Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.03.031

Evaluation of The Effect of Crude Extracts of Fenugreek on Resistant Isolates of Candida species (In vitro study)

Murtadha Abdullah Abbood[1*], Azhar A. F. AL- Attraqchi[2], Hayder B. Sahib[3]
[1]Ph.D. Medical Microbiology, Al-Nahrain University/College of Medicine, Iraq, Karbala
[2]Ph.D. Medical Microbiology, Al-Nahrain University/College of Medicine Iraq, Baghdad
[3]Ph.D. Pharmacology, Al-Nahrain University/College of Pharmacy,Iraq, Baghdad

***Corresponding author:** Murtadha Abdullah Abbood , Ph.D. Medical Microbiology, Al-Nahrain University/College of Medicine, Iraq, Karbala, Email. Murtadaalgaber88m@gmail.com

Submitted: 15 November 2022; Accepted: 16 December 2022; Published: 11 January 2023

ABSTRACT

Background: The recent emergence of fungal resistance strains has caused concern in medical settings. Medicinal plants continue to be viable sources of bioactive chemicals with therapeutic potential. These compounds can be extracted in different techniques using various solvents that give rise to a wide variety of extracted bioactive compounds that act as anti-fungal.

The research aimed to evaluate the effect of fenugreek seed extracts on resistant isolates of Candida spp. isolated from sever COVID-19 patients.

Methodology: The study was conducted from August 2021 to November 2022 at Al-Imam Al-Hussein Medical City and Al-Hayat Respiratory Diseases Units. Under a specialist's physician's supervision, severe COVID-19 cases were collected. The collected 455 sputum samples were examined directly and cultured on Sabouraud′s Dextrose agar (SDA) media; growth colonies were distinguished and used Grams stain with the API system before the antifungal susceptibility test was performed in accordance with clinical and laboratory standards institute (CLSI 2020) by disc diffusion method to differentiate the resistance microorganism.

The extraction process was conducted using the soxhlet technique (100 grams of seed powder and 800 milliliters of solvents (chloroform, methanol, and water) for eight hours. Electrical rotatory evaporators were used to evaporate the extract to get the concentrated crude extracts. FTIR and GC-MS instruments used to detection of bioactive compounds in crude fenugreek seed extracts(aqueous, methanol, and chloroform).

Then, different concentrations of each extract (25, 50, 100, and 150 mg/ml) and their effect against the tested resistance study isolated were examined by well diffusion method and Minimum inhibitory concentration was measured.

Results: A 455 were enrolled in this study. Patients' ages ranged from 20 to 91 years (mean 52.23, SD 15.009). This study indicated that more than half of the samples were males [(262) 57.6%] and [(193) 42.4%] were females.

The FTIR and GC-MS showed the methanolic extract potent the most bioactive compounds, followed by the chloroform and water extracts.

Evaluation of antimicrobial effects at 50 mg/ml, the methanolic extract showed the greatest effect, with a mean inhibition zone of 9.33 mm and a significant value of 0.01; at 100 mg/ml, the chloroform extract showed the next greatest effect, with a mean inhibition zone of 10.33 mm and a significant value of 0.005. At 150 mg/ml, the aqueous extracts showed the least effect, with a mean inhibition zone of 8.33 mm and a non-significant value of 0.024.

Conclusions: Candida spp. were most frequent isolated yeast from sputum of patients with severe COVID-19. Methanol extract was the most effective anti-candida, followed by chloroform extract, and the aqueous extract was the least effective. The most effective anti-candida drug is ketoconazole.

Keywords: *Candida spp., Fenugreek, FTIR and GC-MS*

INTRODUCTION

Fenugreek, also known as Trigonella foenum-graecum, is a spice bean crop used as a medicine as far back as Hippocrates and ancient Egypt [1]. It is a leguminous plant belonging to the family Fabaceae, which is extensively cultivated as a semiarid crop in Northern Africa, the Mediterranean, India, and Canada [2]. The seed of this plant contains numerous bioactive compounds that act as antimicrobials, especially antifungals [3, 4]. There are many different extraction method that used in extraction of these bioactive compounds the most used one the soxhlet extraction method [5]. Many solvent used in the extraction techniques like water, ethanol, methanol, chloroform these used according to polarity of solvents to dissolved polar and non-polar compounds [6]. The bioactive compounds measured by using gas chromatography mass spectrometry (GC-MS) and fourier transform infrared spectroscopy (FTIR) [7, 8]. The fenugreek seed extract have anti-microbial effect against many microbes like fungi and bacteria[9, 10], fenugreek seed extract, which revealed antimicrobial activity as anti-candida [11], Fenugreek seed extract in methanol solvent has potential antimicrobial effect [12].

The emergence of antimicrobial resistance to currently available antimicrobial necessitates searching for other antimicrobial medicines. Because of the evidence of the rapid global spread of antimicrobial-resistant clinical isolates [13], the search for novel antimicrobial drugs is of the utmost urgency. Nevertheless, the history of the quick and widespread establishment of resistance to newly introduced anti-microbial drugs suggests that even new families of anti-microbial medicines will have a short lifespan. In clinical settings across the globe, Candida species. are developing resistance to azoles and echinocandins[14]. Resistant clinical isolates spread quickly around the world, it is essential to find new antimicrobial drugs [15].

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. This is due to medicinal plants' antibacterial capabilities, which make them suitable medication sources[16].

The research showed that it is possible to use a wide range of herbal plant species to kill bacteria, parasites, fungi, and viruses and minimize their cytopathogenic effects. This means that it could be used to treat diseases all over the world [17]. Green plants are a source of potent chemotherapeutic agents and excellent natural antimicrobial agents. There are reports on the utilization of plant byproducts with antimicrobial characteristics against a variety of harmful bacteria and fungi[18].

