Research Article

Attributes of bioactive compounds isolated from commercial brands of fenugreek (*Trigonella foneum-graecum*) in relation to organic solvent systems and their potential as antioxidants and biological activity

Tahir Mehmood^{1*}, Maria Ahmed¹, Zahra Jabeen², Abdul Karim¹, Muhammad Ashraf Shaheen¹ and Faiza Siddique¹

1. Department of Chemistry, University of Sargodha, 40100 Sargodha-Pakistan

2. Department of Biochemistry, Government College University, Faisalabad-38000-Pakistan

*Corresponding author's email: tahiruosbiochem@yahoo.com

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Abstract

The present study is about potential variations of bioactive compounds from different commercial brands of fenugreek; in relation to multiple organic solvent systems, antioxidants and antimicrobial assays. Total phenolic contents, total flavonoid contents, individual phenolic acids, and DPPH assays were checked whereas phenolics analysis was performed by HPLC spectrophotometrically. Antimicrobial activity of different extracts was ascertained by disc diffusion method. The crude extract yields for different commercial brands of fenugreek ranged from 35.53-62.77 g/100g dry weight. Maximum yield was obtained from 100% Local Fenugreek with methanol while minimum was obtained from 100% local fenugreek chloroform. A considerable amount of TPC and TFC 24.4- 42.60 mg of Gallic acid equivalents per g of sample dry weight (mg GAE/g DW) and 0.75-1.87 as mg of catechin equivalents per gram of sample dry weight (mg CE/g DW) respectively was observed. The amount of DPPH and reducing power assay was 0.927-3.78 mg/mL and 4.20-7.76 mg/mL respectively. Different brands of fenugreek had significant amount of phenolics acids ranging from 14.36-1035.44 mg/kg, where Gallic acid was found in all extracts. Antimicrobial activity was tested against microorganisms and were found to be considerable significant. These finding indicates that fenugreek has strong potential to be used as pharmaceuticals, antioxidants and preservatives.

Keywords: Antioxidant activity; Biological activity; Extraction efficacy of solvents; Total phenolics; Total flavonoids

Introduction

Lots of changes have been observed in modern civilization which, no doubt provide

many significant benefits but on other hand, has brought a greater risk to human life, for instance use of chemicals, pesticides,

environmental pollutants, and others cause increased level of free radical production in the body. In living organisms oxidation reactions produced by free radicals cause the oxidation of bio-molecules like lipid, DNA, RNA and protein etc [1, 2]. Whereas the food issues are concerned, the shelf life of the fresh and processed food decreases by oxidative reactions. Thus, the human health is affected due to toxicity from the environmental factors and also from the preserved food stuff [3]. Antioxidants have the ability to retard the damage by free radicals in biological systems as they capture or quench those free radicals that are involved in causing damage to human body and food. They are also very important in increasing the shelf life of food which is affected by oxidative reaction. Synthetic antioxidants are mostly used in food processing and pharmaceuticals industries, but they have many side effects such as liver damage and carcinogenic effect [4]. So, there is need to explore and isolate natural antioxidants in high amount, which have very fewer or no side effects and can be used in medicine instead of the synthetic antioxidants [5].

Plants and fruits are the most important, more safer and compatible sources of natural antioxidants. Epidemiological evidence in the literature have shown that the intake of natural products have been responsible for the lower prevalence of chronic diseases, for example, cancer and heart diseases. Natural products, in addition to the vitamins and minerals, contain phytochemicals such as flavonoids and other phenolics, which may exert therapeutic effects in human diseases [6]. Many phytochemicals possessed the antioxidant activity which helps to protect the cells against the oxidative damage caused by free radicals [7]. Despite, there is great interest in determining the role of phytonutrients, as the synthetic molecules have some side effects, and there is

increasing trend for natural active compounds. In this scenario, beside nutritional value, functional foods have a potentially positive effect on the health [8, 9].

