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Gas chromatography mass spectrometry analysis and *in vitro* antibacterial activity of essential oil from *Trigonella foenum-graecum*

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ABSTRACT

Objective: To evaluate the antibacterial activity of essential oil from *Trigonella foenum-graecum* seeds powder, and identify the compounds from the extracted oil.
Methods: The seeds powder of *Trigonella foenum-graecum* was subjected to Clevenger extractor. Seven strains of bacteria were used to test antibacterial activity of the extract. The activity against bacteria was tested by disk diffusion method using Whatman No. 1 filter paper. Gas chromatography mass spectrometry analysis was performed with an Agilent7890/5975B-gas chromatography/mass selective detector.
Results: The hydrodistillation of seeds powder yielded 0.285% (v/w) of oil. Disk diffusion of the oil showed bactericidal activity against both Gram negative and Gram positive bacteria of tasted strains. The inhibition zone ranged from (8 ± 0) mm to (15.0 ± 0.7) mm depending on microbial strains. Gas chromatography mass spectrometry analysis showed 14 different compounds. The total compounds represented 80.96% of the oil.
Conclusions: The antibacterial activity is due to the effects of different biological active compounds present in the extract. Identification of the compounds may help to develop new effective antimicrobial agent(s). Further researches on purification, characterization and toxicology of the active compounds are needed.

1. Introduction

Infectious diseases and drug resistance have been becoming a serious concern for successful treatment. Food safety, food packaging and storage shelf life are burning issues in food industry. Scientists are looking for new, effective, safe and environment friendly resources for preventing and curing of infection as well as flavoring and preserving food materials. Medicinal plants have been providing huge number of highly effective drugs. These plants are attractive for discovery of new molecular entities due to their largely untapped chemical diversity [1]. For primary health care, about 80% of the world's population rely on traditional medicines (plant extracts). In India, almost 95% of the prescriptions were based on Unani, Ayurveda, homeopathy and Siddha, the traditional systems of treatment [2]. Plants essential oils are natural compounds that have multi-purpose usages [3]. In pharmaceutical industry, the oil has been used due to its anticancer, antinociceptive, antiphlogistic, antiviral, antibacterial and antioxidant properties [4]. It has other uses in food and cosmetic industry [3,5,6].

Trigonella foenum-graecum L. (*T. foenum-graecum*) is an annual, self pollinating, diploid legume plant underneath the family of Fabaceae. It is found in Eastern Mediterranean to Central Asia and Ethiopia, and produced in bulk in Pakistan, India and China [7]. *Trigonella* seeds or Fenugreek is known generally as the dried ripe seeds of *T. foenum-graecum*. It has pungent aromatic properties and is often used to flavor homes [8,9]. In India, Egypt and Yemen fenugreek seeds are used as condiment and food among the general population. Leaves are consumed widely in India as a green, leafy vegetable. They are rich source of calcium, iron, β-carotene

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and other vitamins [10]. Use as herbal medicine in many parts of the world is the traditional application of *T. foenum-graecum*. Alkaloid, yellow coloring matter, tannic acid, diosgenin, vitamin A, fixed and volatile oils are the components of fenugreek seeds [11].

This study aimed to extract essential oil, identify the compounds from the oil using gas chromatography mass spectrometry (GC–MS) analysis and evaluate the antibacterial activity of the oil.

2. Materials and methods

2.1. Essential oil extract preparation

The healthy and mature seeds of *T. foenum-graecum* were collected from the plants at Jessore District in Bangladesh in February 2014. Seeds were washed for 20 min with running tap water and finally rinsed two times with distilled water. Then the seeds were placed in oven at 40 °C for 4 days for drying and avoiding daylight exposure to forestall loss of active components. Dried seeds were grinded and kept in air tight vials. Hydrodistillation of essential oil from seeds powder was conducted using Clevenger extractor [12]. It yielded 0.285% (v/w) of the oil. Obtained essential oil was dried over anhydrous sodium sulfate (Na_2SO_4). Finally it was stored at −4 °C for the test of antimicrobial activity and GC–MS analysis.

2.2. Test organisms

A total of seven organisms (bacteria) were tested for the antimicrobial activity. Among them *Sarcina lutea* (IFO 3232) (*S. lutea*) and *Bacillus subtilis* (IFO 3026) (*B. subtilis*) are Gram positive. The other organisms *Xanthomonas campestris* (IAM 1671) (*X. campestris*), *Escherichia coli* (IFO 3007) (*E. coli*), *Klebsiella pneumonia* (ATTC 10031) (*K. pneumonia*), *Proteus vulgaris* (MTCC 321) (*P. vulgaris*) and *Pseudomonas denitrificans* (KACC 32026) (*P. denitrificans*) are Gram negative.