METHODOLOGY

Study Location and Period

The study was conducted from August 2021 to June 2022 at the central medical laboratory in Karbala city and the medical research laboratory at the College of Medicine at Al-Nahrain University.

Ethical approval
Approvals depended on the ethics guidelines of the institutional review board of Al-Nahrain University/College of Medicine and the Iraqi Ministry of Health for conducting the dissertation.

Research Population
Cases and data were collected from Al-Imam Al-Hussein Medical City/Al-Hayat Respiratory Diseases Units under the supervision of a specialist doctor. They were diagnosed with severe cases of COVID-19.

Types of Samples
Sputum samples.

Inclusion Criteria
Patients with (severe and critical cases)severe cases of COVID-19.

Those patients underwent antibiotics and were not responded.

Exclusion Criteria
Contaminated samples excluded by cytologist.

Patients with a severe case of COVID-19 under antibiotic or antifungal treatment.

Isolation and Identification Candid spp.
Sputum samples collected from the same entity of patients mentioned previously, following direct examined and stained smears with Gram stains, were streaked directly on SDA plates and incubated at 37 °C for 24 to 48 hrs. The growth was identified basing on their morphological and colonies characteristics. An analytical profile index for Candida (API C) was used to confirm the identification of the isolates[19].

Analytical Profile Index for Candida spp.(API Candida)
The analytical profile index for Candida spp. is a standardized tool for diagnosing isolated Candida spp. The API strip consists of 10 wells containing dehydrated substrates, enabling the performance of 12 biochemical identification tests. After incubation for 18 – 24hrs, results were appeared by spontaneous color changes and read visually according to the standards scale, and identification was obtained by consulting the standards lists of profiles supplied in the package[20].

Antifungal Susceptibility Test
Five antifungal discs have been used with the disc diffusion method [19, 21], to identify the sensitive and resistant isolates of Candida spp. The zone of inhibition was measured, and the results were interpreted as susceptible (S), intermediate (I), and resistant (R).

The inoculum was prepared by suspending distinct colonies of the same morphological characteristics obtained from the sub-culture on SDA to ensure purity and viability in the same cultivation circumstances. The colonies were suspended in 5 mL of 0.85% NaCl, and the turbidity was adjusted with a spectrophotometer by adding NaCl or inoculum until it reached 0.5% McFarland standard. This procedure was done by using a disposable cotton swab, which dipped into the inoculum suspension and, rotated well, pushed directly onto the side wall of the tube. The MHA plats were inoculated by rotating the above-described cotton swab at different angles over the entire agar surface to ensure an even distribution of inoculum. Plates were left to be well absorbed before placing the antifungal discs.

Antifungal discs were applied onto an inoculated MHA plate. Disc pressed down to contact with the inoculated surface, and were distributed in adequate distance more than 24 mm from center to center. Four discs on a 100 mm plate were used to prevent overlapping among inserted discs in zone of inhibition. The plates were inverted and placed in an incubator at 37 °C within 15 minutes after the discs were placed for 24 – 48hrs [22]. The results were read as zone of inhibition in millimeters according to CSLI 2020 [23].

Extraction Procedure
For the extraction process (100 grams of seed powder were dissolved in 800 milliliters of solvent (chloroform, methanol, and water), the seed to solvent ratio was 1:8 [24]. Each mixture was applied in soxhlet apparatus for 10 to 18 hours to obtain the desirable extracts [25].

Then the raised product was evaporated by a rotatory evaporator at 40 °C. The yielded extract was collected and weighed. This process was repeated three times to obtain adequate extract quantities for each solvent.

The yielded extract substance was weighed and stored in a sterile dark container with label and kept at 4 °C in the refrigerator till used.

Detection of Bioactive Components

The existence of organic and inorganic chemicals in a sample is determined using fourier transform infrared (FTIR) [26], and gas chromatography-mass spectrometry (GC-MS) as quantitative assays of functional group contents [27].

Fourier Transform Infrared

The procedure involved mixing (1–2 mg) of the substance of interest with (200 mg) of potassium bromide. The combination was grounded, and then the mixture was mechanically pressed to produce a disc, which was then transferred to the IR chamber and examined. Diffuse reflectance was measured directly on powdered materials, and the results were recorded [28].

Gas Chromatography-Mass Spectrometry(GC-MS)

Separation conditions for numerous compound types are provided, along with derivative and un-derivative compounds were done by GC-MS through injection of the samples 1µl in an analytical column under special requirements such as the pressure of 11.933 psi, GC inlet line temperature of 250 ˚C, Aux heater temperature of 310 ˚C, carrier gas He 99.99%, injector temp 250 ˚C, scan range m/z 50-500, injection type splitless.

GC-MS Oven Program: Time amounted to about 34 minutes.

Ramp1: (2˚C hold to 55 ˚C/min.)
Ramp2: (55 ˚C to 180 ˚C 7˚C/min.)
Ramp3: (180˚C to 280˚C 8˚ C/min.)
Ramp4: (280˚C hold to 2˚C/min.)

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 on Candida spp.[29]. The organisms were reactivated by cultivating them on SDA and NA and incubating them at 37 °C for 24 and 48 hours, respectively. Microbial concentrations were used at 0.5 McFarland standards. The tested microorganisms were spread using cotton swabs on MHA plates. 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). DMSO/DW was used as a negative control. Nystatin (100 units) was used as a positive control for Candida spp. The plates were incubated at 37°C for 48 hours. Later on, inhibition zones were measured in millimeters. This process was carried out in triplicate for each extract and concentration, and the means of inhibition zones were recorded.

