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The biological activities of seeds extracts for *fenugreek* and *black cumin* and its inhibitory influences toward some pathogens

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Objective The aim of this study is about antibacterial activity of fenugreek and black cumin against some pathogens.
Methods The bioactive components of the seeds of fenugreek (*Trigonella Foenum-graecum* L.) and black cumin *(Nigella sativa)* are scrutinized by Gas Chromatography-Mass Spectrometry (GC/M technique, beta-D-glucopyranoside, methyl (35.60%), alpha-D-methyl (28.31), and diethyl phthalate (5.94%) such components found in methanolic fenugreek seed extract. On the other hand, the major components of methanolic black seed extracts are 9,12-octadecadienoic acid (Z,Z)-(50.16%), l-(+)-ascorbic acid 2,6-dihexadecanoate (10.75%), propylene glycol monooleate (9.61%), diethyl phthalate (3.32%), and phenol, 2-methyl-5-(1-methylethyl)-(0.17%). In this study, four types of pathogenic bacteria are involved *Streptococcus agalactiae, Proteus mirabilis, Enterococcus cloacae*, and *Escherichia coli*, which subjected to antibacterial tests using methanolic fenugreek and black cumin seed extracts at concentrations 50 and100 mg/ml.
Results The highest percentage inhibiting of methanolic fenugreek seed extract versus *Streptococcus agalactiae* is 13.5 mm at each conc. 50, 100 mg/ml. Furthermore, *E. coli* is the lowest effectiveness of black cumin seed extract, which reached 9.5, 7.5 mm at conc. 50 and 100 mg/ml, comparison with control.
Conclusion In this study, the presence of bioactive compounds as beta-D-glucopyranoside, alpha-D-mannopyranoside, methyl, diethyl phthalate,12-octadecadienoic acid (Z,Z)-, and l-(+)-ascorbic acid 2,6-dihexadecanoate in seeds of fenugreek and black cumin, which is associated with inhibiting effect against some pathogenic bacteria. The variations in chemical compound models can be used to distinguish among medicinal plants.
Keywords fenugreek, black cumin, GC-MS, antibacterial

Introduction

Fenugreek (*Trigonella Foenum-graecum* L.) and black cumin (*Nigella sativa)*, plants are widely dispersed all over the world and which owned by the family Fabaceae and Ranunculaceae, respectively. Fenugreek and black cumin are annual indigenous herbs, which spread by the cultivation on the eastern beaches of the Mediterranean region, Europe, Central and Western Asia and most urbane in India, Egypt, Morocco, and Iraq.[1–6] Fruit of fenugreek has moisture 8–10%, protein 15–28%, fat 6–12%, carbohydrates 35–45%, fiber 8–6%, ash 4–8%, and 0.3% essential oil. As well as, their seeds include vitamin A, niacin, thiamine, riboflavin, tryptophan, flavonoids, alkaloids exist as trigonelline, choline, gentianine, and carpaine. Seeds contain saponins present as main diosgenin. The essential oil of the fenugreek seeds is b-pinene, camphor, b-caryophyllene, and neryl acetate.[6] The seeds of *Black cumin* contain 30–35% stable oil and 0.5% of essential oil. Moisture 4%, protein 22%, fat 41%, fiber 8%, carbohydrate 17%, and ash 4.5%. Additional compounds are alkaloids (nigellicines and nigeledine), sterols, tannins, vitamins, and glucosides. The major components of the essential oil are *p*-cymene, thymoquinone, *a*-pinene, *b*-pinene. On the other hand, the major quinines are thymoquinone, dithymoquinone, and thymohydroquinone.[5,7] As a result of all of these existing compounds, they are considered to be old curative plants that have a vital role in human health, which give the plants many crucial activities, including antibacterial, antioxidant, antifungal, anticancer, hypoglycemic, and anti-inflammatory effects. It has been also universally used as a spice in a conventional food or it has been interfered to prepare for some functional foods.[1–4,6] The estimation of the biologically active constituents of medicinal plants is preferred by one of the chromatographs analysis methods and the most famous in this area is (gas chromatography-mass spectrometry (GC-MS) techniques, which depends on its work for the estimation of bioactive volatile compounds and essential oils.[8] Considering its therapeutic benefit, *fenugreek* and *black cumin* are chosen for their seeds methanolic extracts, which is a goal to verify its antimicrobial activity versus some gram (positive and negative) bacteria.

