Biomedicine and Chemical Sciences 2(2) (2023) 68-75

1-BCS-2005-458

Content lists available at:
www.journals.irapa.org/index.php/BCS/issue/view/23

Biomedicine and Chemical Sciences

Journal homepage: https://www.journals.irapa.org/index.php/BCS

Evaluation of the Effect of Crude Extracts of Fenugreek (*Trigonella Foenum Graecum*) on Resistant Isolates of *Klebsiella Pneumoniae*, and *Pseudomonas Aeruginosa*: *In Vitro* Study

Murtadha Abdullah Abbood*, Azhar A. AL-Attraqchi & Hayder B. Sahib

Medical Microbiology, College of Medicine, Al-Nahrain University, Baghdad - Iraq

ARTICLE INFO

Article history:

Received on: February 07, 2023
Revised on: March 13, 2023
Accepted on: March 23, 2023
Published on: April 01, 2023

Keywords:

Crude extract
Fenugreek
K. pneumonia
P. aeruginosa

ABSTRACT

The recent appearance of resistant bacterial species has generated alarm. Medicinal plants are still a good way to get bioactive substances with medical promise, and they can use to get a wide range of antibacterial substances. As an alternative to chemical agents, evaluated the effect of crude fenugreek seed extracts on resistant isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. This study was conducted from 2021 to 2022 in Karbala. Sputum samples were collected from 455 patients with severe COVID-19, samples were examined directly and cultured on microbiological culture media, and growing colonies were distinguished and preparing smears staining with Grams stain, API system for biochemical and fermentation differentiation, then disc diffusion method to distinguish the resistant isolates in accordance with clinical and laboratory standards institute 2020. Extraction method using the Soxhlet method for eight hours. Electric rotatory evaporators were used to evaporate the remaining solvents, then crude extracts were collected. The GC-MS is used to determine bioactive compounds. Determination of antimicrobial effects by well diffusion method using different concentrations of each extract as (25, 50, 100, and 150mg/ml). The isolated microorganisms were P. aeruginosa comprising 21 (4.6%) including 4 (0.9%) as resistant isolates. However, 50 (11.0%) of the isolates were K. pneumoniae, with 6 (1.3% being resistant) the remaining were other microorganisms**.** Methanolic extract indicated the heights contains of bioactive compounds followed by chloroform and aqueous respectively. Fenugreek seed methanolic extract at a MIC of 100 mg/ml significant effect on tested microorganisms, with inhibition zones of 10.67 and 9.67 mm, respectively. The methanolic extract contains the most bioactive compounds and the most effective antibacterial agent, followed by chloroform and aqueous extract respectively. *P. aeruginosa* was the most resistant isolate.

1. Introduction

Antibiotic resistance to currently available antibiotics necessitates the search for alternative antibacterial medications. Because of the rapid global spread of antibiotic-resistant clinical isolates (Nadimpalli et al., 2018). Bacterial resistance to currently available antibiotics has necessitated the development of novel antibacterial drugs. Because resistant clinical isolates spread rapidly around the world, finding new antimicrobial drugs is critical (Aslam et al., 2018). Because medicinal herbs are inexpensive and viable to get, they are used as the primary form of health care by around 80% of people globally. People have been fascinated with plant-based therapies since the dawn of civilization. Medicinal herbs have been developed from medicinal plants since ancient times to treat a variety of ailments (Habeeb Rahuman et al., 2022). There is a constant and urgent need to make new antibacterial drugs with

***Corresponding author:** Murtadha Abdullah Abbood, Medical Microbiology, College of Medicine, Al-Nahrain University, Baghdad – Iraq

E-mail: **murtadaalgaber88m@gmail.com**

How to cite:

Abbood , M. A., AL-Attraqchi, A. A., & Sahib, H. B. (2023). Evaluation of the Effect of Crude Extracts of Fenugreek (Trigonella Foenum Graecum) on Resistant Isolates of Klebsiella Pneumoniae, and Pseudomonas Aeruginosa: In Vitro Study. *Biomedicine and Chemical Sciences, 2*(2), 68–75.

DOI: **https://doi.org/10.48112/bcs.v2i2.458**

different chemical structures and ways of working to treat new and old infectious diseases (Gorlenko et al., 2020).

Humans had sought out herbs in nature since ancient times, when humans first began to seek treatment for illness. Humans first relied on their innate knowledge of when and how to employ medicinal herbs (Idrees et al., 2020). A wide range of herbal plant species were used to kill bacteria (Zia-Ul-Haq et al., 2021). Fenugreek, or *Trigonella foenum-graecum*, is cultivated from the Mediterranean region to northern India. Fenugreek, a member of the family *Fabaceae*, has been used as a medicine since at least 4000 BC. Fenugreek's uses and benefits were first documented in writing around 1500 B.C. (Sarwar et al., 2020). The fenugreek seed extract has an anti-microbial effect against many microbes (Ceylan et al., 2022; Hadi & Mariod, 2022) fenugreek seed extracts with different solvents have an antibacterial effect against *K. pneumoniae* and *P. aeruginosa* (Benyagoub et al., 2022).