RESULTS AND DISCUSSIONS

Distribution of Age and Sex

A total of 477 cases suffering from severe COVID-19 were identified by physicians, of which 455 were enrolled in this study. Patients' ages ranged from 20 to 91 years (mean 52.23, SD 15.009). This study indicated that more than half of the samples were males [(262) 57.6%] and [(193)42.4%] were females. (Figure NO.1)

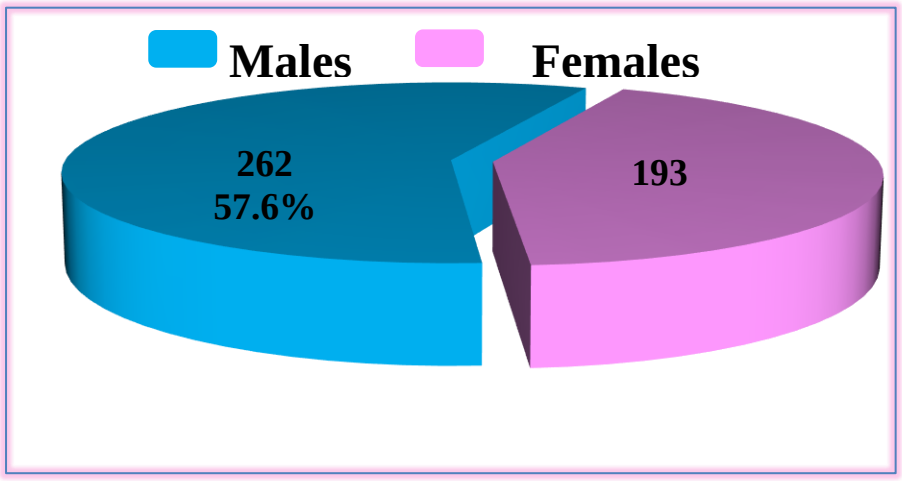

FIGURE 1: Sex distribution for patients.

Regarding to sex distribution that showed in the figure (4-1) the males were (262) 57.6% and (193) 42.4% were females. This result agree with Media S. et al. 2022/Iraq [30] that the sex distribution were males 94 (53.71%) and females were 81(46.29 %) among patients with COVID-19. While it nearly agrees with Somayeh Y. et al.(2022/Iran[31] who found that the percentage of infected males with COVID-19 was 64 (60.4%) while females was 42 (39.6%). Shastri et al. (2020)/Indian [32], Thushara G. et al. (2020)/ USA [33], and Catherine G. et al. (2020)/ Italy, China, Spain, France, Germany, and Switzerland are among the countries involved in this study [34], that proved that males were more affected with COVID-19 than females for multifactorial reasons like hormonal differences in both sexes, and because their immune systems work better than males', females are somewhat protected from COVID-19 and have the fewest signs of the disease.

Microbial Growth and Antimicrobial Sensitivity

According to the sputum sample, culture characteristics, and disc diffusion method for antimicrobial sensitivity, Candida spp. accounted for 166 (36.5%) of the total growth, with 8 (1.8%) isolates being resistant. Table (1) shows the microbes found in the cultured sputum samples.

TABLE 1: Microbial growth and anti-microbial sensitivity

Types of Growth	**Anti-Microbial Sensitivity**			Total NO.(%)
	Sensitive NO. (%)	**Intermediate NO. (%)**	**Resistant NO. (%)**	
Candida spp.	145 (31.9)	13 (2.9)	8 (1.8)	166 (36.5)
Bacterial growth	NA*	NA*	NA*	248 (54.5)
No Growth	0	0	0	41(9.0)
*NA: Not Applied				

Antifungal Sensitivity Profile of Isolated Candida species

According to the API Candida, the results of Candida spp. isolates were 128 (77%) C. albicans isolates and 38 (33%) non-albicans Candida isolates, as shown in figure(2).

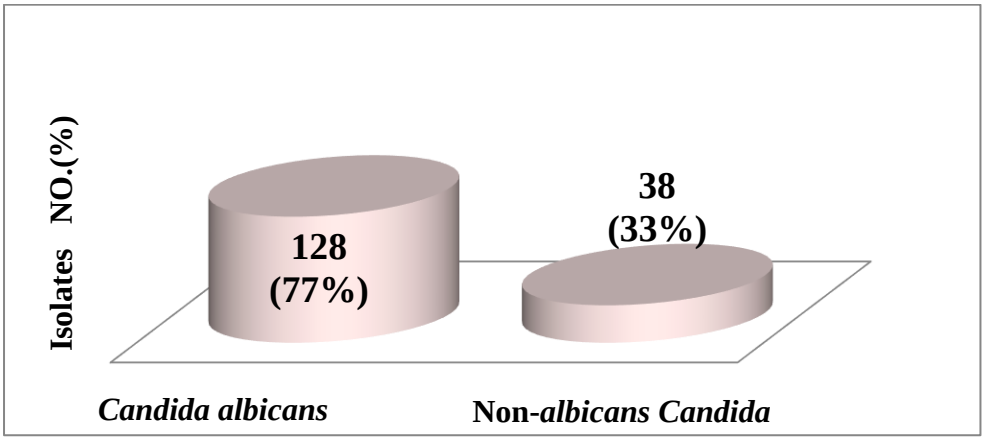

FIGURE 2: Candida spp isolated from sputum samples

Regarding the CLSI 2020 antifungal susceptibility test, four standard antifungal discs were used. The results showed that Candida spp. isolates were sensitive to antifungals; the highest sensitivity was to amphotericin-B 113 (68.1%) isolates, followed by ketoconazole 112 (67.5%), fluconazole 103 (62%), and the lowest was to nystatin 98 (59%) isolates, as shown in table (2).

