GC-MS Analysis and Antibacterial Activity of *Trigonella foenum-graecum* against Bacterial Pathogens

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ABSTRACT

Objective: The purpose of this study was to investigate the chemical composition of essential oil through GC-MS and look for new antibacterial drug agents from *T. foenum graecum*. **Methods:** Bacterial strains *Sarcina lutea* IFO 3232, *Bacillus subtilis* IFO 3026, *Xanthomonas campestris* IAM 1671, *Escherichia coli* IFO 3007, *Klebsiella pneumoniae* ATTC 10031, *Proteus vulgaris* MTCC 321 *and Pseudomonas denitrificans* KACC 32026 were used to measure the antibacterial activity by disc diffusion assay and therefore the chemical composition of essential oil through GC-MS. **Results:** GC–MS analysis of the essential oil from *T. foenum graecum* revealed the presence of fourteen different compounds of that Decane, 5, 6-bis (2, 2 dimethylpropylidene), (E,Z)-(19.58%); Hexadecanoic acid, methyl ester (18.81%); 5, 10-Diethoxy-2, 3, 7, 8-tetrahydro-1H,6H-dipyrrolo[1, 2-a;1', 2'-d] pyrazine (5.81%) and Octadecanoic acid, methyl ester (3.28%) were the major compounds and therefore the minor compounds were Oxiraneoctanoic acid, 3-octyl, methyl ester; Hexadecanoic acid, ethyl ester; 6-Octadecenoic acid; Cis-Calamenene; 2-Pentadecanone, 6, 10, 14-trimethyl; 1, 2, 3, 4 Tetrahydroisoquinolin-6-ol-1-carboxylic acid and Murolan-3, 9(11)-diene-10-peroxy. The organic extracts (300 µg/disk) of *T. foenum-graecum* displayed antibacterial activity against *Bacillus subtilis* IFO 3026, *Sarcina lutea* IFO 3232, *Xanthomonas campestris* IAM 1671, *Proteus vulgaris* MTCC 321 *and Pseudomonas denitrificans* KACC 32026 with their respective zones of inhibition of 6.5 ± 0 to 8 ± 1.7 mm and the essential oil (150 µl/disk) displayed far greater potential antibacterial activity compare to those extracts against all experimental bacteria with their respective zones of inhibition of 8 ± 0 to 15 ± 0.7 mm. **Conclusion:** This experiment showed a great potential of antibacterial activity against gram positive and gram negative bacteria. The results of this study counsel that *T. foenum-graecum* might have potential use as antibacterial agents.

Key words: *T. foenum-graecum*, Antibacterial activity, Disc diffusion assay, GC-MS.

Key Messages: Essential oil of *T. foenum-graecum* could have a possible use in food and pharmaceutical industry as antibacterial agent.

INTRODUCTION

Infectious diseases caused by infective microorganisms like bacteria, fungi, viruses or parasites represent a vital pathological state and one in every of the most causes of morbidity and mortality are included in the list of the ten leading causes of death worldwide.[1,2]

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In the recent years, the emergence of resistant Gram negative and Gram positive bacteria presents a serious challenge for the antimicrobial medical aid and drastically narrows the treatment choices of human infections.[3] So, there's an imperative ought to resolve newer, safer and more effective natural or artificial antibacterial drug molecules so as to fight the emergence of those new resistant.

Natural sources are a serious source of latest medicine. From the ancient times yet plants are used as different disease curative agent and nowadays around 70% of the drugs used as medicine supported natural compound as a result

of they contain parts of therapeutic worth.[4,5] World Health Organization reported that 80% of the world populations especially in developing countries have used herbal products to satisfy their primary health care.[4] Considering the high cost of the synthetic drugs and their facet effects, wide varieties of natural plants are often thought of as a secure very important supply for anti-microbial agents.[6]

T. foenum-graecum is an annual, self pollinating, diploid legume plant underneath the family of Fabaceae and contained several nutrients.[7-9] The genus name *'Trigonella'* comes from Latin acceptation 'little triangle' indicating the triangular shape of the small yellowish-white flowers.[10]

T. foenum-graecum relieves congestion, fights against infection, useful in lowering fever, accustomed treat organic process ulcer, flush out harmful toxins from abdomen, beneficial in colic, digestion, flatulence, dysentery, diarrhoea, dyspepsia, dropsy, enlargement of liver and spleen and rickets.[10,11] It is accustomed stop blackheads, pimples, wrinkles and effective treatment of skin issues like burns, boils, eczema, employed in hairdressing preparations as a cosmetic, helps in weight loss.[11]