The present COVID-19 epidemic may contribute to the global growth in antibiotic resistance. Antimicrobial stewardship defected due to many COVID-19 patients using antibiotics for prevention, which all contributed to the rise in resistance (Nadeem et al., 2020). More than 2.8 million cases of MDR-TB occur annually in the United States, resulting in at least 35,000 annual fatalities and $20 billion in annual healthcare costs (Prevention, 2013), 80% of COVID-19 hospitalized patients received antibiotics. Antibiotics and antifungals save lives, but they also cause resistance in humans. Half of the hospitalized patients were administered azithromycin and ceftriaxone (Tanne, 2022).

There are numerous approaches to overcoming antimicrobial resistance; one of the most important is the use of natural herbal extracts (Nadeem et al., 2020). The fenugreek seed extract contained various bioactive compounds that act as antimicrobial (Sarwar et al., 2020). The bioactive compounds vary depending on the solvents and extraction techniques used (Farahmandfar et al., 2019). According to the WHO study, the important organisms that developed resistance to antibiotics were (*Enterococcus faecium, Staphylococcus aureus, K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species*), which ranked highest because they pose a significant threat to humans (Lomazzi et al., 2019).

The aim of the study is to evaluate the effect of crude fenugreek seed extracts (methanolic, chloroform and aqueous) on resistant isolates (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) isolated from sever COVID-19 cases.

2. Materials and Methods

The experimental study was designed on resistant bacterial isolates from the sputum of the severe cases of COVID-19 in Iraq (Karbala)/Imam AL-Husain Medical City/AL-Zahraa hospital (Al-Hayat Unit for Chest & Respiratory Diseases) in the period from August 2021 to June 2022.

Inclusion Criteria

Resistance isolates of *P.* aeruginosa and *K. pneumoniae.*

Extraction Procedure

For the extraction process (100) grams of seed (Provide fenugreek seeds from the local market in Iraq), seed grinding to powder (Bhanger et al., 2008) and dissolved in 800 milliliters of solvent either (chloroform, methanol, and water), the seed to solvent ratio was (1:8). Each mixture was applied in soxhlet apparatus (WuboLab/china) for 10 to 18 hours to obtain the desirable extracts (Handa et al., 2008). Then the raised product was evaporated by a rotatory evaporator (MERC/ UK) at 40 °C. The yielded extract was collected and weighed. This process was repeated three times to obtain adequate extract quantities for each solvent.

Detection of Bioactive Components

The existence of organic and inorganic chemicals in a sample was determined using gas chromatography-mass spectrometry (GC-MS) Agelint/USA as quantitative assays of functional group contents.

Bacterial Isolation and Identification

Sputum samples were cultivated on different media and incubated for 24hrs at 37 °C after that, the identification occurs. Blood agar (Oxoid/UK) plates were investigated for morphological characteristics of colonies, including color, size, the appearance of colonies, and hemolytic zones surrounding colonies. MacConkey agar (Oxoid/UK) plates were evaluated for Gram-negative bacteria, like morphological features and lactose fermentation of growing colonies (Tille, 2015).

Evaluation of Antimicrobial Activity of Fenugreek Seed Extract

The antimicrobial activity of each crude extract (Chloroform, Methanol and aqueous) was achieved using the agar well diffusion method against *K. pneumoniae* and *P. aeruginosa*. The organisms were reactivated by cultivating them on nutrient agar (Oxoid/UK) and incubating them at 37 °C for 24 hours. Microbial concentrations were used at 0.5 McFarland standards (BioMerieux/France). The tested microorganisms were spread using cotton swabs on Muller-Hinton agar plates (Oxoid/UK). Wells of 5 mm were punctured using sterilized inverted tips onto these plates. All the wells were filled with 50 µl of (Chloroform, Methanol, and aqueous) crude extracts of fenugreek seed of the concentrations (50, 100, and 150 mg/ml). Dimethyl sulfoxide (Romil/UK) with distilled water was used as a negative control. Colistin (50µg) for bacteria. The plates were incubated at 37°C for 24 hours. Later on, inhibition zones were measured in millimeters and minimum inhibitory concentration was noted. This process was carried out in triplicate for each extract and concentration, and the means of inhibition zones were recorded.

