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Chemical Characterization and Antibacterial Activity of Ethanolic Extract of *Trigonella foenum-graecum* (Fenugreek) Seeds

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The aims of the present study were to test the antibacterial activity and chemical composition of *Trigonella foenum-graecum* extract. The extract was obtained using 50% aqueous - ethanol extraction solution to extract *T. foenum-graecum* seeds. The extract was prepared and evaluated for antimicrobial activity against six bacterial strains by determining minimum inhibitory concentration (MIC). The results revealed that the 50% aqueous - ethanol extract is potent in inhibiting bacterial growth of both gram-positive and gram negative bacteria. The chemical composition of fenugreek was analyzed by gas chromatography/mass spectroscopy (GC/MS). The hydroxymethyl furfural, gingerone, α-curcumene, bergamotene, and gingerol were the highest abundant compounds out of total 31 compounds were identified in the fenugreek extract.

Key words: GC-MS, Phytochemicals, Antibacterial.

Plants are an essential part of human society since the civilization started. Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. In the last decades, various plant extracts have been the focus of great interest from researchers because they represent natural resources of new antibacterial agents with possibly novel mechanisms of action. The potential use of these products as an alternative for the treatment of several infectious diseases has been extensively screened. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[5]. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds.

Trigonella foenum-graecum, known as fenugreek, has long been used as a spice and an herbal remedy across the Middle East. People

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harvest and roast dried seeds of the plant for food flavoring and medicinal purposes. Various components of fenugreek are responsible for its beneficial effects, including blood sugar regulation and cholesterol reduction.[1]

Trigonelline is known to have some hypoglycemic effect. The effect of an alkaloid extract of fenugreek dried seeds (*Trigonella foenum-graecum L.*) on blood glucose, serum insulin, serum lipid profile and lipid peroxidation in addition to histological and histochemical study of liver and kidney in streptozotocin induced diabetic albino rats have been studied [2]. Further investigations concerning the protective effect of *Trigonella foenum-graecum* L. on the histological structures and function of liver, kidney and pancreas of induced diabetes were studied [3-6].

A growing interest for the rapid extraction from different matrices and for the precise analyses of annual plants compounds increased the need for optimization of the experimental design, to obtain better recoveries, low solvent consumption and reduced extraction times [7-9]. In the latest years the interest for the study of the organic compounds from plants and their activity has increased. A lot of extraction methods and analytical methods as spectrophotometry, high performance liquid chromatography, capillary electrophoresis, gas chromatography (GC) with flame ionization detection (FID), gas chromatography–mass spectrometry (GC–MS) are developed for plant active compounds study. The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC/MS an ideal technique for qualitative and quantitative for volatile and semi-volatile compounds. In addition, the use of a proper extraction method is needed.

MATERIALS AND METHODS

Preparation of extracts

The Fresh fenugreek seeds, was purchased from a local market at Riyadh, Saudi Arabia. About 100 g of fenugreek seeds were crushed in a mortar. Exactly 10 g of fenugreek seeds powder were soaked in 100 ml of 50% ethanol water with agitation at 40°C. The EtOH:H_20 extract was then filtered, evaporated under steam of nitrogen using sample concentrator model Techne DB.3 (Techne, UK). The yield of the aqueous-ethanol extract was 1.8 g. Aliquot of the extract was resolved in ethanol to a final concentration of 1.0 mg/mL.

Analysis and identification of compounds

The chemical composition of fenugreek extract was identified according to Priya *et al.,* 2011. Chemical identification of components was assigned by matching their mass spectra with Wiley and NIST library data, standards of the main components and comparing their Kovats Retention Indices (KRI) with reference libraries (Abdulmalik *et al*. 2005; Zaldivar and Ingram 1999) and from the literature. The component concentration was obtained by semi-quantification by peak area integration from GC peaks and by applying the correction factors.