Trigonella foenum-graecum also called Fenugreek (locally known as Methi) is widely grown in Pakistan, India, Egypt, and Middle Eastern countries [10]. It belongs to the family *Fabaceae*. It often used as a condiment in Mediterranean countries and Indian sub-continental location. Fenugreek contains a gum or mucilage which is called galactomannan which acts as a thickener or stabilizer or additive in food [11]. Fenugreek (*Trigonella foenum-graecum*) is considered to be the oldest medicinal plant, and had been used as a tonic for treatment of edema of legs, lactation stimulant, in treatment of baldness, anti-diabetes and cholesterol reducing properties, as an antibacterial, anti-inflammatory and antiulcerogenic [12]. Recent research has also shown that fenugreek helps in lowering the blood glucose level and plays a significant role for treatment of diabetes. Diabetic rats treated by supplementation of fenugreek seeds showed elevation in their antioxidant potential [13]. Fenugreek has a great role in pharmacological aspect that may include anti-inflammatory, antiviral, antitumor, hypertensive, anti-microbial and antioxidant activity [14].

The functional constituents in natural sources are affected by varieties, cultivation, maturation, storage, and processing. The nature of extracting solvent also plays an important role in resulting extracts yields for their antioxidant activities and other important bioactive compounds [15, 16]. Multiple solvents system plays a vital role in extraction of maximum bioactive compounds from selected plants it also avoids fruitless effort in future by avoiding solvent that extract minimum compound [17]. As fenugreek is reported to be rich

source of valuable metabolites and antioxidants such as phenolic acid and flavonoids [18], it would be interesting to evaluate the extract, by using different solvents systems, from different commercial brands of fenugreek that includes shan, national and local variety for their different antioxidant attributes.

Materials and methods

Sample collection

Three different samples of fenugreek were collected. Two samples were marketed by shan and national while the third sample of fenugreek was bought from the local market.

Preparation of extracts

The samples were dried after cleaning, crushed into fine powder, placed separately in polythene bags and marked individually and then placed in refrigerator. Extraction of all samples was done by different solvents having different ratios e.g. 100%, 80% and 50% methanol and chloroform. Ratio of sample plus solvent are kept as 1/10 (w/v). Extraction was done for 12 hours thrice by using orbital shakers. Extract was separated by the process of filtration by keeping residue left behind. These extracts were concentrated by using vacuum rotary evaporator at reduced temperature under vacuum. After that extract was kept in refrigerator at a temperature of 4 °C and then used for further analysis [19].

Assessment of total phenolic contents (TPCs)

Quantity of TPC was calculated using FC (Folin-Ciocalteu) reagent as described by Kalpna *et al.* [20]. The result expressed as GAE (gallic acid equivalents) mg/g of dry weight of sample. Readings were taken in triplicate and the results were averaged. Results were reported on the basis of dry matter.

Determination of total flavonoid contents (TFCs)

TFCs were determined by the procedure followed by Zhishen *et al.* [21]. The results

were expressed as catechin equivalent (10-500 ppm) ($R^2=0.9913$) CE mg/g of dry weight. Assay was performed in triplicate.

Determination of reducing power (RP)

The reducing power of CFPE was determined as reported earlier by Zhuang *et al.* [22] with slight modifications. In this assay, different concentrations of extracts (2-10mg/mL) were mixed with 3.5 mL phosphate buffer (0.2 M, pH 6.6) and 2 mL of 1 % potassium ferricyanide. For 20 min, the mixture was incubated at room temperature. Following the addition of 2.5 mL of 10 % TCA (trichloroacetic acid) the mixture and the mixture was centrifuged for 10 min at 3000 rpm. 2.5 mL of supernatant was collected by pipette and mixed with 2.5 mL of distilled water. To this mixture 0.5 mL of 0.1 % ferric chloride was added and then absorbance of the mixture calculated at 700 nm with a spectrophotometer.

DPPH radical scavenging assay

The antioxidant activity of *Trigonella foenum-graecum* was assessed by DPPH (1, 1 diphenyl-2-picrylhydrazyl) radical scavenging assay described by Zhuang *et al.* [22]. BHA that is synthetic antioxidant was used as a positive control. Calculation was made at IC₅₀, concentration of extract that is scavenged/ neutralized 50% of DPPH free radicals.

A_{control} = the absorbance of control which contain all reagents except sample

A_{extract} = the absorbance of extracts

% scavenging activity = $(A_{\text{control}} - A_{\text{extract}})/A_{\text{control}} \times 100$

The % scavenging activity at 50% is IC₅₀

High performance liquid

chromatography (HPLC) analysis

Individual phenolics profile was analyzed by using HPLC. Separation of phenolic acids was carried out on HPLC using ODS (C18) reversed phase column (250 × 4.6 mm) [23]. The mobile phase used was acidified acetonitrile (99.5%) at a constant flow rate of 1 mL/min in isocratic mode. With the help of a microsyringe 20-μL sample was injected into the column. The detection was

carried out at 280 nm. Phenolics compounds were identified by matching their relative retention times with those of the pure standards of targeted compounds. On the basis of peak area measurement, the contents of an individual compounds were calculated.