3. Results and Discussion

Microbial Growth and Antimicrobial Sensitivity

According to the sputum sample, culture characteristics, and disc diffusion method for antimicrobial sensitivity. The K. pneumoniae were 50 (11.0%) of the isolates, with 6 (1.3% being resistant). While the P. aeruginosa growth comprised 21 (4.6%) isolates, including 4 (0.9%) resistant isolates. Table (1) shows the bacteria found in the cultured sputum samples, the result interpretation regarding Clinical and

Laboratory Standards Institute (CLSI 2020) standards as sensitive (S), intermediate (I) and resist (R) (Pollack et al., 2018)

Table 1
Microbial growth and anti-microbial sensitivity

Types of Growth	Anti-Microbial Sensitivity			Total NO.(%)
	Sensitive NO. (%)	Intermediate NO. (%)	Resistant NO. (%)	
P. aeruginosa	14 (3.1)	3 (0.7)	4 (0.9)	21 (4.6)
K. pneumoniae	30 (6.6)	14 (3.1)	6 (1.3)	50 (11.0)
Others microorganisms	NA*	NA*	NA*	343 (75.4)
No Growth	-	-	-	41(9.0)
Total	44 (9.7)	17 (3.7)	10 (2.2)	455(100)

*NA: Not Applied.
Sensitive, Intermediate, and Resistant are classified according to (CLSI 2020) standard.

Regarding to the microbial growth and susceptibility test results are mention in above table K. pneumoniae were formed 50 (11%) this nearly agree with (Tiri et al., 2020)/ Italy, who showed the co-infection rate with K. pneumoniae were 15% in severe and critical cases, and disagree with Li et al., (2020), who documented that K. pneumoniae were (30.8%) of a total of 159 critical COVID-19 patients, this discrepancy may due to therapy protocol used and sample size differences. It also agree with Ghanizadeh et al., (2021), that documented of 70 K. pneumoniae from Sever and critical patients with COVID-19. P. aeruginosa isolates were 21(4.6%) which is fully agree with Qu et al., (2021), who proved a concomitant infection of P. aeruginosa with COVID-19 as 21(5.1%) in 408 severe cases of COVID-19, and also agree with Qu, et al. (2021) in the United States, who documented an antimicrobial resistance in P. aeruginosa among COVID-19 patients. These co-infections could result from hospitalized COVID-19 patients receiving broad-spectrum antibiotics as prophylaxis, resulting in a biological imbalance.

Regarding to *K. pneumoniae* antibiotic susceptibility test, the highest resistance rate for amoxicillin/ clavulanic acid and azithromycin was seen and this result agreed with de Souza et al., (2022) l, that proved a highly resistant of *K. pneumoniae* to ampicillin, amoxicillin/clavulanic acid and azithromycin were seen, and agree with Li et al., (2020), who found that COVID-19 patients primarily had a secondary bacterial infection caused by Gram-negative bacteria, with *Acinetobacter baumannii* and *K. pneumoniae* being the most common bacteria and showing the highest antimicrobial resistance rates as show in table (2).

Table 2
Antibiotic susceptibility profile of *K. pneumonia*

Antibacterial	Symbol	Sensitive NO. (%)	Intermediate NO. (%)	Resistant NO. (%)
Amoxicillin/ Clavulanic acid	AMC	30 (60)	7 (14)	13 (26)
Ampicillin	AMP	29 (58)	15 (30)	6 (12)
Azithromycin	AZM	36 (72)	0 (0)	14 (28)
Cefotaxime	CTX	28 (56)	19 (38)	3 (6)
Ceftriaxone	CRO	28 (56)	18 (36)	4 (8)
Gentamycin	GEN	33 (66)	12 (24)	5 (10)
Levofloxacin	LEV	40 (80)	7 (14)	3 (6)
Meropenem	MEM	37 (74)	10 (20)	3 (6)
Piperacillin/ Tazobactam	PTZ	43 (86)	7 (14)	0 (0)
Trimethoprim/ sulfamethoxazole	SXT	34 (68)	12 (24)	4 (8)

Sensitive, Intermediate, and Resistant are classified according to (CLSI 2020) standard.

Regarding the antibiotic susceptibility test for isolated *P. aeruginosa* that showed highest resistant to cefotaxime and gentamycin, followed by trimethoprim/sulfamethoxazole and ceftriaxone. These bacteria were resistant to different antibiotics like gentamycin, trimethoprim/sulfamethoxazole and amikacin, Mahmoudi, (2020) authenticated the highest resistance of these bacteria towered tobramycin and gentamicin. This partial agreement with the later study could be attributed to the genetic differences between the isolated strains and the different tested antibiotic used as show in table (3).