Materials and Methods

A collection of samples

The seeds of the fenugreek and black cumin are collected from local markets in the holy city of Kerbala. They are cleaned, grinded, and stored in the refrigerator until use. This experiment is conducted in the laboratories of the Faculty of Agriculture, University of Kerbala.

Chemicals

Methanol, diethyl sulfoxide (DMSO) (India). The media of Mueller Hinton Agar and Mueller Hinton Broth (Hi-media) is obtained from the equipped company.

Bacterial isolates

Strains of *Streptococcus agalactiae, Proteus mirabilis, Enterococcus cloacae*, and *Escherichia coli* are isolated from the patients with diarrhea or injuries, it has used the selective media to investigate or detect bacteria as MacConkey Agar and XLD Agar. The bacteria are also identified by the API 20E test of the intestinal family. It is got from the Research laboratory of the Department of Health Observation at a Kerbala city, Iraq. The nutrient agar (Hi-media) is utilized in the development of bacteria for 24 h at 37°C.

Elaboration of alcoholic extracts of seeds of the fenugreek and black cumin

A 20 g of powdered seeds, added to 70% methanol at a room temperature in shaker flask for 72 h. The remaining layer is treated with a double layer cloth and then centrifuged at 6000 g cycles for 3 min to obtain a pure filtrate and dried on the oven at 45°C to remove the excess solvent and save it in the refrigerator at a temperature of 4°C.[9–12]

Limitation of bioactive compounds by GC-MS technique

Bioactive compounds is diagnosed for each raw alcohol extracts after it has been dissolved 0.1 g of each extract in 10 ml of 99% ethanol to get a solution that can be injected with a device GC-MS (QP2010, Shimadzu Company, Japan).[11] It is examined at the Food Safety Unit, Department of Science, College of Agriculture, the University of Basra by Dr. Dhia F. Al-Fekaiki. Under the following circumstances: in auto-injector unit (AOC-20i+s). Injection volume 1 µL, viscosity comp. time: 0.2 s, pumping times: 5, injection port dwell time: 0.3 s, washing volume: 8 µL, solvent selection: only C; in the GC-2010 unit, column oven temp.: 40°C, injection temp.: 280°C, injection mode: split, flow control mode: pressure: 49.5 kPa, total flow: 34.0 mL/min, column flow: 1 mL/min, linear velocity: 36.1 cm/s, purge flow: 3.0 mL/min, split ratio: 30.0, high pressure injection: OFF, carrier gas saver: OFF, splitter hold: OFF, oven temp. program.

Elaboration of two concentrations for antibacterial from seed extracts

Elaboration of McFarland Solution: The solution is prepared with blending 0.5 ml of 1.75% barium chloride $BaCl_2.2H_2O$ and it is subjoined to 99.5 ml of 1% sulfuric acid H_2SO_4. The turbidity is tantamount to 1×10^8 colony formation unit (CFU)/ml. The colonies of the bacterial pathogen are transported by a loop to 4 ml of the peptone water and the conveyance continues until the turbidity of peptone water is equal to the turbidity of McFarland suspension.[13] It is elaborated two concentrations from a raw seed extracts by dissolving 1 g of raw extracts in 10 ml of DMSO to obtain a concentration of 100 mg/ml, and dissolving 1 g of extracts in 20 ml of DMSO to get a concentration of 50 mg/ml.[11]

Antibacterial Activity: The inoculant suspension of pathogens is spread on a Muller–Hinton agar plate, and allowed to dry for approximately 15 min. The steel device sterile borer is dug with a 25 µL perforation. The samples diffusion the drill. Then, the dishes are transferred to the incubator at a temperature of 37°C for 24 h. Then, it is measured by the inhibition zone (mm), the reduction of 4 mm the size of the hole. The cup method is used which is described by Ref. 14.