Microorganisms

Six bacterial strains used in this study, including *Staphylococus aureus* ATCC 25923, *Bacillus cereus* ATCC 7004 as Gram positive bacterium. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 53657, *Proteus vulgaris* ATCC 29905 *and Salmonella typhi* ATCC 0650 as Gram negative bacterium. These organisms were obtained from ATCC (American Type and Collection Center). The bacteria rejuvenated in Mueller-Hinton broth MHB (Difco, USA) at 37°C for 18 h and then stocked at 4°C in Mueller-Hinton Agar MHA (Sigma, USA).

Antibacterial assay

The method reported by Baqir *et al.,* 1985[12] has been adopted. The tests were run in triplicate. Petri plates (23×23 mm) were prepared with Trypticase soy agar and an adequate amount of inoculum was flooded onto each plate, excess inoculum was removed and the plates were dried for 30 min at 37°C. Holes (6 mm diameter) were made in the inoculated agar and filled with samples of plant extracts, plates were incubated for 24 h at 37°C. Inhibition zones when present were measured in millimeter (Table 2).

Antimicrobial activity assay

The Antimicrobial activities were determined by Kirby Bauer Disc diffusion method described by Bauer *et al.,*. 1966[13]. The extracts were prepared and the sterile blotting paper disc (5 mm) was soaked in the diluted extract in two different final concentrations (50 µl and 100 µl/disc). The prepared disc were dried in controlled temperature

(at 37 °C overnight) to remove excess of solvent and used for study.

RESULTS AND DISCUSSION

The chemical composition of Fenugreek seeds is given in Table 1. These seeds are a rich source of fiber and protein. The fiber may be further classed as gum (gel fiber) and neutral detergent fiber. The protein fraction contains the amino acid 4-hydroxyisoleucine, which has been proven to stimulate insulin production. Whole Fenugreek seeds also contain 4.8% saponins. Fenugreek seed saponins are of steroidal nature (type furostanolsaponins) with diosgenin as the principal steroidal saponin.

Fenugreek 50% aqueous-ethanol were screened for their antimicrobial activity at two different concentration (50 ul and 100 ul) against *S. aureus, B. cereus, E. coli, P. aeruginosa, K. pneumonia* and *S. typhi*. The results were given in table 2 showed that; the extract inhibited all the tested bacteria with varied level of inhibition. These results suggesting a broad antimicrobial activity *T. foenum-graecum* leaves extracts in a concentration-dependent manner. Among various concentration, maximum in vitro inhibition of the tested bacterial strains *B. cereus, P. aeruginosa, K. pneumonia* and *E. coli* was achieved in solvent extract in 100 ul concentration with zone of inhibition 9±0.2, 8±0.8, 7±0.7, and 6±0.8 mm respectively. The most resistant microorganisms were *S. typhi* and *S. aureus* with 5±0.2 and 5±0.4 mm zone of inhibition.

The MIC of the solvent extract and fractionation residues were determined using broth dilution technique. The results of MIC of the solvent *T. foenum-graecum* leaves extracts with concentrations ranging from 0.25-2 mg/ml-1. Values are absorbance at 600 nm. Values are means of three independent replicates. The extract was found to inhibit the growth with maximum activity against *P. aeruginosa* and *B. cereus* whereas poor activity was noted against *E. coli, S. typh* and *K. pneumonia.* This result suggests support the earlier findings of Omoloso and Vagi, 2001 who reported maximum activity of T. foneum-graecum against 26 bacterial pathogens[15]. Also, a previous study by Premanath *et al.,* (2011) indicated that the solvent extract of *T. foenum-graecum* showed broad spectrum of antibacterial activity against pathogenic bacteria.[16] Sudharsan *et al.,* 2011 observed a strong activity of *T. foenum-graecum* against *B. subtilis* and *P. aeruginosa* where as weak activity in *S. aureus* and *E. coli.*[17] On the other hand Dash *et al.,* 2011 reported that the methanol and acetone extracts of fenugreek showed antimicrobial activity against *Pseudomonas* spp. whereas acetone extract of spices exhibited highest activity against *E. coli.* Acetone extract of Fenugreek showed no activity against *Salmonella typhi*.[18]