Antibacterial activity

The Antibacterial activity of the extracts was determined by disc diffusion method. Nutrient agar medium was prepared and autoclaved. 100 μ L inoculums was added in medium while it was liquid and cool, mixed and then poured into petri plates. After that 6 mm wicks paper discs were laid flat on medium and 100 μ L extract was put on each disc. For 24h, the petri plates were then incubated at 37°C to check the growth of bacteria. The extracts showing the antibacterial activity inhibited the bacterial growth by forming clear zone of inhibition, measured in millimeters [24]. The bacterial strains that used were *Bacillus Substilis*, *Streptococcus aureus*, *Salmonella typhae* and *E.coli*. Ciprofloxacin was used as standard.

Statistical analysis

Statistical analysis data was expressed as mean values at 95 % confidence interval. Analysis of variance was performed using ANOVA procedures. Significance difference for values with respect to means of samples and solvents were determined. $P > 0.05$ means no significance difference.

Analysis was done by using SPSS 20 software [25].

Results and discussion

Extraction yields

The extraction yields for antioxidant components from different commercial brands of fenugreek with different solvent systems include methanol (100%, 80% and 50%) and chloroform (100%, 80%, and 50 %). The extract yields ranged from 35.53-62.77%. Maximum yield was obtained from 100% local methanol while minimum is obtained from 100% local chloroform. The range from different commercial brands varies like for shan 37.27-55.20, national 37.33-62.40, and local 35.53-62.77. Based on the results, the extraction efficacy of different solvents is 100% methanol > 0% methanol > 50% methanol > 80% chloroform > 50% chloroform > 100% chloroform. The higher yield from local 100% methanol showed that methanol has greater efficacy to recover antioxidant components from commercial brands of fenugreek. Results showed that effect of different solvents and sample type on ($p < 0.05$) % extraction yield is significant. So it can be considered that there is a significant difference ($p < 0.05$) in the extracted yields in relation to the solvent system and fenugreek different commercial brands that has been used. The present result has shown greater yield with methanol (Table 1) that is comparable to [26].

Table 1. Extract yields (%) of fenugreek

Solvents	% Composition of solvents	Yield (%)		
		Shan	National	Local
Chloroform	100%	37.27 ± 1.05^A_a	37.33 ± 0.05^B_a	35.53 ± 0.15^B_a
	80%	44.66 ± 1.39^A_b	38.47 ± 0.35^B_b	39.40 ± 0.26^B_b
	50%	38.60 ± 0.95^A_a	42.46 ± 1.10^B_a	40.46 ± 0.35^B_a
Methanol	100%	55.20 ± 1.00^A_c	62.40 ± 0.26^B_c	62.77 ± 0.94^B_c
	80%	54.20 ± 0.10^A_d	55.37 ± 0.15	55.60 ± 0.10^B_d
	50%	53.43 ± 0.30^A_e	51.57 ± 0.12^B_d	52.50 ± 0.30^B_e

Values are means $\pm$ SD (n=3) of three separate experiments. Different small letter in subscript within the same column show significant ($p < 0.05$) differences of means among *Citrus* species. Different superscript letters within the same row show significant ($p < 0.05$) differences of means among extraction solvents

Total phenolic contents

Total phenolic contents from different brands of fenugreek (Shan, National, Local) by using different solvents (100%, 80%, 50% methanol and 100%, 80%, 50% chloroform) ranged from 27.8-42.60 % of dry weight (DW). The lowest value observed was 27.8 % for national 50% chloroform and highest value was for shan 100% methanol (42.60 g/100g). Different TPC values have been observed from different brands of fenugreek like shan 27.8-42.60, national 25.0-41.2 and local 24.4-40.1. The order of TPC by using different solvents was 100% methanol > 80% methanol > 50% methanol > 100% chloroform > 80% chloroform > 50% chloroform. The present trend of solvent

trend showing higher yields in methanol (Methanol has a polarity index of 5.1. Mostly methanol is used for extraction various polar compounds but certain group of non-polar compounds are fairly soluble in methanol if not readily soluble) is comparable to Birjees *et al.* [27]. Leena *et al.* [28] also reported the highest TPC content in methanol followed by chloroform. The values were observed comparatively higher as compare to the present trend. Other factors that contribute to the recovery of phenolic contents may include polarity and solubility of compounds and solvents as well. TPC that has been observed from different fenugreek brands was found to be in order of local < national < shan (Table 2).