Table 3
Antibiotic susceptibility profile of *Pseudomonas aeruginosa*

Antibacterial	Symbol	Sensitive NO. (%)	Intermediate NO. (%)	Resistant NO. (%)
Amoxicillin/ Clavulanic Acid	AMC	16 (76.2)	3 (14.3)	2 (9.5)
Ampicillin	AMP	12 (57.1)	5 (23.8)	4 (19.0)
Azithromycin	AZM	17 (81.0)	0 (0.0)	4 (19.0)
Cefotaxime	CTX	12 (57.1)	3 (14.3)	6 (28.6)
Ceftriaxone	CRO	14 (66.7)	2 (9.5)	5 (23.8)
Gentamycin	GEN	10 (47.6)	5 (23.8)	6 (28.6)
Levofloxacin	LEV	17 (81.0)	2 (9.5)	2 (9.5)
Meropenem	MEM	16 (76.2)	4 (19.0)	3 (14.3)
Piperacillin/ Tazobactam	PTZ	15 (71.4)	4 (19.0)	2 (9.5)
Trimethoprim/ sulfamethoxazole	SXT	11 (52.4)	5 (23.8)	5 (23.8)

Sensitive, Intermediate, and Resistant are classified according to (CLSI 2020) standard.

The GC-MS Analysis of Fenugreek Seed Extracts: The bioactive compounds of fenugreek seed extracts (aqueous, methanol, and chloroform) revealed that methanol had the most dissolved bioactive seventeen compounds), followed by aqueous ten compounds and chloroform was the last one with eight compounds), as shown in the figures (1,2&3).