TABLE 2: Antifungal sensitivity profile for Candida spp.

Antifungal	Symbol	Sensitive NO. (%)	Intermediate NO. (%)	Resistant NO. (%)
Amphotericin B	**AMB**	113 (68.1)	49 (29.5)	4 (2.4)
Fluconazole	**FLC**	103 (62.0)	58 (35.0)	5 (3.0)
Ketoconazole	**KT**	112 (67.5)	51 (30.7)	3 (1.8)
Nystatin	**NS**	98 (59.0)	64 (38.6)	4 (2.4)

Regarding to the microbial growth and susceptibility test results are mention in table (4-1), for Candida spp. represented 166 (36.5%) of the total growth results which is nearly agree with Segrelles C. et al. (2021)/Spain [35] who found the patients with severe COVID-19-associated pneumonia were formed more likely to have systemic candidiasis**.**

Regarding to Candida spp., Candida albicans represents 128(77%) of the total candida isolates, this result agree with Somayeh Y. et al.(2022/Iran [31], they found that Candida albicans was the most commonly isolated species of the genus Candida (69%), and also agree with Ioannis V. et al. (2020)\Greece [36] they proved that Candida spp. infection was found in severe case of COVID-19 patients.

Regarding to anti-Candida susceptibility test the resistance results of AMB, FLC, KT and NS were [4(2.4%), 5(3.0%), 3(1.8%) and 4(2.4%)] respectively, these result nearly agree with Somayeh Y. et al.(2022/Iran [31] who demonstrated that Candida spp. isolated from COVID-19 patients were sensitive to the majority of anti-candidal agents. While disagree with Dhara N. et al.(2021)/USA [37], that was 26 (20%) of patients had fluconazole resistance Candida spp. because the most patients enrolled in their study represented 14% of the total cases pre used of fluconazole as prophylaxis therapy.

Fourier Transform Infrared (FTIR) Assay

The infrared spectrum of the most active subfraction of fenugreek seed extracts in water, methanol, and chloroform showed that the methanolic extract had the highest concentration of [carboxylic acid, alcoholic, double-bond, and long-chain fatty acids] as 34 peaks, followed by chloroform, which contained 22 peaks. On the other hand, the fenugreek seed aqueous extract had the lowest number of active compounds, with only seven peaks, as shown in figures (3) , (4), and (5), respectively.