Table 2. Total phenolic and flavonoid contents of extracts from fenugreek

Solvents	TPC (GAE mg/g DW)			TFC (CE mg/g DW)		
	Shan	National	Local	Shan	National	Local
100% chloroform	35.2±0.00 ^a _A	34.3±0.02 ^b _A	32.1±0.00 ^a _A	1.86±0.0 ^a _A	1.86±0.1 ^a _A	1.85±0.02 ^a _A
80% chloroform	33.2±0.01 ^a _B	32.3±0.02 ^b _B	31.9±0.02 ^a _B	1.85±0.0 ^a _B	0.91±0.0 ^a _B	1.7±0.1 ^a _B
50% chloroform	27.8±0.05 ^a _A	25.0±0.01 ^b _A	24.4±0.03 ^a _A	0.87±0.0 ^a _C	0.75±0.1 ^a _C	1.31±0.59 ^a _C
100% methanol	42.60±0.04 ^a _D	41.2±0.01 ^b _D	40.1±0.06 ^a _D	1.22±0.0 ^a _B	1.74±0.1 ^a _B	0.96±0.1 ^a _B
80% methanol	40.5±0.03 ^a _E	41±0.04 ^b _E	39.9±0.11 ^a _E	1.87±0.0 ^a _A	1.75±0.1 ^a _A	1.77±0.0 ^a _A
50% methanol	38.4±0.03 ^a _F	36±0.04 ^b _F	37.1±0.07 ^a _F	1.65±0.3 ^a _B	1.78±.47 ^a _B	1.46±0.03 ^a _B

Values are means ± SD (n=3) of three separate experiments. Different small letter in subscript within the same column show significant ($p < 0.05$) differences of means among *Citrus* species. Different superscript letters within the same row show significant ($p < 0.05$) differences of means among extraction solvents.

Total flavonoid contents (TFC)

Total Flavonoid contents (TFC) observed in different commercial brands of fenugreek is given in Table 2. Values of TFC extracted from different brands of fenugreek ranged from 0.75-1.87 CE mg/g of dry weight. The maximum value was observed with 80% methanol from shan and lowest value observed from 50% chloroform national. The value from different commercial brands of fenugreek extracts were shan 0.87-1.87, national 0.75-1.86, local 0.96-1.85. The order of solvents ability for TFC extraction was 100% chloroform > 80% methanol > 50% methanol > 80% chloroform > 100%

methanol > 50% chloroform. The order of TFC values of commercial brands of fenugreek was shan > national > local. TFC values varied prominently among different commercial brands of fenugreek. The present values of TFC are comparable to the values that were observed by Leena *et al.* [28] and Birjees *et al.* [27], results of TFC observed in case of methanol were quite higher as compare to the present values.

DPPH radical scavenging activity

IC₅₀ (In term of DPPH scavenging activity) for different commercial brands of fenugreek ranged from 0.927-3.78mg/mL shown in Table 3. The highest value was

noted for local 80% chloroform 3.78, while lowest value was observed in case of Shan 100% chloroform Table 3. IC₅₀ (mg/ml) values for different commercial brands of fenugreek varied significantly with respect to the solvents that has been used. 80% local chloroform considered to be on the higher range comparatively. Collectively, the order of solvents for antioxidant potential is 50% methanol > 80% methanol > 80% chloroform > 100% methanol > 50%

chloroform > 100% chloroform. The effect of extraction solvents on radical scavenging activity is significant ($p < 0.05$). The order of commercial brands of fenugreek to scavenge the free radical is local > national > shan. Local Fenugreek has shown highest radical scavenging activity showing significant ($p < 0.05$) variations among other commercial brands. Anupma *et al.* [29] observed highest radical scavenging activity in methanol which is comparable to the present trend.