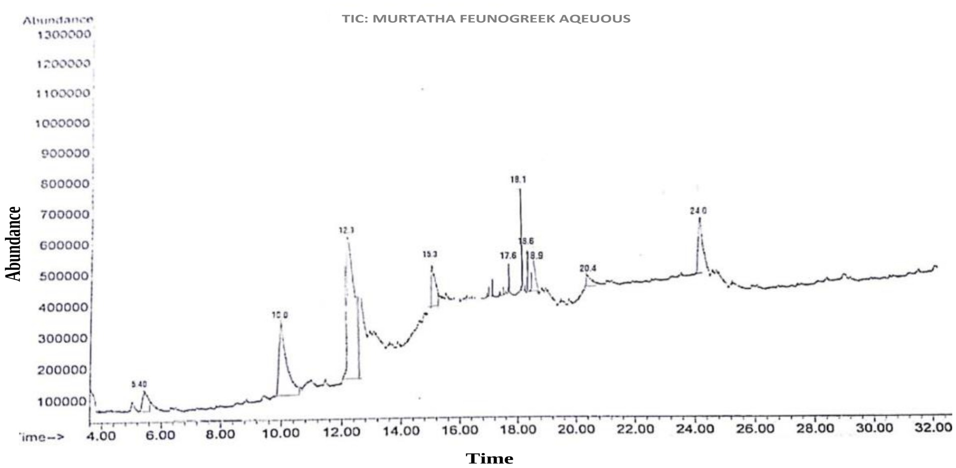

Fig. 1. GC-MS of fenugreek seed aqueous extract

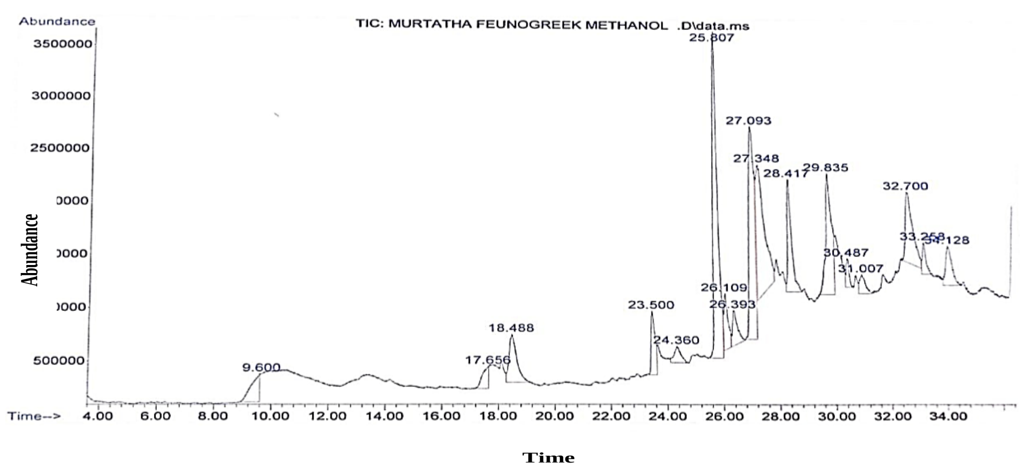

Fig. 2. GC-MS of fenugreek seed methanol extract

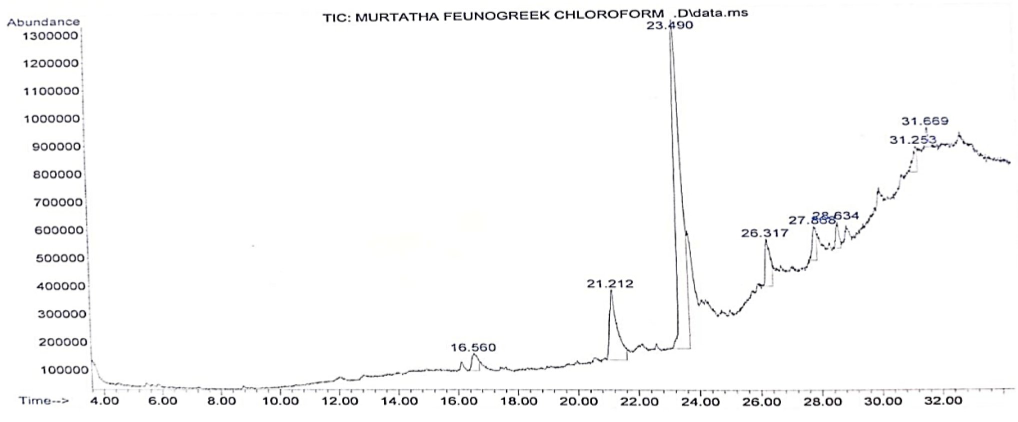

Fig. 3. GC-MS of fenugreek seed chloroform extract

Regarding to GC-MS analysis of fenugreek seed aqueous extract that appeared to contain 10 bioactive compounds and this result agree with Navarro, et al., (2020), who said that GC-MS analysis of an aqueous extract of fenugreek seeds were identified by 15 peaks of bioactive compounds. Regarding GC-MS analysis of fenugreek seed methanolic extract that appears to contain (17) bioactive compounds, this result nearly agrees with Keskes et al., (2018), the findings of this study were 20 bioactive compounds by using hexane as a solvent and agrees with Akbari *et al.,* (2019), in which found 17 compounds was seen at the retention time 37.43min. Regarding to GC-MS analysis of fenugreek seed chloroform extract that appeared contain (8) of bioactive compounds, this result agree with Chatterjee *et al.,* (2010), that found 9 bioactive compounds using maceration method for extraction.

Anti-Klebsiella Activity of Fenugreek Seed Extracts

Results of an experiment evaluating the minimum inhibitory concentration (MIC) of fenugreek crude extracts (aqueous, methanol, and chloroform) against resistant isolates of *K. pneumoniae* at concentrations of (25, 50, 100, and 150 mg/ml) are shown in the table (4). At a 100 mg/ml concentration, the MIC of the methanolic extract resulted in a mean inhibition zone size of 10.67 mm, with a significance level of 0.001. The next MIC was for the chloroform extract, and it had an inhibition zone of 10.33 mm and a significant value of 0.002 at a concentration of 150 mg/ml. On the other hand, the MIC of aqueous extracts exhibited the lowest effect on them at the MIC concentration of 150 mg/ml, with a mean inhibition zone of 9.67 mm and a P value of 0.003.

Table 4
The effect of fenugreek seed extracts against *K. pneumonia*

Concentrations	Inhibition Zone in millimeters					
	Aqueous Extracts		Methanolic Extracts		Chloroform Extracts	
	Mean(SD)	*P.* Value	Mean(SD)	*P.* Value	Mean(SD)	*P.* Value
25 mg/ml	1.67(0.57)	0.714	3.33(0.57)	0.271	2.33(0.57)	0.564
50 mg/ml	3.67(0.57)	0.208	6.0 (0.0)	0.059	4.0 (0.0)	0.157
100 mg/ml	7.33(0.57)	0.021	10.67(0.57)	0.001*	7.67(1.15)	0.016
150 mg/ml	9.67(0.57)	0.003*	15.33(0.57)	0.0001*	10.33(0.57)	0.002 *
Positive Control	25.0(0.00)	-	25.0 (0.00)	-	25.0 (0.00)	-
Negative Control	0.0 (0.00)	-	0.0 (0.00)	-	1. (0.00)	-
*Significant *P.* Value <0.01						

Regarding results of fenugreek seed extract against *K. pneumoniae* that inhibited the growth of testing bacteria in different solvent types and concentrations as in mentioned previously in above table. These result agree with those obtained by Dharajiya et al., (2016), which indicated that fenugreek extracts to have a significant effect as an antimicrobial agent due to the presence of different phytochemicals, and agree with Raut et al., (2022), who saw fenugreek seed extracts a good activity against resistant *K. Pneumoniae* than normal organisms.