Nandagopal *et al.,* (2012) reported that the presence of alkaloids, anthracene glycosides, flavonoids, phenolics, saponins, tannin, volatile oils and phenolics in the fenugreek were responsible for the antibacterial activity.[19] Previous study by Isaac and Chinwe (2001) revealed that alkaloids along with tannins and saponins are responsible for antibacterial activity of the extract of Tetracapidium conophorum.[20] . This study pays attention for more concern and research to recognize the active compounds responsible for

Table 1. Proximate Composition (%) of Fenugreek Seeds (13)

Component	Whole Seeds
Moisture	9.0
Ash	3.0
Lipids	8.0
Protein	26.0
Starch	6.0
Total Fiber	48.0
Gum	20.0
Neutral Detergent Fiber	28.0

Table 2. Zone of inhibition activity (in millimeter) of different 50% aqueous-ethanol of Trigonellafoenumgraecum (50 and 100 µl concentration) against various microorganisms

Bacteria	Activity	
	100 ul	50 ul
S. aureus	5±0.4	5±0.4
B. cereus	9±0.2	6±0.0
E. coli	6±0.8	4±0.8
Ps. aeruginosa	8±0.8	4±0.8
K. pneumonia	7±0.7	3±0.7
S. typhi	5±0.2	5±0.2

the biological activity of the Fenugreek. Additional studies should be undertaken to explain the accurate mechanism of action by which extracts exert their antimicrobial effect.

A total of 34 compounds were recorded in solvent extracts as indicated in Table 4. 31 components were identified. Most of them playing a role in the odour of natural extracts. Some of these compounds are reported for the first time in fenugreek seeds. The major compounds characterized were Hydroxymethylfurfural(16.3%), Gingerone (12.0%), α-Curcumene (8.7%), Bergamotene (4.81%), and Gingerol (4.85). The structure of these compounds given in Table 5. Furfural is phenolic compound and one of the major flavour compounds. Furfural compounds was found to inhibit the cell growth and fermentation and used as antioxidant[11,21]. Furthermore, furfural derivatives have also been used for therapeutic purposes. For instance, Hydroxymethylfurfural is a potential candidate for treating sickle cell anemia [10]. Gingerol, has been found to possess many