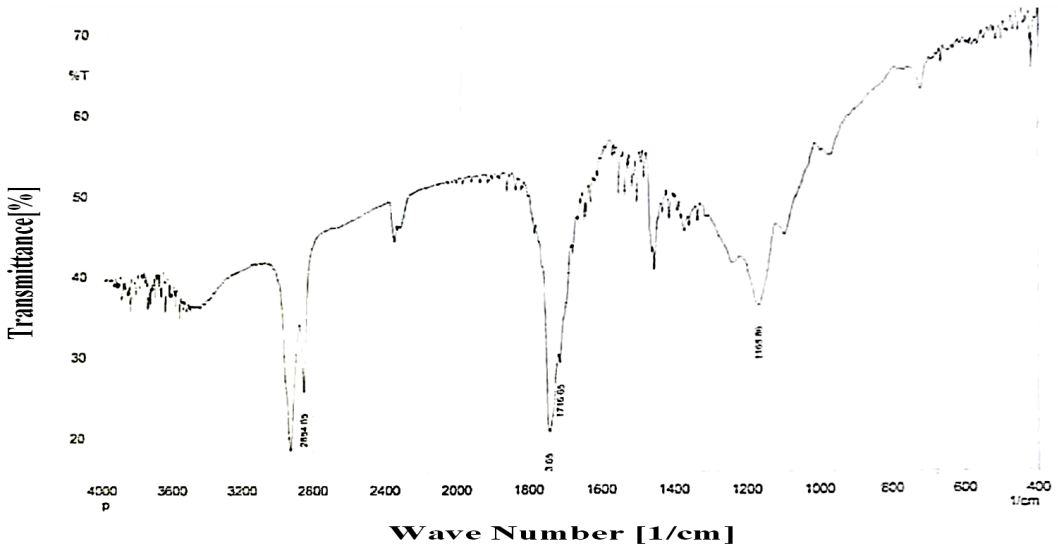

FIGURE 3: The FTIR of fenugreek seed aqueous extract

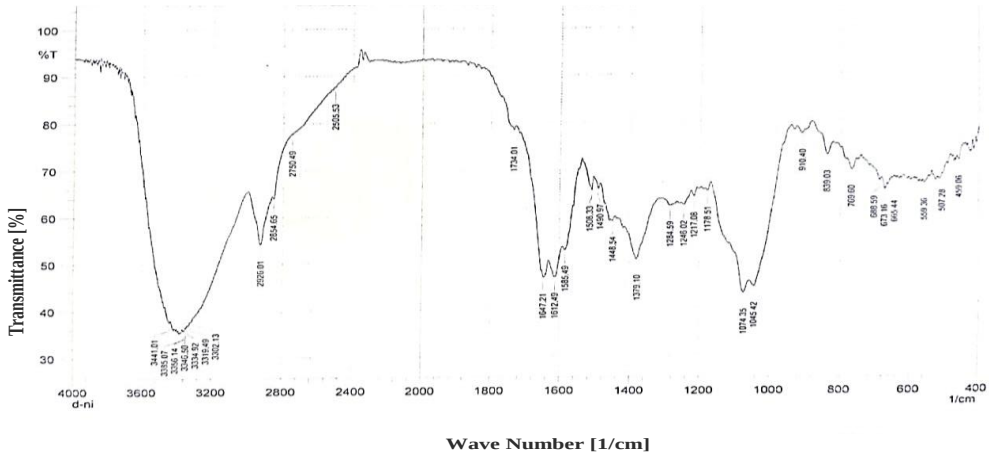

FIGURE 4: The FTIR of fenugreek seed methanol extract

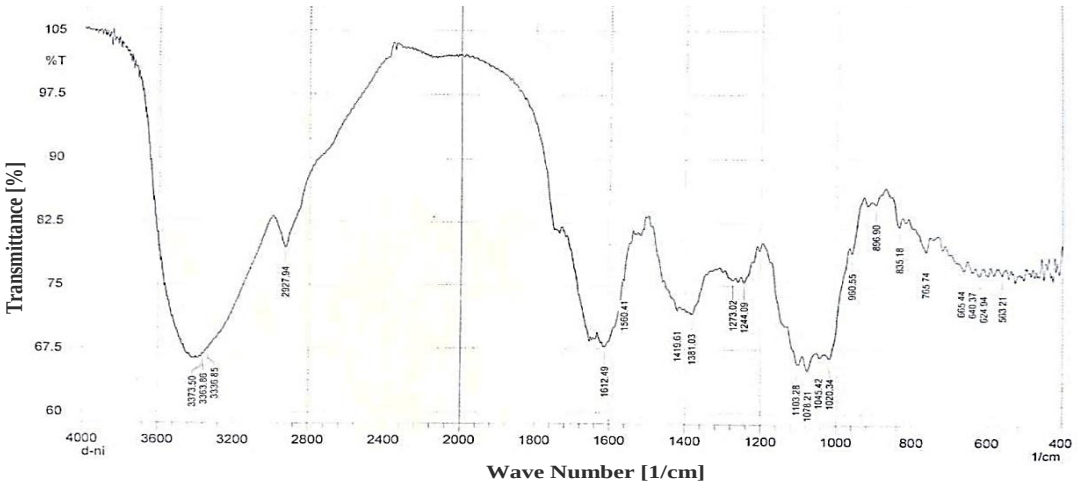

FIGURE 5: The FTIR of fenugreek seed chloroform extract.

J Popul Ther Clin Pharmacol Vol 30(3):e291–e302; 11 January 2023.

These results agree with G. M. El-Bahy (2005)/Egypt[38]; Which reported nearly comparable peak results, when methanol reported the highest peak count, chloroform reported the second highest, and aqueous extracts reported the lowest, and agree with Sweeta Akbari et al. (2019)/ Malaysia[39], who documented peaks number in fenugreek seed aqueous extract of 9 peaks and partially agree with Medhat I. et al. (2010)/Egypt[40], who documented 12 peaks in fenugreek seed aqueous extract, this partial agreement may due to the use of different method and solvent for extraction or due to intra seed variation.

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 compounds seventeen compounds), followed by aqueous ten compounds and chloroform was the last one eight compounds), as shown in the tables (3), (4) and (5) below.

TABLE 3: GC-MS of fenugreek seeds aqueous extracts

Aqueous Extract			
No.	**Compound**	**Minutes**	**Area%**
1.	2-Trimethylsilyloxybenzaldehyde	5.4	2.41
2.	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	10.0	26,27
3.	Hexa-1,3,5-trienyl]cyclohex-1-en-carboxaldehyde	12.3	1.97
4.	Nonane dioic acid	15.3	1.24
5.	9-Octadecenal	17.6	11.2
6.	Linoleic acid	18.1	52.9
7.	2-Pentadecanone	18.6	3.55
8.	Azelaic acid	18.9	15.7
9.	Vitamin E	20.4	0.68
10.	Isopropyl linoleate	24.0	1.05

TABLE 4: GC-MS of fenugreek seeds methanol extracts

NO.	**Compound**	**Minutes**	**Area%**
1.	Cyclopentanone	9.6	3.1
2.	2-Butenoic acid	17.6	1.84
3.	Benzoic acid	18.4	4.78
4.	Hexadecanoic acid, methyl ester	23.5	3.43
5.	1,2-Benzisothiazole	24.36	1.37
6.	6-Octadecenoic acid, methyl ester	25.8	19.88
7.	Heptadecanoic acid, 16-methyl ester	26.1	2.72
8.	Palmidrol[Valeric acid]	26.39	2.3
9.	dimethylphosphor amidothioate	27.09	16.81
10.	Butanoic acid	27.34	13.14
11.	2-Bromoethyl	28.41	6.28
12.	2-Decanone	29.83	10.11
13.	Vitamin E	30.4	1.04
14.	Oleic Acid	31.0	1.42
15.	13-Octadecenal	32.7	7.31
16.	Cyclopropane	33.25	1.37
17.	Succinic acid	34.12	3.28

TABLE 5: GC-MS of fenugreek seeds chloroform extracts

NO.	**Compound**	**Minutes**	**Area%**
1.	Benzopyran	16.5	3.22
2.	Heptadecanoic acid, 16-methyl ester	21.2	15.69
3.	6-Octadecenoic acid, methyl ester	23.4	63.26
4.	Oleic Acid	26.31	6.89
5.	2-Pentadecanone	27.86	4.08
6.	7-Hexadecenoic acid, methyl ester	28.63	2.93
7.	1,2-Benzisothiazole	31.25	3.05
8.	Cyclopropane	31.66	1.4

Regarding to GC-MS analysis of fenugreek seed aqueous extract that appeared to contain 10 bioactive compounds and this result nearly agree with G. Deokar et al. (2019)\ India[41] who certified 12 peaks of aqueous FTIR assay of fenugreek seed, and also agree with Joaquin N et al. (2022)\ Spain [42] who said that GC-MS analysis of an aqueous extract of fenugreek seeds were identified by 15 peaks of bioactive compounds. The current results disagree with Bayan Y.et al.(2015)/ Iraq [43] who found 38 bioactive compounds and this discrepancy may because using different extraction method (maceration method with heating) with citric acid and sugar powder before GC-MS analysis which yielded many peaks of detected compounds.