Rate	Temperature (°C)	Hold time (min)
–	40	1
10	200	1
10	300	2

Equilibrium time :1 min [GC Program], [GCMS-QP2010 Ultra], ion source temp: 200°C, interface temp: 280°C, solvent cut time: 2.50 min, detector gain mode: relative, detector gain: 0.69 + 0.10 kV, threshold: 0, If (Group 1—event 1-start time: 3.00 min, end time: 30.00 min, ACQ Mode: Scan, event time: 0.30 s, scan speed: 1666, start m/z: 20.00, end m/z: 500.00. After it getting a mass spectrum each compound is identified with curves separated by a NIST08 database.

Results and Discussion

The outcome of fenugreek and black cumin extracts are 17.5 g/100 g, 14.15 g/100 g dry matter of plants, respectively, the yield of seed extracts are based on many factors, including the method of extraction, a genetic condition, environmental conditions, and period of harvest, topographical origins.[6,16] Fig. 1 and Table 1 show that bioactive compounds in crud seed of fenugreek methanolic extract by GC-MS, is noticeable that 30 peaks have been separated from the ingredients of fenugreek seed extracts,[11] compounds have a dampening effect on the growth of bacteria as indicated in Table 1, which is based on a (*National Center for Biotechnology Information(NCBI). PubChem Compound Database*) under a classification paragraph. The major constituents are beta-D-glucopyranoside (35.60%), methyl, alpha-D-mannopyranoside, methyl (28.31%), and diethyl phthalate (5.94%), which is made of phenols and flavonoids components.[6] It belongs to, its antimicrobial, antibacterial effects, as well as it is used in the medication of different diseases.[6,15] Fig. 2 and Table 2 show that biological components in the raw seed of black seed methanolic extracted by GC-MS that be detected it 40 peaks is split for constituents. It relies on *NCBI. PubChem Compound Database*) under a classification paragraph that diagnosed 14 components, which inhibiting effectiveness toward pathogenic bacteria, which are significant constituents through the area occupied by the separated components, including emersol (50.16%), l-(+)-ascorbic acid 2,6-dihexadecanoate (10.75%), diethyl phthalate(3.32%), propylene glycol monooleate (9.61%), phenol, 2-methyl-5-(1-methylethyl)-(0.17%), which belong to phenols and flavonoids components.[6,11] These components also show their antibacterial and antimicrobial agents, which are found to have multiple therapeutic properties such as antioxidants, anti-inflammatory, anticancer, etc.[6,17]

Four types of bacteria including, *Streptococcus agalactiae, Proteus mirabilis, Enterococcus cloacae*, and *Escherichia coli* are subjected to this study, which are shown in Table 3.There is no significant differences $P < 0.05$ among seed methanolic extracts and pathogens.The fenugreek seed extract got the highest percentage of inhibition in both concentrations 50 mg/ml and 100 mg/ml, it reached (13.50, 10.5, 9.5, and 9.0) mm, (13.5, 13.0, 13.5, and 10.5) mm, respectively, which compared to the black cumin level reached (13.05, 10.0, 11.50, and 9.50) mm,(11.50, 11.50, 10.50, and 7.50), respectively. *S. agalactiae* mostly affected the inhibitory ability of all extracts, it is 13.5 mm at conc. 50 mg/ml for both extracts while reaching (13.5 and 11.5) mm at conc. 100 mg/ml, in a similar study.[11] It is found that bacteria are the mostly discouraged by the alcoholic cumin and fennel seed extracts. On the other hand, we got the