Furthermore, it's helpful for head colds, influenza, catarrh, constipation, bronchial complaints, asthma, emphysema, pneumonia, pleurisy, tuberculosis, sore throat, laryngitis, hay fever and sinusitis.[10]

The plant *T. foenum-graecum* possesses numerous medicative properties: antidiabatic antiplasmodic, hypolipidemic, medication, anthelmintic, medicament, inhibitor, antifungal, etc helps to beat several physiological complications.[6,11]

In this research, we examined the antibacterial activity of different organic extracts and essential oil from *T. foenum-graecum* and analyzed the chemical composition of the essential oil from *T. foenum-graecum* through GC-MS for the discovery of potential antibacterial agents that might be used for the management of bacterial infectious diseases.

MATERIALS AND METHODS

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. Seed were washed for fifteen minutes 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. It yields 0.245 % (v/w) of the oil. The 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.

Test organisms

Total seven organisms (bacteria) were subjected for the test of antimicrobial activity. Among them *Sarcina lutea (*IFO 3232) and *Bacillus subtilis* (IFO 3026)*, are gram positive. The other organisms Xanthomonas campestris* (IAM 1671), *Escherichia coli* (IFO 3007)*, Klebsiella pneumonia*e (ATTC 10031)*, Proteus vulgaris* (MTCC 321) *and Pseudomonas denitrificans* (KACC 32026) *are gram negative. All* organisms were obtained from the Microbiology laboratory of Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh.

Determination of antibacterial activity of essential oil

The antibacterial activity was carried out by disc diffusion method.[5] Six (6) mm diameter disks of Whatman No 1 filter paper were used. Briefly, 150 µl suspension of individual test microorganism was spread homogenously on each plate of MS agar media. Each disk was employed to soaked 150 µl of essential oil and placed on the microbial lawns. Four disks were placed on each plate. The plates were incubated at 37°C for 24 hrs and checked the inhibition zones in millimeters (mm). The tests were replicated three times and the data were presented in average.

Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analyses were carried out with an Agilent 7890/5975B-GC/MSD (Palo Alto, CA, USA), equipped with a HP-5 MS capillary column (30m x 0.25 mm, i.d.; 0.25 mm) and a HP 5975B mass selective detector. The sample was diluted as 1/10 in ether. One micro litter (1 µl) of diluted sample was injected manually with as plit ratio of 40:1. Seventy (70 eV) electro volt energy 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 gradually increased to 250°C at a 3°C/min rate and held at this temperature for 4 min. Temperature 220°C and 250°C were Injector and MS transfer line temperatures accordingly. Helium gas at flow rate of 1 mL/min was used as carrier. The components were identified based on the comparison of the irrelative retention time and their mass spectra with those in the NIST98 GC–MS library.

RESULTS

Antibacterial activity of plant extract and essential oils

The *in vitro* antibacterial activity of essential oil against the employed bacteria was qualitatively assessed by the presence or absence of inhibition zones. The essential oil exhibited antibacterial activity against all two Gram-positive and five Gram-negative bacteria at the concentrations of 150 µl diluted with methanol. The essential oil exhibited a potent inhibitory effect against *S. lutea, B. subtilis, E. coli*, *P. denitrificans*, *K. pneumoniae and X. campestries* with diameter of inhibition zones ranging from of 8 ± 0 to 15 ± 0.7 mm, as shown in table. Essential oil showed the strongest antibacterial effect against *P. denitrificans* (15 ± 0.7), *P.vulgaris* (15 ± 0), *K. pneumonia* (15 ± 0), *B. subtilis* (14 ± 2.8) and good activity against *X. campestris* (12.5 ± 0.7) and moderate activity against *S. lutea* (9 ± 1.4) and *E. coli* (8 ± 0) (Figure 1).