Regarding GC-MS analysis of fenugreek seed methanolic extract that appears to contain (17) bioactive compounds, this result nearly agrees with Henda K. et al. (2018) / Tunisia [3], the findings of this study were 20 bioactive compounds by using hexane as a solvent and nearly agrees with Sweeta A. et al. (2019) / Malaysia [39] in which found 17 compounds was seen at the retention time 37.43min, while the current results were so far from these obtained by Abd-Alrahman, S. et al.(2013)/ Saudi Arabia [44] who obtained 20 bioactive compound when use nearly polarity solvent (ethanol). While the study result was disagree with Neda A. et al. (2020)\Iran [45] who found 8 bioactive compounds in fenugreek alcoholic extract, this difference may due to used extract of fenugreek leaves, also the current results disagree with those by Sherif H. et al.(2013) / in the common study at Egypt and Saudi Arabia [46] they obtained 34 bioactive compounds. This discrepancy may due to their use of a solvent mixture (water and ethanol) resulting more bioactive compounds.

Regarding to GC-MS analysis of fenugreek seed chloroform extract that appeared contain (8) of bioactive compounds, this result agree with Suchandra C. et al. (2010)/ India [47] that found 9 bioactive compounds using maceration method for extraction, while it disagrees with Sharma, J. et al. (2017)/ India [48] who gained 20 bioactive compounds. This discrepancy arises from the fact that various extraction techniques were put to use, the retention time of bioactive compounds was lengthened in GC-MS, or there is more intra-seed variation that gives rise to more peaks of bioactive compounds.

Anti-Candida 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 against resistant isolates of Candida spp.; At 50 mg/ml, the methanolic extract showed the greatest effect, with a mean inhibition zone of 9.33 mm and a significant value of 0.01; at 100 mg/ml, the chloroform extract showed the next greatest effect, with a mean inhibition zone of 10.33 mm and a significant value of 0.005. At 150 mg/ml, the aqueous extracts showed the least effect, with a mean inhibition zone of 8.33 mm and a non-significant value of 0.024. These are shown in table (6).

TABLE 6: The effect of fenugreek seed extracts against Candida spp.

	Aqueous Extracts		Methanol Extract		Chloroform Extract	
	Inhibition Zone per millimeters					
Concentration	Mean (SD)	P. Value	Mean (SD)	P. Value	Mean (SD)	P. Value
25 mg/ml	1.33 (0.57)	0.753	5.33 (0.57)	0.129	3.0 (0.0)	0.432
50 mg/ml	3.0 (0.0)	0.432	9.33 (0.57)	0.014 *	5.33 (0.57)	0.129
100 mg/ml	5.67 (0.57)	0.093	15.0 (0.0)	0.001 *	10.33(0.57)	0.005 *
150 mg/ml	8.33 (0.57)	0.024	18.33(0.57)	0.0001 *	13.67(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)	-	0.0 (0.00)	-
* Significant P. Value ≤ 0.01						

According to the anti-candida effect of fenugreek seed extracts in table (6) that appear a significant effect of tested extracts against Candida spp. at different concentrations of methanolic and chloroform solvents. These results agree with Tomasz B. et al. (2017)\ Poland [49], which authenticated that fenugreek extract had a protection against fungal pathogens.

The current study certified that methanolic was the strongest, followed by chloroform, and finally the aqueous extract, these results indicate the ability of solvent types and polarity to dissolve unique bioactive compounds that act in various ways to inhibit many microorganisms, as in Candida spp. Jolly O. et al (2022)\ Kenya [50] proved that ethanol and methanol solvents were the strongest in thier anti-microbials effects when used in the extraction of herbals bioactive compounds, and also agree with Hussein A. et al. (2021)\Iraq [51] and Riti T. et al.(2021)\ India [52] results who documented the aqueous fenugreek seed extract has a broad antifungal activity against fungi, and also agree with Eiman S. et al.(2022)\Sudan [53] who examined the antifungal activity of fenugreek seed extract using the paper disc diffusion method and showed a clear effect on Candida albicans, and also agree with Fathi M. et al. (2015)/ Libya[4] who documented that aqueous (hot, cold and boiled)extracts of fenugreek seed not effect on C. albicans.

Regarding the zone of inhibition, the result of methanol and chloroform fenugreek seed extracts at the concentration(50 and 100 mg/ml) 9.33 and 10.33mm respectively, these results agree with Maryam J. et al. (2021) \ Iraq [54] who proved that fenugreek seed extract have an inhibitory effect against Candida spp. with mean zone of inhibition 10.47 mm and they demonstrated that alcoholic extract had the larger zone of inhibition.

CONCLUSIONS

1. Methanol extract of fenugreek seed was the most effective antifungal, followed by chloroform extract, and the aqueous extract was the least effective.
2. Candida spp. were most frequent isolated yeast from sputum of patients with severe COVID-19.
3. The most effective anti-candida drug is ketoconazole.