The zone of inhibition of methanolic extract at 100 mg/ml, was (10.67 mm), and this of chloroform extract at 150 mg/ml, was (10.33 mm), these results agree with Ahmed et al., (2022), who documented that fenugreek seed extract at a concentration of 40% had a zone of inhibition mean(14±1.7 mm) against *K. pneumoniae*, and with Mir et al., (2021), who found fenugreek seed extract inhibited the growth of *K. pneumoniae* with inhibition zone (11mm).

Anti- Pseudomonas Activity of Fenugreek Seed Extracts: Fenugreek seed crude extracts (aqueous, methanol, and chloroform) were tested at concentrations of (25, 50, 100, and 150 mg/ml) for their ability to inhibit the growth of resistant isolates of *P. aeruginosa*, and the MIC results were recorded. The MIC for the methanolic extract was established at 100 mg/ml with a mean inhibition zone size of 9.67 mm and a significance level of 0.001, and the MIC for the chloroform extract was established at 100 mg/ml with a mean inhibition zone size of 6.33 mm and a significance level of 0.008. However, these bacteria were resistant to all concentrations of aqueous extracts, so they showed no significant effect on the bacteria with *P.* values above 0.01. The outcomes are displayed in table in table (5).

Table 5
The effect of fenugreek seed extracts against *P. aeruginosa*

Concentrations	Inhibition Zone in millimeters					
	Aqueous Extracts		Methanolic Extracts		Chloroform Extracts	
	Mean (SD)	*P.* Value	Mean (SD)	*P.* Value	Mean (SD)	*P.* Value
25 mg/ml	0.67(0.57)	0.834	2.67(0.57)	0.249	2.00(0.0)	0.463
50 mg/ml	2.33(0.57)	0.345	6.00(0.0)	0.01	4.33(0.57)	0.052
100 mg/ml	3.00(0.0)	0.041	9.67(0.57)	0.001 *	6.33(0.57)	0.008 *
150 mg/ml	4.67(0.57)	0.053	13.67(0.57)	0.0001 *	8.33(0.57)	0.002 *
Positive Control	25.0(0.0)	-	25.0(0.0)	-	25.0(0.00)	-
Negative Control	0.0(0.0)	-	0.0(0.0)	-	0.0(0.00)	-

*Significant *P.* Value <0.01

Regarding the result of fenugreek seed extracts, which revealed a significant effect against resistant isolates of *P. aeruginosa*, these results are agree with (Al-Timimi, 2019) who demonstrated that an extract of fenugreek seeds had antibacterial properties, (Walli et al., 2015), who found that aqueous extracts of fenugreek seed not effect on *P. aeruginosa*.

Regarding the zone of inhibition results are agree with Othman, (2021), who found the diameter of inhibition zone (12mm), and proved the effectiveness of fenugreek extract against selected clinical isolate of *P. aeruginosa* with inhibition zone mean (16.5 mm), and with (Al-Timimi, 2019), who certified diameter of the inhibition zone of ethanolic and aqueous extracts were (16 and 13mm) respectively.

The current results disagree with those of (Al-abdeen et al., 2010), who documented that there is no effect of fenugreek extract on *P. aeruginosa* when compared with standard antibiotic discs, this discrepancy may be due to the use of different extraction techniques (maceration) and solvents (hot-boiled distilled water, ethanol, benzene, chloroform, hexane, and petroleum ether), as well as the low concentration of the extract.

4. Conclusions

Fenugreek seed methanolic extract contains the most bioactive compounds. It was the most effective antibacterial agent, followed by the chloroform extract, while the aqueous extract it showed the least effect. Regarding the microorganism isolated from COVID-19 patient's sputum with severe symptoms. *Pseudomonas aeruginosa* was the most resistant isolate followed by *K. pneumoniae*.

Competing Interests

The authors have declared that no competing interests exist.

References

Ahmed, R. M., Alshafei, N., & Mohamed, H. A. (2022). Green method of synthesis silver nanoparticles as using fenugreek seeds extract (Trigonella foenum-graecum) and its application as antibacterial agent. University of Bahri Journal of Veterinary Sciences, 1(1), 8-14.

Akbari, S., Abdurahman, N. H., Yunus, R. M., Alara, O. R., & Abayomi, O. O. (2019). Extraction, characterization and antioxidant activity of fenugreek (Trigonella-Foenum Graecum) seed oil. Materials Science for Energy Technologies, 2(2), 349-355. **https://doi.org/10.1016/j.mset.2018.12.001**

Al-abdeen, S. S. Z., Faraj, B. M., & Nasrulla, O. J. (2010). Antibacterial effects of fenugreek (Trigonella foenum-graecum). Basrah journal of veterinary research, 9(2).