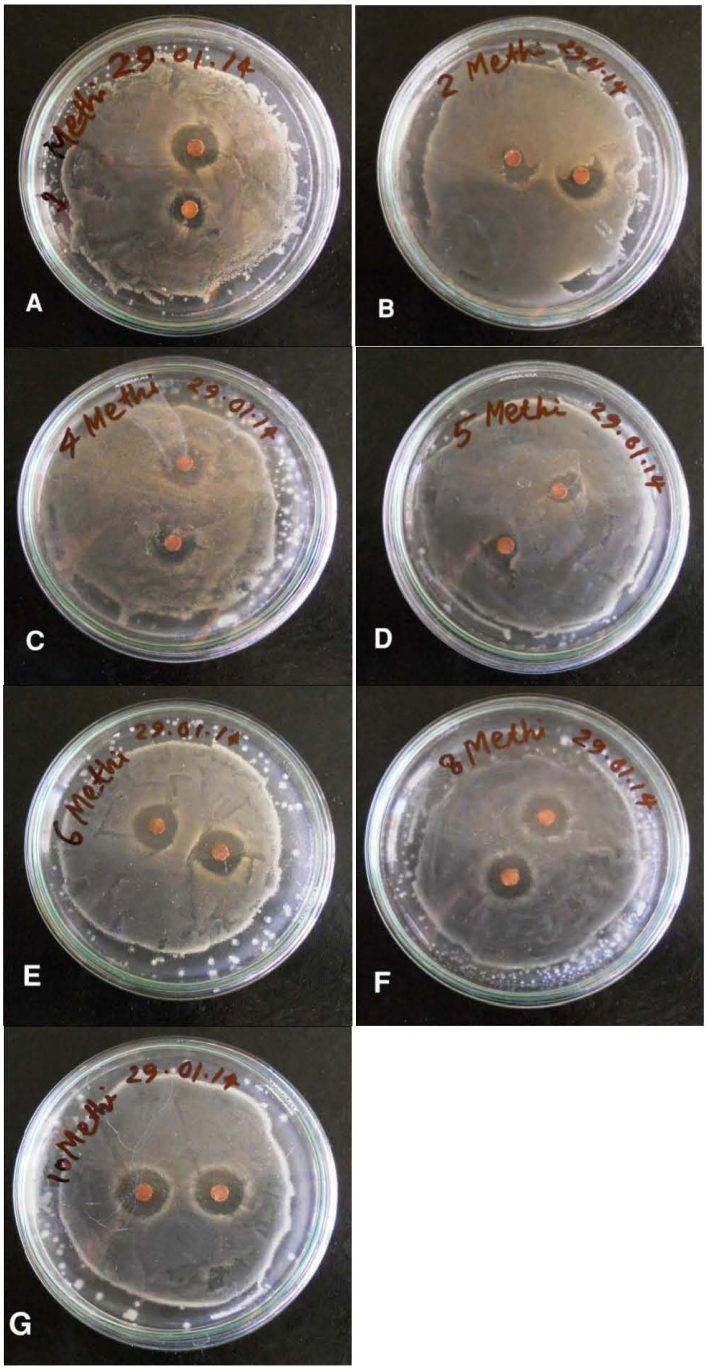

Figure 1: Zone of inhibition of *T. foenum-graecum* essential oil against (A) *B. subtilis* (B) *S. lutea* (C) *X. campestris*, (D) *K. pneumoniae*, (E) *E. coli*, (F) *P. denitrificans* and (G) *P. vulgaris*

Table 1: Chemical composition of essential oil of *T. foenum-graecum*

Peak no	Retention time	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, 6,10,14trimethyl-	$C_{18}H_{36}O$
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-	$C_{19}H_{36}O_3$