Table 3. DPPH radical scavenging activity and reducing power

Solvents	DPPH (IC ₅₀ mg/mL)			Reducing power (EC ₅₀ mg/mL)		
	Shan	National	Local	Shan	National	Local
100% chloroform	0.927±0.05 ^A	1.91±0.00 ^{bA}	1.87±0.03 ^{cA}	7.76±0.05 ^{aA}	6.37±0.04 ^{aA}	5.51±0.03 ^{aA}
80% chloroform	1.66±0.03 ^B	2.87±0.07 ^{bB}	3.78±0.06 ^{cB}	6.49±0.00 ^{aA}	6.19±0.02 ^{aA}	6.56±0.67 ^{aA}
50% chloroform	2.82±0.83 ^B	2.5±0.01 ^{bB}	2.07±0.02 ^{cB}	5.09±0.00 ^{aB}	6.08±0.04 ^{aB}	6.13±0.03 ^{aB}
100% methanol	2.03±0.04 ^C	2.46±0.05 ^{bC}	2.46±0.03 ^{cC}	5.04±0.00 ^{aC}	4.20±0.00 ^{aC}	5.35±0.02 ^{aC}
80% methanol	2.23±0.20 ^B	2.34±0.05 ^{bB}	3.21±0.2 ^{cB}	6.13±0.00 ^{aA}	5.04±0.04 ^{aA}	7.24±0.07 ^{aA}
50% methanol	2.68±0.01 ^B	3.45±0.49 ^{bB}	2.67±0.04 ^{cB}	6.41±0.02 ^{aA}	6.34±0.01 ^{aA}	7.24±0.07 ^{aA}

Values are means ± SD (n=3) of three separate experiments. Different small letter in subscript within the same column show significant ($p < 0.05$) differences of means among *Citrus* species. Different superscript letters within the same row show significant ($p < 0.05$) differences of means among extraction solvents

Reducing power

The EC₅₀ for different commercial brands of fenugreek is given in (Table 3). The EC₅₀ values ranged from 4.20-7.76. The lowest value has been observed in 100% methanol national and highest value has been observed in chloroform 100% (7.76). The IC₅₀ values for commercial brands of fenugreek are shan 5.04-7.76, national 4.20-6.37 and local 5.35-7.24. The ability to extract solvents in order to recover the reducing agent from commercial brand of fenugreek is in order of 50% methanol > 100% chloroform > 80% chloroform > 80% methanol > 50% chloroform > 100% methanol. The reducing potential for commercial brand of fenugreek is shan > local > national. According to Bukhari *et al.* [30] methanol extract of fenugreek show higher value that is comparable to the present work.

Phenolic acids profile

With the help of HPLC, qualitative and quantitative analysis of phenolic acid is performed. Comparison has been made between commercial brands of fenugreek and standard phenolic acids. Ten phenolic acids have been used that include gallic acid, caffeic acid, chlorogenic acid, protocatechuic acid, 3-hydroxy benzoic acid, syringic acid, 4-hydroxyl benzoic acid, p-coumeric acid, 3,5 dihydroxyl benzoic acid and vanillic acid. Total phenolic acids were ranged between 18.36-1035.99. The range of gallic acid and protocatechuic acid is 10.90-96.89 mg/kg and 2441-277.70 mg/kg of extract respectively. Gallic acid was present in almost all the samples except few ones. The highest value of gallic acid was found in 100% methanol (shan 96.89) and lowest value was found in 50% chloroform (national 10.90). Higher amount of all present phenolic acids was found in shan 80% (275.70 mg/kg) as shown in Table 4.

These phenolic acids play a great role as antioxidant and has a great medicinal value. Gallic acid is found in a frequent amount that can easily be absorbed by human body and it has a positive influence on the cancerous cell and prevent their growth. P-coumaric acid plays a significant role in prevention of stomach cancer by inhibiting the activity of carcinogenic nitrosamines [31].

Biological activity

Fenugreek (*Trigonella foenum-graecum*) has antibacterial activity which was tested against different bacterial strains like *Bacillus Subtilis*, *Streptococcus aureus*, *Salmonella typhae* and *Escherichia-coli* (Table 5 and 6).

For *Bacillus Subtilis*, values of antibacterial activity for different commercial fenugreek brands were ranged from 10.47-24.30 mm. The maximum value was observed for methanol 100% (24.30 mm) extract. While minimum value was observed for chloroform (10.77 mm). The antibacterial values of different samples include shan fenugreek (10.77-24.30), national fenugreek (11.37-23.33), and local fenugreek (10.47-23.30). Values of antibacterial activity against *E. coli* ranged from 10.43-24.27 mm. . The maximum value was observed for methanol 100% shan (24.30 mm) extract.