Al-Timimi L. A. N. (2019). Antibacterial and Anticancer Activities of Fenugreek Seed Extract. Asian Pacific journal of cancer prevention : APJCP, 20(12), 3771–3776. **https://doi.org/10.31557/APJCP.2019.20.12.3771**

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M. K. F., & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. Infection and drug resistance, 11, 1645–1658. **https://doi.org/10.2147/IDR.S173867**

Benyagoub, E., Nabbou, N., Bouchikhi, F., & Milioudi, M. N. (2022). Chromatographic analyses and in vitro screening of the antimicrobial activity of tannins extract of fenugreek seeds grown in the Taghit region (Southwest of Algeria). International Journal on Nutraceuticals, Functional Foods and Novel Foods. **https://doi.org/10.17470/NF-022-0049**

Bhanger, M. I., Bukhari, S. B., & Memon, S. (2008). Antioxidative activity of extracts from a Fenugreek seeds (Trigonella foenum-graecum). Pakistan Journal of Analytical & Environmental Chemistry, 9(2), 6.

Ceylan, Ş., Camadan, Y., Saral, Ö., & Batur, Ö. Ö. (2022). Chemical Composition, Antimicrobial, and Antioxidant Activities of Medicinal Plants Nutsedge and Fenugreek. BioResources, 17(3).

Chatterjee, S., Variyar, P. S., & Sharma, A. (2010). Bioactive lipid constituents of fenugreek. Food Chemistry, 119(1), 349-353. **https://doi.org/10.1016/j.foodchem.2009.05.076**

de Souza, C. M., da Silva, Á. P., Júnior, N. G. O., Martínez, O. F., & Franco, O. L. (2022). Peptides as a therapeutic strategy against Klebsiella pneumoniae. Trends in pharmacological sciences. **https://doi.org/10.1016/j.tips.2021.12.006**

Dharajiya, D., Jasani, H., Khatrani, T., Kapuria, M., Pachchigar, K., & Patel, P. (2016). Evaluation of antibacterial and antifungal activity of fenugreek (Trigonella foenum-graecum) extracts. Int J Pharm Pharm Sci, 8(4), 212-217.

Farahmandfar, R., Esmaeilzadeh Kenari, R., Asnaashari, M., Shahrampour, D., & Bakhshandeh, T. (2019). Bioactive compounds, antioxidant and antimicrobial activities of Arum maculatum leaves extracts as affected by various solvents and extraction methods. Food science & nutrition, 7(2), 465-475. **https://doi.org/10.1002/fsn3.815**

Ghanizadeh, A., Najafizade, M., Rashki, S., Marzhoseyni, Z., & Motallebi, M. (2021). Genetic diversity, antimicrobial resistance pattern, and biofilm formation in Klebsiella pneumoniae isolated from patients with coronavirus disease 2019 (COVID-19) and ventilator-associated pneumonia. BioMed research international, 2021. **https://doi.org/10.1155/2021/2347872**

Gorlenko, C. L., Kiselev, H. Y., Budanova, E. V., Zamyatnin Jr, A. A., & Ikryannikova, L. N. (2020). Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics?. Antibiotics, 9(4), 170. **https://doi.org/10.3390/antibiotics9040170**

Habeeb Rahuman, H. B., Dhandapani, R., Narayanan, S., Palanivel, V., Paramasivam, R., Subbarayalu, R., ... & Muthupandian, S. (2022). Medicinal plants mediated the green synthesis of silver nanoparticles and their biomedical applications. IET nanobiotechnology, 16(4), 115-144. **https://doi.org/10.1049/nbt2.12078**

Hadi, S. T., & Mariod, A. A. (2022). Antioxidant and antimicrobial activity of fenugreek (Trigonella foenum-graecum) seed and seedoil. In Multiple Biological Activities of Unconventional Seed Oils (pp. 111-117). Academic Press. **https://doi.org/10.1016/B978-0-12-824135-6.00011-8**

Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). Extraction technologies for medicinal and aromatic plants. Earth, Environmental and Marine Sciences and Technologies.

Idrees, S., Hanif, M., Ayub, M., Hanif, A., & Ansari, T. (2020). Medicinal Plants of South Asia. Novel Sources for Drug Discovery, 1-699.