Table 3. Identified compounds of 50% aqueous-ethanol extract of fenugreek

#	RT (min.)	Name	Formula	% of Total	RI (iu)
1	4.06	-	-	2.16	
2	4.107	-	-	2.45	
3	4.189		-	0.87	
4	5.237	Acetoxyacetic acid, 1-naphthyl ester	$C_{14}H_{12}O_4$	7.59	1884
5	5.278	(Propylsulfanyl)cyclopentane		5.16	
6	6.357	2-Ethyl-2-hexen-1-ol	$C_{16}H_{30}O_2$	1.34	
7	6.437	5-Isopropyldihydro-3(2H)-furanone	$C_7H_{12}O_2$	0.74	
8	6.659	5-Hydroxymethylfurfural	$C_6H_6O_3$	16.29	1176
9	7.538	4-(Hydroxyamino)-2-pyrimidinamine	$C_{10}H_{18O}$	0.75	1224
10	8.54	4-Vinylguaiacol	$C_9H_{10}O_2$	1.36	
11	9.452	Eugenol	$C_{10}H_{12}O_2$	1.25	1337
12	9.769	2-Propylphenol	$C_9H_{12}O$	3.52	1197
13	9.999	Hippeastrine	$C_{17}H_{17}NO_5$	0.78	2569
14	10.463	Vanillin	$C_8H_8O_3$	1.52	1403
15	10.602	Piperidine1-propenyl	$C_8H_{15}N$	1.94	1039
16	12.369	α-Curcumene	$C_{15}H_{22}$	8.68	1472
17	12.71	α-Bergamotene	$C_{15}H_{24}$	4.81	1407
18	12.903	Valencen	$C_{16}H_{26}$	2.06	
19	13.05	β-Bisabolene	$C_{15}H_{24}$	1.25	1500
20	13.488	Cedrene	$C_{15}H_{24}$	2.91	1415
21	13.58	Homovanillyl alcohol	$C_9H_{12}O_3$	0.60	1542
22	14.709	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	0.33	1683
23	15.245	Carbanilic acid, N-methyl-, ethyl ester	$C_{10}H_{13}NO_2$	3.56	1308
24	16.14	D-Germacrene	$C_{15}H_{24}$	1.32	1480
25	16.291	Elemol	$C_{15}H_{26}O$	1.56	1535
26	16.486	Gingerone	$C_{11}H_{14}O_3$	12.03	1648
27	16.88	β-Eudesmol	$C_{15}H_{26}O$	1.40	1644
28	17.773	Methyl 2,5-octadecadiynoate	$C_{19}H_{30}O_2$	2.03	2112
29	18.324	N-(4-Oxotricyclo[3.3.1.1[3,7]]dec-2-yl) acetamide	$C_{12}H_{17}NO_2$	0.52	
30	18.946	Methyl 2,5-octadecadiynoate	$C_{15}H_{22}O$	1.50	1763
31	19.944	Pyrimido[5,4-E][1,2,4]triazine-5,7 (6H,8H)-dione, 6-methyl-3-propyl	$C_9H_{11}N_5O_2$	0.92	1944
32	20.331	Diepicedrene-1-oxide	$C_{15}H_{24}O$	1.44	1551
33	21.543	Cedrenol	$C_{15}H_{24}O$	0.59	1604
34	29.508	Gingerol	$C_{17}H_{26}O_4$	4.82	2396

interesting pharmacological and physiological activities, such as anti-inflammatory, analgesic, and cardiotonic effects. Gingerone results from the thermal degradation of gingerols during extraction[22]. Curcumene and Bergamotene are sesquiterpene hydrocarbons. Fenugreek extract were found to contain small amounts of other important sesqui-terpenes (D-Germacrene, Valencen, and Cedrene). This in line with other investigators[23].

CONCLUSION

This study pays attention for more concern and research to recognize the active compounds responsible for the biological activity of the Fenugreek. 50% aqueous - ethanol extract is potent in inhibiting bacterial growth of both gram-positive and gram negative bacteria. The extract inhibited all the tested bacteria with varied level of inhibition. *B. cereus, Ps. Aeruginosa, K. pneumonia* and *E. coli.* were highly inhibited while *S. typhi,* and *S. aureus* were highly resistant. The hydroxymethylfurfural, gingerone, α-curcumene, bergamotene, and gingerol were the highest abundant compounds out of total 31 compounds were identified in the fenugreek extract

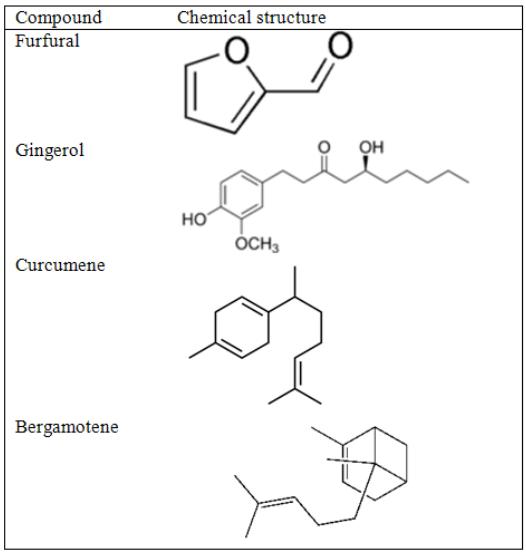

Fig. 1. Chemical structure of the most abundant compounds in ethanolic extract of fenugreek

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