While minimum value was observed for chloroform 50 % (10.77mm) local. The antibacterial values of different samples include shan (11.43-24.27), national (11.37-23.47) and local (10.43-23.30).

Maximum antibacterial activity against *Staphlococcus aureus* was observed for methanol 100% Shan (23.17 mm) extract. While minimum value was observed for chloroform 50% National (10.23mm). The antibacterial values of different samples include Shan (10.40-23.17), National (10.23-22.37), and Local (10.40-21.40). 80% methanol Local extract was found to be on top rank related to antibacterial activity against *Salmonella tyaphimurium*. While minimum value was observed for chloroform 50% National (10.23 mm). The antibacterial values of different samples include shan (10.40-23.17), national (10.37-23.50) and local (10.37-26.48).

Collectively, the order of solvents used for antibacterial activity against selected bacterial strain is: 100% methanol > 100% chloroform > 80% methanol > 80% chloroform > 50% methanol > 50% chloroform showing significant effect ($p < 0.05$). The results are also comparable to those reported by Mehmood *et al.* [31] fenugreek (*Trigonella foenum-graecum*) has an antibacterial activity.

Table 4. Individual phenolic acids (mg/kg of extract) profile *Trigonella foenum-graecum* of extracts from different commercial brands of fenugreek

Extracts	samples	Phenolic Acids (mg/kg)										Total amount of phenolic acids (mg/kg)
		Gallic acid	Caffic acid	Chlorogenic acid	Protocatechuic acid	3-hydroxy benzoic acid	Catechin	4-hydroxyl benzoic acid	p-coumaric acid	3,5 dihydroxyl benzoic acid	Ferulic acid	
100% Chloroform	Shan	91.89	87.22	—	—	—	5.5	—	—	—	—	184.61
	National	19.53	87.71	61.9	—	—	—	—	—	—	—	169.14
	Local	18.36	—	—	—	—	—	—	—	—	—	18.36
80% Chloroform	Shan	13.05	744.24	—	277.70	—	—	—	—	—	—	1035.44
	National	63.48	39.65	—	—	—	—	—	—	—	—	103.13
	Local	22.72	—	—	244.15	—	125.78	—	—	—	—	392.65
50% Chloroform	Shan	76.98	—	—	—	—	—	—	—	183.84	288.79	549.61
	National	10.90	—	105.14	—	—	122.77	—	—	—	—	238.81
	Local	23.02	—	—	—	—	—	—	—	—	—	23.02
100% Methanol	Shan	96.89	88.22	—	—	—	6.5	—	—	—	—	191.61
	National	19.53	85.71	62.9	—	—	—	—	—	—	—	168.14
	Local	14.36	—	—	—	—	—	—	—	—	—	14.36
80% Methanol	Shan	14.05	764.24	—	275.70	—	—	—	—	—	—	1035.99
	National	64.48	38.65	—	—	—	—	—	—	—	—	103.13
	Local	23.72	—	—	245.15	—	126.78	—	—	—	—	395.65
50% Methanol	Shan	77.98	—	—	—	—	—	—	—	184.84	287.79	550.61
	National	12.90	—	107.14	—	—	125.77	—	—	—	—	245.81
	Local	24.02	—	—	—	—	—	—	—	—	—	24.02

Table 5. Antibacterial activity of different commercial brands of fenugreek (*Trigonella foenum graecum*) against *Escherichia coli* and *Bacillus subtilis*