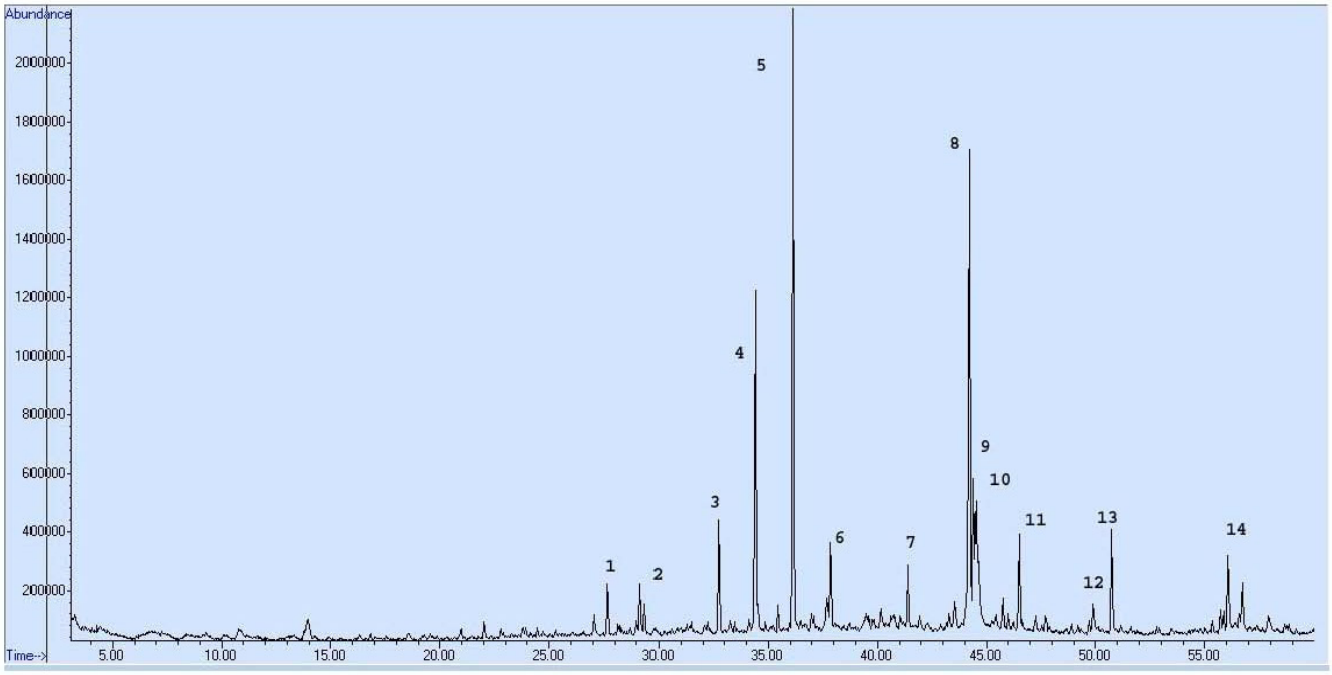

Figure 2: GC-MS chromatograph of essential oil extract of *T. foenum-graecum*

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

GC–MS analysis of the oil led to the identification of 14 different compounds. List of identified are shown in Table 1. The major compounds detected in the essential oil were Decane, 5, 6-bis (2, 2 dimethylpropylidene), (E, Z)-(19.58%); Hexadecanoic acid, methyl ester (18.81%); Octadecanoic acid, methyl ester (3.28%); 5, 10-Diethoxy-2, 3, 7, 8-tetrahydro-1H, 6H-dipyrrolo [1, 2-a; 1', 2'-d] pyrazine (5.81%) and the minor compounds were Hexadecanoic 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 (Figure 2).

DISCUSSION

Fenugreek seeds are rich source of polyphenols (apigenin, kaempferol, quercitin gylcosides) [12]Flavonoids (vitexin, tricin, naringenin, quercetin and tricin 7-O-b-D-glucopyranoside).[13] Alluri and Majumdar studied methanol extract of *T. foenum-graecum* seeds powder. They found antimicrobial inhibition zones against bacteria (*Staphylococcus aureus, Bacillus cereus,* Methicillin resistant *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*) in range from 7.3 ± 0.15 mm to 16.9 ± 0.28 mm and fungus (*Candida albicans Trichophyton rubrum* and *Aspergillus flavus*) in range from 8.1 ± 0.21 mm to 19.6 ± 0.28 mm depending on different concentration of extract.[14] Omezzine and co-wprkers[15] reported inhibitory activity of organic solvent extract from *T. foenum-graecum* against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *lycopersici.* Our study interprets similar result with previous work for common microbe *E. coli*. We found inhibitory zone ranging from 8 ± 0 mm to 15 ± 0.7 mm against *Pseudomonas denitrificans*, *Proteus vulgaris*, *Escherichia coli*, *Bacillus subtilis, Xanthomonas campestris, Sarcina lutea* and *Klebsiella pneumonia.* The antimicrobial activity may be due to the combine effect of phenolic compounds, alkaloids, tannins, flavonoids, terpenoids present in oil.

We studied GC-MS analysis of essential oil obtain from hydro distillation of seeds powder and obtained fourteen picks**,** each for individual compound. Kenny *et al*[16] studied solid liquid sequential extraction (hexane, dichloromethane (DCM), methanol and water) of *T. foenum-graecum* seeds powder. They quantified 18 phenolic compounds by using UPLC-MS.

CONCLUSION

The essential oil showed a great potential of antibacterial activity against gram positive and gram negative. Results of our study recommend the possibility of using the essential oil of *T. foenum-graecum* as natural antibacterial in food or pharmaceutical industry. However, further research is needed in order to establish the real application of *T. foenum-graecum* essential oil in food or pharmaceuticals.

It can be concluded that the plants investigation have opened up a new perspective in pharmaceutical research and they can be used for the development of potential, novel antibacterial agents for the treatment of bacterial diseases. In this direction, *T. foenum-graecum* can be pursued further as it is a promising source of antibacterial activity.

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