2.3. Determination of antibacterial activity of essential oil

The antibacterial activity was tested out by disc diffusion method [13]. Disks with 6 mm in diameter of Whatman No. 1 filter paper were used. Briefly, 150 μL suspension of individual test microorganism was spread homogenously on each plate of mannitol salt agar media. Each disk was soaked with 150 μL of essential oil and placed on the microbial lawns. Two disks were placed on each plate. The plates were incubated at 37 °C for 24 h and the inhibition zones in mm were checked. Commercial antibiotic disks of gentamicin (10 μg/disc), chloramphenicol (30 μg/disc), ciprofloxacin (5 μg/disc), erythromycin (15 μg/disc), co-trimoxazole (25 μg/disc), nalidixic acid (30 μg/disc), vancomycin (30 μg/disc), azithromycin (30 μg/disc), tetracycline (30 μg/disc), cefuroxime (30 μg/disc), cloxacillin (1 μg/disc), ceftazidime (30 μg/disc), ampicillin (25 μg/disc), cefotaxime (30 μg/disc) were also tested for their activity against these microbes. The tests were replicated three times and the data were presented in average.

2.4. GC–MS analysis

GC–MS analyses were carried out with an Agilent7890/5975B-GC/MSD (Palo Alto, CA, USA) equipped with a HP-5 MS capillary column (30 m × 0.25 mm, i.d. 0.25 mm) and a HP 5975B mass selective detector. The reaction was carried out according to Sun and coworkers [4]. The sample was diluted as 1/10 in ether. One micro litter of diluted sample was injected manually with split ratio of 40:1. Then 70 eV was used for electron ionization for GC–MS detection. At first the oven temperature was kept at 50 °C for 3 min. Then the temperature was gradually increased to 250 °C at a 3 °C/min rate and held at 250 °C for 4 min. Temperature 220 °C and 250 °C were injector and MS transfer line temperatures respectively. Helium at flow rate of 1 mL/min was used as carrier gas. The components were identified based on the comparison of the retention time and the mass spectra with those in the NIST98 GC–MS library and those in the literature data [14].

2.5. Statistical analysis

All the experiments were carried out in triplicates. Results were expressed as mean ± SE of three independent experiments (n = 3).

3. Results

3.1. Antibacterial activity of essential oil

The essential oil exhibited antibacterial activity against both Gram negative and Gram positive bacteria of tested strains. Disks (6 mm) containing 150 μL essential oil were subjected to seven microbial strains individually. Among the tested microbial strains, strong inhibition effect was found against *P. denitrificans* [(15.0 ± 0.7) mm], *P. vulgaris* [(15 ± 0) mm], *E. coli* [(15 ± 0) mm] and *B. subtilis* [(14.0 ± 2.8) mm]. *X. campestris* [(12.5 ± 0.7) mm] is susceptible to the oil, whereas *S. lutea* [(9.0 ± 1.4) mm] and *K. pneumonia* [(8 ± 0) mm] are relatively less susceptible (Figure 1). The commercial antibiotic disks gentamicin, chloramphenicol, ciprofloxacin, erythromycin, co-trimoxazole, nalidixic acid, vancomycin, azithromycin, tetracycline, cefuroxime, cloxacillin, ceftazidime, ampicillin, cefotaxime were also tested against the microbes. It was found that *B. subtilis* is susceptible to all tested antibiotics. Cefuroxime, cloxacillin and ceftazidime were less effective to the tested organisms (Table 1).

3.2. Chemical composition of essential oil of T. foenum-graecum

The GC–MS analysis of the *T. foenum-graecum* essential (volatile) oil led to identification of 14 different compounds. The total compounds percentage was 80.96%. Decane, 5,6-bis (2,2 dimethylpropylidene), hexadecanoic acid, methyl ester, dihydro methyl jasmonate, Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), 5-Fluoro-1,1,3,3-tetramethyl-1,3-dihydroisobenzofuran, octadecanoic acid, methyl ester, oxiraneoctanoic acid, 3-octyl-, methyl ester, cis- were major compounds (Table 2); and the minor compounds were hexadecanoic