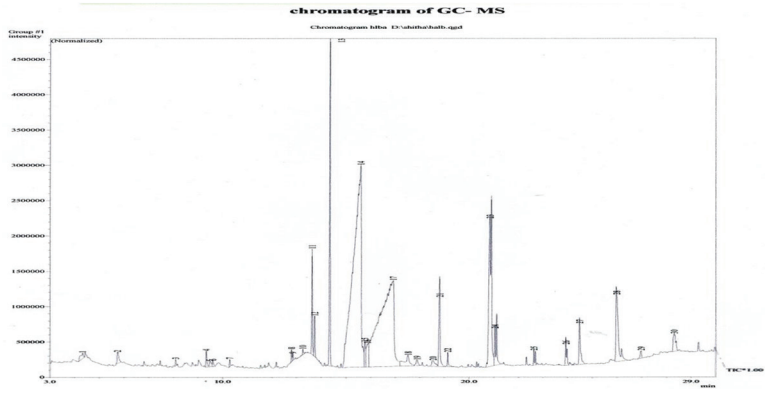

Fig. 1 **Chromatogram of fenugreek seed extracts by GC-MS.**

Table 1. **Constitution of fenugreek seed extracts by GC-MS**

No.	Peak no.	Compound	Molecular weight	RT	Area %	Synonyms	Similarity %	*Effectiveness
1	4	Benzofuran, 2, 3-dihydro	120	9.334	0.36	Coumaran	91	Antibiotic, antibacterial
2	5	2-Furancarboxaldehyde, 5-(hydroxymethyl)	126	9.480	0.24	5-Oxymethylfurfurole	92	Antibiotic, antibacterial
3	8	Decane, 1-chloro	176	12.790	0.22	*n*-Decyl chloride	91	Antibiotic, antibacterial
4	9	1-Undecanol	172	12.841	0.11	Undecanol	89	Antibiotic, antibacterial, antimycotics
5	13	Diethyl Phthalate	222	14.346	5.94	Unimoll DA, or Phthalol	97	Antibiotic, antibacterial, antioxidant[a]
6	14	Beta-D-glucopyranoside, methyl	194	15.597	35.60	Methyl a-D-galactoside	85	Antibiotic, antibacterial, antimycotics[b]
7	16	2-O-Methyl-D-mannopyranosa	194	15.870	1.26	Alpha-D-mannopyranoside, methyl	79	Antibiotic, antibacterial, antimycotics
8	17	Alpha-D-mannopyranoside, methyl	164	16.921	28.31	Methyl pentofuranoside	80	Antibacterial agent[c]
9	19	Ethyl 2-isocyanato-4-methyl valerate	171	17.888	0.28	Ethyl 1-methylnipecotate	70	Antibiotic, antibacterial agents, antimycotics
10	27	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	320	24.487	1.53	Glycerol beta-palmitate	83	Antioxidants, antibacterial, antibiotic
11	29	Nonanedioic acid, bis (2-ethylhexyl) ester	412	26.975	0.25	Azelaic acid, di(2-ethyl-hexyl) ester	71	Antibacterial, antiviral, anti-inflammatory agents

[a]National Center for Biotechnology Information. PubChem Compound Database; CID = 6781, https://pubchem.ncbi.nlm.nih.gov/compound/6781 (accessed Nov. 24, 2017). [b]National Center for Biotechnology Information. PubChem Compound Database; CID = 2108, https://pubchem.ncbi.nlm.nih.gov/compound/2108 (accessed Nov. 24, 2017). [c]National Center for Biotechnology Information. PubChem Compound Database; CID = 2108, https://pubchem.ncbi.nlm.nih.gov/compound/2108 (accessed Nov. 24, 2017).