Keskes, H., Belhadj, S., Jlail, L., El Feki, A., Damak, M., Sayadi, S., & Allouche, N. (2017). LC-MS–MS and GC-MS analyses of biologically active extracts and fractions from Tunisian Juniperus phoenice leaves. Pharmaceutical Biology, 55(1), 88-95. **https://doi.org/10.1080/13880209.2016.1230139**

Li, J., Wang, J., Yang, Y., Cai, P., Cao, J., Cai, X., & Zhang, Y. (2020). Etiology and antimicrobial resistance of secondary bacterial infections in patients hospitalized with COVID-19 in Wuhan, China: a retrospective analysis. Antimicrobial Resistance & Infection Control, 9(1), 1-7. **https://doi.org/10.1186/s13756-020-00819-1**

Lomazzi, M., Moore, M., Johnson, A., Balasegaram, M., & Borisch, B. (2019). Antimicrobial resistance–moving forward?. BMC public health, 19, 1-6. **https://doi.org/10.1186/s12889-019-7173-7**

Mahmoudi H. (2020). Bacterial co-infections and antibiotic resistance in patients with COVID-19. GMS hygiene and infection control, 15, Doc35. **https://doi.org/10.3205/dgkh000370**

Mir, I. H., Sharma, M. K., & Singh, A. (2021). Antimicrobial activity of some medicinal plant extracts against multidrug resistant bacteria. Journal of Advanced Scientific Research, 12(01 Suppl 1), 42-47.

Nadeem, S. F., Gohar, U. F., Tahir, S. F., Mukhtar, H., Pornpukdeewattana, S., Nukthamna, P., ... & Massa, S. (2020). Antimicrobial resistance: more than 70 years of war between humans and bacteria. Critical Reviews in Microbiology, 46(5), 578-599. **https://doi.org/10.1080/1040841X.2020.1813687**

Nadimpalli, M., Delarocque-Astagneau, E., Love, D. C., Price, L. B., Huynh, B. T., Collard, J. M., ... & Guillemot, D. (2018). Combating global antibiotic resistance: emerging one health concerns in lower-and middle-income countries. Clinical Infectious Diseases, 66(6), 963-969. **https://doi.org/10.1093/cid/cix879**

Navarro del Hierro, J., Reglero, G., & Martin, D. (2020). Chemical characterization and bioaccessibility of bioactive compounds from saponin-rich extracts and their acid-hydrolysates obtained from fenugreek and quinoa. Foods, 9(9), 1159. **https://doi.org/10.3390/foods9091159**

Othman, D. T. I.-A. (2021). Antimicrobial Activity of Trigonella foenum-graecum (fenugreek) oil against β-lactamase producers Escherichiacoli, Klebsella pneumoniae, and Pseudomonas aeruginosa using Gas chromatograph/mass spectrometery and molecular docking Sudan University of Science & Technology].

Pollack, R. A., Findlay, L., Mondschein, W., & Modesto, R. R. (2018). Laboratory exercises in microbiology. John Wiley & Sons.

Prevention, C. (2013). Antibiotic resistance threats in the United States, 2013. Threat Report, 50-52.

Qu, J., Cai, Z., Liu, Y., Duan, X., Han, S., Liu, J., ... & Yang, L. (2021). Persistent bacterial coinfection of a COVID-19 patient caused by a genetically adapted Pseudomonas aeruginosa chronic colonizer. Frontiers in cellular and infection microbiology, 129. **https://doi.org/10.3389/fcimb.2021.641920**

Raut, N., Bhoyar, S. K., Zambre, D., Raut, S., Zode, R., & Umekar, M. (2022). Effect of green synthesized fenugreek metal-ion nanoparticles on resistant microorganism. Multidisciplinary International Research Journal of Gujarat Technological University, 4(1), 113-119

Sarwar, S., Hanif, M. A., Ayub, M. A., Boakye, Y. D., & Agyare, C. (2020). Fenugreek. In Medicinal Plants of South Asia (pp. 257-271). Elsevier. **https://doi.org/10.1016/B978-0-08-102659-5.00020-3**

Tanne, J. H. (2022). Covid-19: Antimicrobial resistance rose dangerously in US during pandemic, CDC says. In: British Medical Journal Publishing Group.

Tille, P. (2015). Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences.

Tiri, B., Sensi, E., Marsiliani, V., Cantarini, M., Priante, G., Vernelli, C., ... & Cappanera, S. (2020). Antimicrobial stewardship program, COVID-19, and infection control: spread of carbapenem-resistant Klebsiella pneumoniae colonization in ICU COVID-19 patients. What did not work?. Journal of clinical medicine, 9(9), 2744. **https://doi.org/10.3390/jcm9092744**

Walli, R. R., Al-Musrati, R. A., Eshtewi, H. M., & Sherif, F. M. (2015). Screening of antimicrobial activity of fenugreek seeds. Pharm Pharmacol Int J, 2(4), 1-4.

Zia-Ul-Haq, M., Bin-Jumah, M. N., Alothman, S. I., & Henidi, H. A. (2021). Alternative Medicine Interventions for COVID-19. Springer.