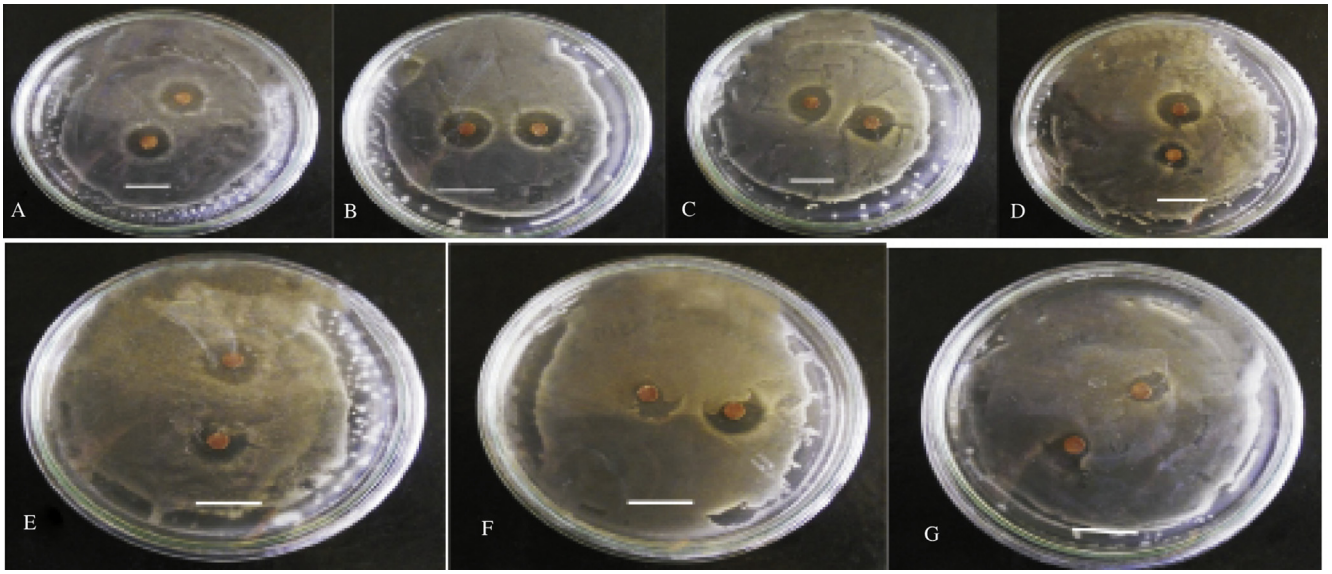

Figure 1. Inhibition zone of *T. foenum-graecum* essential oil against microbes.
A: *P. denitrifican*; B: *P. vulgaris*; C: *E. coli*; D: *B. subtilis*; E: *X. campestris*; F: *S. lutea*; G: *K. pneumonia*. The length of white straight line is 15 mm.

Table 1
Commercial antibiotics against the tested organisms.

Bacterial strain	Inhibition zone (mm)													
	GEN	C	CIP	E	COT	NA	VA	AZM	TE	CXM	COX	CAZ	AMP	CTX
B. subtilis	27.00 ± 0.20	35.0 ± 0.3	29.0 ± 0.3	38.0 ± 0.4	19.0 ± 0.5	22.0 ± 1.1	27.0 ± 0.2	26.0 ± 0.1	28.0 ± 1.2	21.0 ± 0.5	26.0 ± 0.8	21.0 ± 1.1	7.0 ± 0.6	23.0 ± 0.5
S. lutea	33.00 ± 0.40	18.0 ± 0.5	31.0 ± 1.0	31.0 ± 0.2	14.0 ± 0.8	24.0 ± 1.7	16.0 ± 0.7	34.0 ± 0.4	35.0 ± 0.9	12.0 ± 0.7	13.0 ± 0.5	–	17.0 ± 0.9	24.0 ± 0.7
X. campestris	18.00 ± 0.17	20.0 ± 1.2	33.0 ± 0.0	16.0 ± 0.0	8.0 ± 0.2	21.0 ± 0.3	–	28.0 ± 0.3	17.0 ± 0.7	–	–	–	12.0 ± 0.7	25.0 ± 0.2
E. coli	28.00 ± 0.22	24.0 ± 0.6	28.0 ± 9.0	22.0 ± 0.7	21.0 ± 0.6	22.0 ± 0.5	20.0 ± 0.9	29.0 ± 0.8	30.0 ± 1.1	–	21.0 ± 1.1	–	–	33.0 ± 0.0
K. pneumonia	21.00 ± 0.10	25.0 ± 0.9	30.0 ± 8.0	21.0 ± 0.5	24.0 ± 0.3	18.0 ± 0.9	21.0 ± 0.4	26.0 ± 0.6	21.0 ± 0.5	–	–	–	–	31.0 ± 0.3
P. vulgaris	20.00 ± 0.00	23.0 ± 0.3	27.0 ± 0.5	17.0 ± 0.0	21.0 ± 0.9	21.0 ± 0.7	17.0 ± 0.1	22.0 ± 0.2	22.0 ± 0.2	–	16.0 ± 0.2	–	–	21.0 ± 0.4
P. denitrificans	28.00 ± 1.10	26.0 ± 1.3	29.0 ± 0.2	18.0 ± 1.1	16.0 ± 0.2	19.0 ± 0.6	16.0 ± 0.0	21.0 ± 0.3	24.0 ± 0.8	–	17.0 ± 0	–	6.7 ± 0.4	18.0 ± 0.1