Solvents	Zone of inhibition (mm) against <i>Escherichia coli</i>			Zone of inhibition (mm) against <i>Bacillus subtilis</i>		
	Shan	National	Local	Shan	National	Local
100% chloroform	21.83±0.76 ^A _a	21.47±0.45 ^B _a	22.23±0.21 ^C _a	21.13±0.15 ^A _a	21.23±0.21 ^A _a	22.27±0.25 ^A _a
80% chloroform	13.3±0.32 ^A _b	13.43±0.37 ^B _b	12.53±0.47 ^C _b	12.37±0.32 ^A _b	12.47±0.42 ^A _b	12.50±0.43 ^A _b
50% chloroform	11.43±0.37 ^A _c	11.37±0.35 ^B _c	10.43±0.37 ^C _c	10.77±0.80 ^A _c	11.37±0.35 ^A _c	10.47±0.42 ^A _c
100% methanol	24.27±0.25 ^A _d	23.47±0.45 ^B _d	23.30±0.26 ^C _d	24.30±0.26 ^A _d	23.33±0.31 ^A _d	23.30±0.26 ^A _d
80% methanol	17.37±0.32 ^A _e	16.30±0.30 ^B _e	15.48±0.42 ^C _e	17.33±1.70 ^A _e	17.30±0.30 ^A _e	16.48±0.42 ^A _e
50% methanol	12.33±0.30 ^A _f	11.43±0.37 ^B _f	11.33±0.35 ^C _f	13.33±0.31 ^A _f	12.43±0.38 ^A _f	11.05±0.25 ^A _f
Ciprofloxacin	10.3±0.23 A	10.3±0.23 B	10.3±0.23 C	14.3±0.32 A	14.3±0.32 B	14.3±0.32 C

Values are means ± SD (n=3) of three separate experiments. Different small letter in subscript within the same column show significant ($p < 0.05$) differences of means among *Citrus* species. Different superscript letters within the same row show significant ($p < 0.05$) differences of means among extraction solvents

Table 6. Antibacterial activity of different commercial brands of fenugreek (*Trigonella foenum-graecum*) against *Salmonella typhimurium* and *Staphylococcus aureus*

Solvents	Zone of inhibition (mm) against <i>Salmonella typhimurium</i>			Zone of inhibition (mm) against <i>Staphylococcus aureus</i>		
	Shan	National	Local	Shan	National	Local
100% chloroform	21.40±0.36 ^A _a	21.40±0.40 ^B _a	21.23±0.21 ^A _a	21.17±0.15 ^A _a	22.33±0.31 ^A _a	21.40±0.36 ^B _a
80% chloroform	14.37±0.32 ^A _b	14.43±0.38 ^B _b	14.53±14.53 ^A _b	14.16±0.15 ^A _b	14.40±0.36 ^A _b	14.37±0.30 ^B _b
50% chloroform	10.4±0.38 ^A _c	10.37±0.3 ^B _c	10.37±10.37 ^A _c	10.40±0.24 ^A _c	10.23±0.21 ^A _c	10.40±0.40 ^B _c
100% methanol	23.27±0.35 ^A _d	23.50±0.44 ^B _d	23.30±23.30 ^A _d	23.17±0.15 ^A _d	22.37±0.32 ^A _d	21.40±0.36 ^B _d
80% methanol	16.37±0.32 ^A _e	16.37±0.35 ^B _e	26.48±26.48 ^A _e	15.17±0.15 ^A _e	15.4±0.36 ^A _e	14.37±0.32 ^B _e
50% methanol	11.33±0.31 ^A _f	11.40±0.40 ^B _f	11.43±11.43 ^A _f	10.40±0.36 ^A _f	10.3±0.26 ^A _f	10.43±0.40 ^B _f
Ciprofloxacin	9.3±0.31	9.3±0.31	9.3±0.31	9.3±0.31	9.3±0.31	9.3±0.31

Values are means ± SD (n=3) of three separate experiments. Different small letter in subscript within the same column show significant ($p < 0.05$) differences of means among *Citrus* species. Different superscript letters within the same row show significant ($p < 0.05$) differences of means among extraction solvents

Conclusion

It can be concluded that each commercial brand of fenugreek contain a considerable amount of antioxidants. Solvents have a significant role in the extraction of plant constituents. The phenolics and total flavonoid contents were extracted in moderate to high yields. The higher yield of 100% methanol showed that, it has greater efficiency to recover antioxidant components from commercial brands of fenugreek as compared to the extract of chloroform. Extracts from all tested brands contain considerable antimicrobial activity against microorganisms like *S. typhae*, *S. aureus*, *E. coli* and *B. substili*. Above

parameter elucidated that different commercial brands of fenugreeks have good antioxidant potential and substantial contents of phenolics so, our findings suggest, it can be used as a natural source powerful antioxidants and preservatives in food industry and pharmaceuticals .

Authors' contributions

Conceived and designed the experiments: T Mehmood, Performed the Experiments: M Ahmed & Z Jabeen, Analyzed the Data: T Mehmood, A Karim, MA Shaheen & F Siddique, Contributed reagents/ materials/ analysis tools: T Mehmood . Wrote the paper: T Mehmood & F Siddique.

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