon	1000314-35-9	10.185	1.18
2-Methoxy-4-vinylphenol	007786-61-0	11.363	1.92
alpha.-D-Galactopyranoside, methyl	003396-99-4	14.925	3.05
n-Hexadecanoic acid	000057-10-3	19.006	1.82
7-Pentadecyne	022089-89-0	20.694	0.68
Octadecanoic acid	000057-11-4	20.883	0.40
9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	21.427	0.21

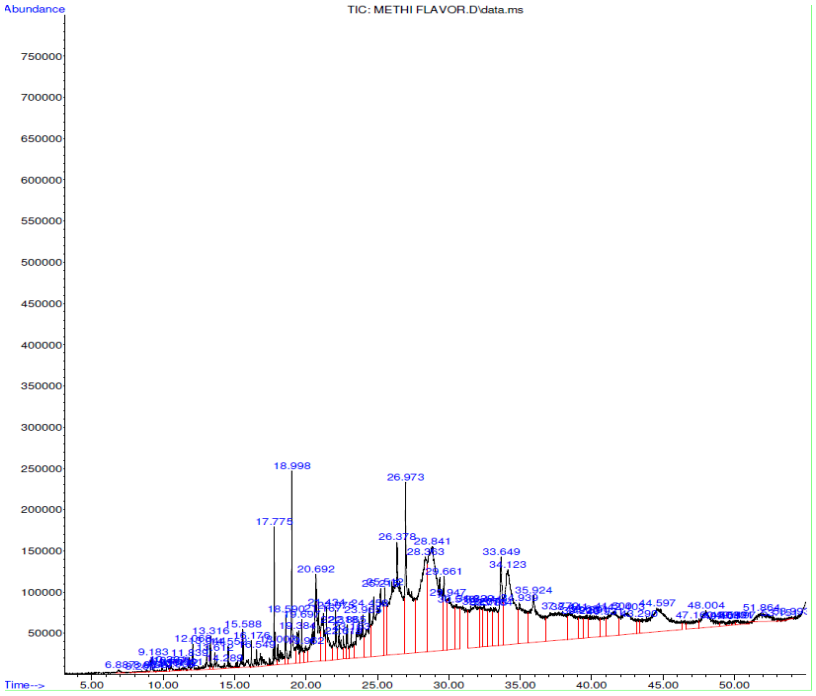

Fig 5: Chromatogram for methi leaves flavor analysis

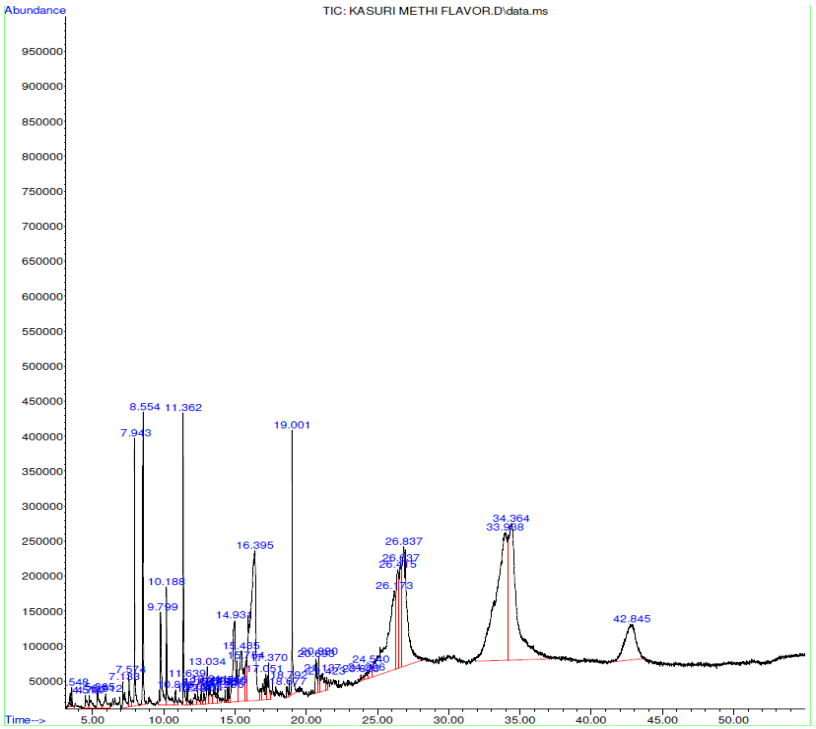

Fig 6: Chromatogram for kasuri methi flavor analysis

4. Conclusion

The chemical compositions of the tropical Indian raw *Trigonella foenum-graecum* and *Trigonella corniculata* show that they can be potential sources of nutraceuticals and flavoring agents. Based on the results represented here, it can be concluded that both the samples i.e. methi and kasuri methi have appeared as rich sources of bioactive compounds and offer immense opportunities for the development of various value added products and other food applications to provide enhanced nutrition and improved health. These results are useful to provide more value addition and usefulness from these leafy vegetables. Apart from the known benefits of methi seeds, methi leaves also have equal potential to be a nutraceutical. The flour from the leaves can be incorporated into processed foods and thus provide health along with taste. Stems also have very good antioxidant properties and good amount of phytochemicals. Kasuri methi, most renowned for imparting flavor to foods, provides a tremendous amount of nutrients helpful in treating and preventing several health problems. The obtained compounds are known to possess potent antioxidant properties and good therapeutic potential which may help in drug development. Furthermore, the high correlation observed between the various assays employed and phenolics content indicates that these phenolics (total, free and flavonoids) are among the predominant sources of antioxidant activity in both the species of Trigonella. It can therefore be concluded that apart from flavor and fragrance, kasuri methi and methi leaves have a lot to offer in terms of health improvement.

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