GEN: Gentamicin; C: Chloramphenicol; CIP: Ciprofloxacin; E: Erythromycin; COT: Co-trimoxazole; NA: Nalidixic acid; VA: Vancomycin; AZM: Azithromycin; TE: Tetracycline; CXM: Cefuroxime; COX: Cloxacillin; CAZ: Ceftazidime; AMP: Ampicillin; CTX: Cefotaxime.

Table 2
Chemical composition of essential oil of *T. foenum-graecum*.

Peak no.	Retention time (min)	Area (%)	Name of the compound	Formula
1	27.678	1.66	1,2,3,4-Tetrahydroisoquinolin-6-ol-1-carboxylic acid	$C_{10}H_{11}NO_3$
2	29.147	1.86	cis-Calamenene	$C_{15}H_{22}$
3	32.743	3.54	5-Fluoro-1,1,3,3-tetramethyl-1,3-dihydroisobenzofuran	$C_{12}H_{15}FO$
4	34.404	10.99	Dihydro methyl jasmonate	$C_{13}H_{22}O_3$
5	36.135	19.58	Decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)-	$C_{20}H_{38}$
6	37.855	2.95	Murolan-3,9(11)-diene-10-peroxy	$C_{15}H_{24}O_2$
7	41.399	1.82	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$
8	44.214	18.81	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
9	44.389	5.81	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a;1′,2′-d]pyrazine	$C_{14}H_{22}N_2O_2$
10	44.622	3.63	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$
11	46.499	2.84	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
12	49.897	1.14	6-Octadecenoic acid	$C_{18}H_{34}O_2$
13	50.742	3.28	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$
14	56.069	3.05	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	$C_{19}H_{36}O_3$

acid; ethyl ester; cis-calamenene; 6-Octadecenoic acid; 2-pentadecanone, 6,10,14-trimethyl; 1,2,3,4 Tetrahydroisoquinolin-6-ol-1-carboxylic acid and Murolan-3,9(11)-diene-10-peroxy.

4. Discussion

Fenugreek seeds are rich source of polyphenols (apigenin, kaempferol, quercetin glycosides) and flavonoids (vitexin, tricin, naringenin, quercetin and tricin 7-O-b-D-glucopyranoside) [15,16]. Alluri and Majumdar studied methanol extract of *T. foenum-graecum* seeds powder [17]. They found antimicrobial activity with inhibition zones against bacteria (*Staphylococcus aureus*, *Bacillus cereus*, methicillin resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*) in range from (7.30 ± 0.15) mm to (16.90 ± 0.28) mm and fungus (*Candida albicans, Trichophyton rubrum* and *Aspergillus flavus*) in range from (8.10 ± 0.21) mm to

(19.60 ± 0.28) mm depending on different concentration of extract [17]. Omezzine and co-workers reported inhibitory activity of organic solvent extract from *T. foenum-graecum* against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Fusarium oxysporum* f. sp. *lycopersici* [18]. Our study interprets similar result to that of previous work for common microbe *E. coli*. We found inhibitory zone ranging from (8 ± 0) mm to (15.0 ± 0.7) mm against *P. denitrificans*, *P. vulgaris*, *E. coli*, *B. subtilis*, *X. campestris*, *S. lutea* and *K. pneumonia* (Figure 1). The antimicrobial activity may be due to the combined effect of phenolic compounds, alkaloids, tannins, flavonoids and terpenoids present in oil.

We performed GC–MS analysis of essential oil obtained from hydrodistillation of seeds powder and obtained 14 peaks, each for individual compound (Table 2). Kenny *et al*. studied solid liquid sequential extraction (hexane, dichloromethane, methanol and water) of *T. foenum-graecum* seeds powder [19]. They quantified 18 phenolic compounds by using ultra-performance liquid-chromatography–mass spectrometry.

It is necessary to introduce new antimicrobial agent to resist antibiotic resistance and for effective control of infections. This study showed the antibacterial activity of essential oil of *T. foenum-graecum* with many biological active compounds. It may help to identify and develop new effective antimicrobial agent. Further study is needed to purify and characterize the active compounds. Toxicological study is also needed before using it as pharmaceutical ingredient.

Conflict of interest statement

We declare that we have no conflict of interest.

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