REFERENCES

1. Yao, D., et al., Advances on application of fenugreek seeds as functional foods: Pharmacology, clinical application, products, patents and market. Critical reviews in food science and nutrition, 2020. **60**(14): p. 2342-2352.
2. Dar, T.A. and M. Uddin, Fenugreek: A Miraculous Medicinal Herb. J. Complement. Med. Alt. Healthcare, 2018. **7**: p. 1-3.
3. Keskes, H., et al., LC–MS–MS and GC–MS analyses of biologically active extracts of Tunisian Fenugreek (Trigonella foenum-graecum L.) Seeds. Journal of Food Measurement and Characterization, 2018. **12**(1): p. 209-220.
4. Walli, R.R., et al., Screening of antimicrobial activity of fenugreek seeds. Pharm Pharmacol Int J, 2015. **2**(4): p. 1-4.
5. Baba, W.N., et al., Some nutraceutical properties of fenugreek seeds and shoots (Trigonella foenum-graecum L.) from the high Himalayan region. Food bioscience, 2018. **23**: p. 31-37.
6. Mashkor, I., Phenolic content and antioxidant activity of fenugreek seeds extract. International Journal of Pharmacognosy and Phytochemical Research, 2014. **6**(4): p. 841-844.
7. Hema, R., S. Kumaravel, and K. Alagusundaram, GC/MS determination of bioactive components of Murraya koenigii. Journal of American Science, 2011. **7**(1): p. 80-83.
8. Karpagasundari, C. and S. Kulothungan, Analysis of bioactive compounds in Physalis minima leaves using GC MS, HPLC, UV-VIS and FTIR techniques. Journal of Pharmacognosy and Phytochemistry, 2014. **3**(4): p. 196-201.
9. Ceylan, Ş., et al., Chemical Composition, Antimicrobial, and Antioxidant Activities of Medicinal Plants Nutsedge and Fenugreek. BioResources, 2022. **17**(3).
10. Hadi, S.T. and A.A. Mariod, Antioxidant and antimicrobial activity of fenugreek (Trigonella foenum-graecum) seed and seedoil, in Multiple Biological Activities of Unconventional Seed Oils. 2022, Elsevier. p. 111-117.
11. Miedzianka, J., et al., Comparative Evaluation of the Antioxidative and Antimicrobial Nutritive Properties and Potential Bioaccessibility of Plant Seeds and Algae Rich in Protein and Polyphenolic Compounds. Applied Sciences, 2022. **12**(16): p. 8136.

12. Rawat, S. and R. Dubey, Anti-Microbial Studies Of Aqueous, Methanolic And Saponins Extract Of Seeds Of Trigonella Foenum-Graecum On Human Vaginal Pathogens Causing Uti Infection. 2022.
13. Nadimpalli, M., et al., Combating global antibiotic resistance: emerging one health concerns in lower-and middle-income countries. Clinical Infectious Diseases, 2018. **66**(6): p. 963-969.
14. Pristov, K. and M. Ghannoum, Resistance of Candida to azoles and echinocandins worldwide. Clinical Microbiology and Infection, 2019. **25**(7): p. 792-798.
15. Aslam, B., et al., Antibiotic resistance: a rundown of a global crisis. Infection and drug resistance, 2018. **11**: p. 1645.
16. Cunha, A.R., et al., Advances in Botanical Research. Advances in Botanical Research, 2021: p. 224.
17. Zia-Ul-Haq, M., et al., Alternative Medicine Interventions for COVID-19. 2021: Springer.
18. Wang, J., A. Sasse, and H. Sheridan, Traditional chinese medicine: from aqueous extracts to therapeutic formulae, in Plant Extracts. 2019, IntechOpen.
19. Mora-Montes, H.M. and L.M. Lopes-Bezerra, Current progress in medical mycology. 2017: Springer.
20. AL-Abedi, S.F., Comparative Study For Candida Spp Isolated And Identification From Goats Milk With Mastitis Between Api Candida Kit Test, Candida Chromogenic Agar And Vitek 2 System.
21. Ioannou, P., et al., Candida spp. isolation from critically ill patients' respiratory tract. Does antifungal treatment affect survival? Germs, 2021. **11**(4): p. 536.
22. Wayne, P., Method for antifungal disk diffusion susceptibility testing of yeasts. CLSI m44-a, 2004. **23**(6).
23. Weinstein, M.P. and J.S. Lewis, The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. Journal of clinical microbiology, 2020. **58**(3): p. e01864-19.
24. Akbari, S., A. Nour, and R. Yunus. Determination of phenolics and saponins in fenugreek seed extracted via microwave-assisted extraction method at the optimal condition. in IOP Conference Series: Materials Science and Engineering. 2020. IOP Publishing.
25. Organization, U.N.I.D., et al., Extraction technologies for medicinal and aromatic plants. 2008: Earth, Environmental and Marine Sciences and Technologies.
26. Shameer, P.M. and P.M. Nishath, Exploration and enhancement on fuel stability of biodiesel: A step forward in the track of global commercialization, in Advanced biofuels. 2019, Elsevier. p. 181-213.
27. Hübschmann, H.-J., Handbook of GC-MS: fundamentals and applications. 2015: John Wiley & Sons.
28. Abidi, N., FTIR Microspectroscopy: Selected Emerging Applications. 2021: Springer Nature.
29. Jasim, A., et al., Studies on antifungal properties of some plant extracts (garlic, fenugreek, ginger) against of clinical isolate Candida species. Int J Sci Eng Technol Res, 2013. **2**(19): p. 2180-5.
30. Muhammed, M.S.A., Detection of Aspergillus spp. and Candida albicans Antigenemia in a Sample of Iraqi Patients with Severe COVID-19, in Medical microbiology. 2022, Al-Nahrain University: Iraq- College of Medicine -Al-Nahrain University. p. 90.
31. Yazdanpanah, S., et al., Assessment of Risk Factors and Clinical Outcomes in Hospitalized COVID-19 Patients with Candida spp. Co-infections: Species Distribution and Antifungal Susceptibility Patterns of Isolates. Mycopathologia, 2022: p. 1-12.
32. Shastri, A., et al., Delayed clearance of SARS-CoV2 in male compared to female patients: high ACE2 expression in testes suggests possible existence of gender-specific viral reservoirs. MedRxiv, 2020.
33. Galbadage, T., et al., Systematic review and meta-analysis of sex-specific COVID-19 clinical outcomes. Frontiers in medicine, 2020. **7**: p. 348.
34. Gebhard, C., et al., Impact of sex and gender on COVID-19 outcomes in Europe. Biology of sex differences, 2020. **11**(1): p. 1-13.
35. Segrelles-Calvo, G., et al., Candida spp. co-infection in COVID-19 patients with severe pneumonia: Prevalence study and associated risk factors. Respiratory Medicine, 2021. **188**: p. 106619.
36. Ventoulis, I., et al., Bloodstream infection by Saccharomyces cerevisiae in two COVID-19 patients after receiving supplementation of Saccharomyces in the ICU. Journal of Fungi, 2020. **6**(3): p. 98.
37. Shah, D.N., et al., Impact of prior inappropriate fluconazole dosing on isolation of fluconazole-nonsusceptible Candida species in hospitalized patients with candidemia. Antimicrobial agents and chemotherapy, 2012. **56**(6): p. 3239-3243.
38. El-Bahy, G., FTIR and Raman spectroscopic study of Fenugreek (Trigonella foenum graecum L.) seeds. Journal of Applied Spectroscopy, 2005. **72**(1): p. 111-116.
39. Akbari, S., et al., Extraction, characterization and antioxidant activity of fenugreek (Trigonella-foenum graecum) seed oil. Materials Science for Energy Technologies, 2019. **2**(2): p. 349-355.