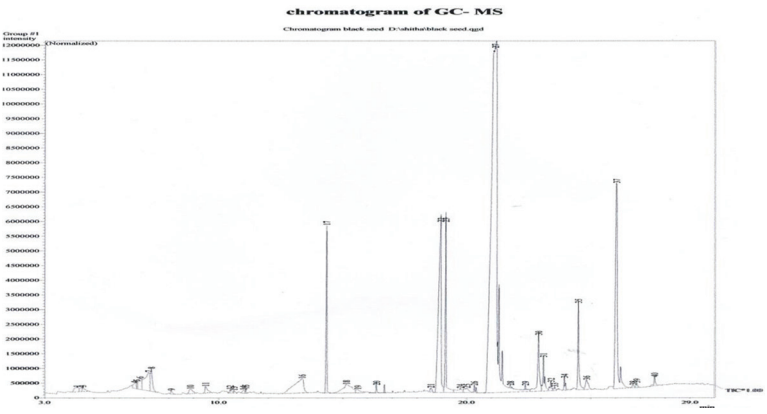

Fig. 2 **Chromatogram of black cumin seed extracts by GC-MS.**

Table 2. **Constitution of black cumin seeds extract by GC-MS**

No.	Peak no.	Compound	Molecular weight	RT	Area%	Synonyms	Similarity%	*Effectiveness
1	3	1, 2-Cyclopentanedione	98	4.533	0.24	2-Pyrazolin-5-one, 3-methyl-	83	Antibiotic
2	4	1, 4-Butanediol, diacetate	114	6.625	0.40	1,4-Dioxin, 2, 3-dihydro-5,6-dimethyl-	77	Antibiotic, antibacterial
3	6	2, 5-Dimethyl-4-hydroxy-3(2H)-furanone	128	6.838	0.85	Pineapple ketone	92	Antibacterial, antioxidant
4	9	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	8.104	0.13	4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-	90	Antibacterial, antioxidant
5	11	2-Furancarboxaldehyde, 5-(hydroxymethyl)	126	9.476	0.41	5-Oxymethylfurfurole	89	Antibiotic, antibacterial
6	12	Phenol, 2-methyl-5-(1-methylethyl)	150	10.472	0.17	Carvacrol	94	Antibiotic, antibacterial, antioxidant[e]
7	17	Diethyl Phthalate	222	14.355	3.32	Diethyl Phthalate	97	Antibiotic, antibacterial, antioxidant[d]
8	22	l-(+)-Ascorbic acid 2,6-dihexadecanoate	228	18.952	10.75	Tetradecanoic acid, or Univol U 316S	89	Antibiotic, antibacterial[b]
9	27	9, 12-Octadecadienoic acid (Z,Z)-	280	21.143	50.16	Emersol or Linoleic acid 95	93	Antibiotic, antibacterial, antioxidant[a]
10	28	10-Undecenoyl chloride	202	21.775	0.11	omega.-Undecylenic acid chloride	77	Antibiotic, antibacterial, anti-inflammatory agents
11	34	Fumaric acid, 2-dimethylaminoethyl nonyl ester	213	23.944	0.37	3-Cyclopentylpropionic acid	89	Antibiotic, antibacterial
12	35	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	358	24.501	2.24	Tegin 515	88	Antibiotic, antibacterial, antioxidant
	36	gamma-Sitosterol	414	24.840	0.64	Clionasterol	88	Antibiotic, antioxidant
13	37	Propylene glycol monoleate	354	26.037	9.61	1-Monolinolein	83	Antibiotic, antibacterial, antioxidant[c]
14	39	Lupeol	426	26.855	0.16	Triterpene or lupeol	89	Antibiotic, antibacterial, antioxidant

[a]National Center for Biotechnology Information. PubChem Compound Database; CID = 5280450, https://pubchem.ncbi.nlm.nih.gov/compound/5280450 (accessed Nov. 24, 2017). [b]National Center for Biotechnology Information. PubChem Compound Database; CID = 54722209, https://pubchem.ncbi.nlm.nih.gov/compound/54722209 (accessed Nov. 24, 2017). [c]National Center for Biotechnology Information. PubChem Compound Database; CID = 5365625, https://pubchem.ncbi.nlm.nih.gov/compound/5365625 (accessed Nov. 24, 2017). [d]National Center for Biotechnology Information. PubChem Compound Database; CID = 6781, https://pubchem.ncbi.nlm.nih.gov/compound/6781 (accessed Nov. 24, 2017). [e]National Center for Biotechnology Information. PubChem Compound Database; CID = 10364, https://pubchem.ncbi.nlm.nih.gov/compound/10364 (accessed Nov. 24, 2017).