40. Ibrahim, M., Molecular spectroscopic study of acid treated fenugreek seeds. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2010. **77**(5): p. 1034-1038.
41. Deokar, G., et al., Impact of hydrothermal isolation method on the recovery of fenugreek seed hemi-cellulose analyzed through FTIR-DSC-SEM interpretations. Int. J. Pharm. Sci. Res., 2019. **10**(10): p. 4677-4684.
42. Navarro del Hierro, J., G. Reglero, and D. Martin, Chemical Characterization and Bioaccessibility of Bioactive Compounds from Saponin-Rich Extracts and Their Acid-Hydrolysates Obtained from Fenugreek and Quinoa. Foods, 2020. **9**(9): p. 1159.
43. Al-Abdullah, B.Y. and W.F. Jazaa, Production of Functional Drinks From Some Medicinal Herbs and Studying their Microbial Tests and Diagnosis The Chemical Components in them by GC-MS Technique. Tikrit Journal for Agricultural Sciences, 2015. **15**(3).
44. Abd-Alrahman, S.H., M.M. Salem-Bekhit, and M. Elhalwagy, Chemical composition and antimicrobial activity of Ziziphus jujuba seeds extract. Journal of pure and applied microbiology, 2013. **7**: p. 379-385.
45. Akhlaghi, N., G. Najafpour-Darzi, and H. Younesi, Facile and green synthesis of cobalt oxide nanoparticles using ethanolic extract of Trigonella foenumgraceum (Fenugreek) leaves. Advanced Powder Technology, 2020. **31**(8): p. 3562-3569.
46. Yakout, S.M., et al., Chemical Characterization and Antibacterial Activity of Ethanolic Extract of Trigonella foenum-graecum (Fenugreek) Seeds. JPure Appl Microbiol, 2013. **7**(2): p. 1373-1378.
47. Chatterjee, S., P.S. Variyar, and A. Sharma, Bioactive lipid constituents of fenugreek. Food Chemistry, 2010. **119**(1): p. 349-353.
48. Sharma, J., et al., Screening of Free Radical Scavenging, Anticancer Potential and GC-MS Analysis of Trigonella foenum graecum Leaves.
49. Bienkowski, T., et al., Content of biogenic elements and fatty acid composition of fenugreek seeds cultivated under different conditions. Chilean journal of agricultural research, 2017. **77**(2): p. 134-141.
50. Akullo, J.O., et al., Effect of aqueous and organic solvent extraction on in-vitro antimicrobial activity of two varieties of fresh ginger (Zingiber officinale) and garlic (Allium sativum). Heliyon, 2022. **8**(9): p. e10457.
51. Salim, H.A., et al., Efficacy of Fenugreek Seeds Extract (-Trigonella foenum graecum Penicillium Rhizoctonia solani) against sp, and Aspergillus ochraceus in vitro.
52. Kapoor, R.T., Comparative Analysis Of Bioactive Components And Antifungal Potential Of Trigonella Foenum-Graecum Seeds. Plant Archives, 2021. **21**(1): p. 2105-2108.
53. Elsiddig, E.S.E.H., Green Synthesis of Silver Nano-particles by Using Fenugreek Seeds Extract and Application as Antifungal Agent. 2022, Sudan University of Science & Technology.
54. Al-Salihi, M.J.O.M. and H.A.M. Al-Saedi, Estimation of The Inhibition Activity of Extracts of Green Tea Leaves, Bitter Melon Fruits, Chili Pepper and Fenugreek Seeds against The Yeasts Candida albicans, C. tropicalis, C. glabrata and C. krusei isolated from the Vagina of Married Women in Kirkuk City/Iraq. Turkish Journal of Physiotherapy and Rehabilitation. **32**: p. 3.

J Popul Ther Clin Pharmacol Vol 30(3):e291–e302; 11 January 2023.