Table 3. **The effect of methanolic extracts against some pathogens**

Type of bacteria	Diameter of inhibition zone (mm) for tested bacteria			Diameter of inhibition zone (mm) for tested bacteria		
	Conc. of fenugreek (mg/ml)			Conc. of black cumin (mg/ml)		
	Control DMSO (10%)	Conc. 50 mg/ml	Conc. 100 mg/ml	Control DMSO (10%)	Conc. 50 mg/ml	Conc. 100 mg/ml
Streptococcus agalactiae	0	13.50	13.50	0	13.50	11.50
Proteus mirabilis	0	10.50	13.00	0	10.00	11.50
Enterococcus cloacae	0	9.50	13.50	0	11.50	10.50
Escherichia coli	0	9.00	10.50	0	9.50	7.50
LSD $P < 0.05$	2.704			4.576		

impression from Table 3 that the diameter clear zone of *E. coli* is the lowest for each extract, it reached (9.0 and 9.5) mm at conc. 50 mg/ml and (1.5 and 7.5) mm at conc.100 mg/ml. These results are in similar with Ref. 11), which find the diameter clear zone reached (0.0) mm of cumin seeds extract. These results harmonize with those scrutinized in earlier studies[15–20] who acquired in their studies that fenugreek and black cumin seed extracts inhibitory ability as a broad-spectrum antibiotic. In this study, it can be noticed that the results of the GC-MS analysis of Figs. 1 and 2 and Tables 1 and 2 of fenugreek, black cumin seed extracts, and its relationship with diameter inhibition zone of bacteria that subjected to examination. This is due

to the consequences of GC-MS assay of fenugreek seed extract that have a number of an active phenolic constituents similar to beta-D-glucopyranoside, methyl, alpha-D-mannopyranoside, methyl, and diethyl phthalate. On the other hand, black seed extract has a number of phenolic components. The most important phenolic components of this study are 9, 12-octadecadienoic acid (Z, Z)-, l-(+)-Ascorbic acid 2, 6-dihexadecanoate, and propylene glycol monoleate, which are attributed to the inhibitory ability for extracts versus pathogen. Furthermore, there have many alkaloids, and terpenes among the components. This agrees with the study of Ref. 22, when it diagnosed a several of chemicals compounds in medicals herbs by GC-MS technique. The different effect of the extracts on the diverse bacteria is due to a species and subspecies of bacteria as well as the category of an active substance found in the extract itself which has a destructive effect of pathogenic bacteria because of interfering with DNA or proteins cell.[21] It also shown in Table 3 that the inhibition results for each *Proteus mirabilis* and *E. coli* which reached (10.5 and 10.0) mm, (9.0 and 9.5) mm, respectively at 50 mg/ml and (13.0 and 11.5) mm (10.5 and 7.5) mm, respectively at 100 mg/ml, these results are consistent with the study of Ref. 23 that showed the highest percentage of inhibition against *Proteus mirabilis,* and *E coli* at 50 mg/ml from alcoholic fenugreek seed extract. Moreover, the best concentration of alcoholic black seed extract to inhibit growth *S. aureus* at (50 and 100) mg/ml.[24]

Conclusion

In this study, the presence of all the above bioactive compounds in seeds of fenugreek and black cumin is associated with inhibiting effect against some pathogenic bacteria. The variations in chemical compounds model can be used to distinguish among plants. We should shed more light on some missing aspects of these chemical components as phenols, flavonoids, alkaloids, fatty acids, etc. and its biological roles in humankind. One of the most important aspects to be studied is the transcriptomic and genetic analysis.

Conflict of Interest

None.

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