University of Thi-Qar Journal of Science (UTJsci)
E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol. 11, No. 2, Dec. 2024

GC-MS Assay to Alkaloid Extract of *T. foenum-graecum* Seeds and Detection Effect of Extract as Antibacterial

Sura Salim Nadhm[1a*] and Alla N. AL- Waheeb[2]
Department of Biology, Collage of Science, University of Thi-Qar, Thi-Qar, 64001, Iraq[1,2]
[a*]Corresponding author: sura.salim@utq.edu.iq

Received: 2024-05-08, Revised: 2024-05-24, Accepted: 2024-10-06, Published: 2024-12-05

***Abstract*- The need for standardized modern analytical procedures becomes urgent due to the rise in drug-resistant for pathogens. These procedures use to isolate new bioactive compounds from medicinal plants. The medicinal plant-derived compounds could provide a new method to fight pathogenic bacteria. This study sheds light on the antimicrobial activity of plant-derived components, their possible mechanisms of action, and their chemical potential. Five different plant extract concentrations were used:12.5, 25, 50, 100, and 200 mg/ ml. The maximum inhibition zone for the alkaloid extracted from seeds of *Trigonella foenum* against *Klebsiella pneumonia* was (21.0 ± 0.63) mm at 200 Mg ml^{1} dose. In comparison, the minimum inhibition zone for *Pseudomonas aeruginosa* was (6.16 ± 0.40) mm at 25 Mg ml^{1}. According to the results of the gas chromatography-mass spectrometry (GC-MS) analysis, the chemical composition analysis of the *Trigonella foenum* extract had a high concentration of organic components with antimicrobial and antioxidant properties.**

***Keywords*-** GC-MS, Antibacterial, *Trigonella foenum*

I. INTRODUCTION

The use of plant extracts to treat various diseases becomes more widespread, especially due to the increase in bacterial resistance. This resistance poses a main threat to the health of individuals. Long-term frequent and indiscriminate use of medications has led to harmful side effects for individuals [1]. Medicinal plants contain many bioactive compounds, like alkaloids, flavonoids, phenolic compounds, steroids, tannins, terpenoids and other secondary metabolites, which have a remarkable effect on parasites and pathogens [2]. Plant-based compounds have unique pharmacological properties, like being affordable, less toxic, with fewer side effects, and less likely to develop resistance [3]. The increased stability and bioavailability of the plant extract enriches the individuals getting to ensure the thoroughness of product manufacturing. So, there are good numbers of plant - based pharmaceutical excipients in the markets. In addition, their abilities to exert a wide range of effects depends on their properties and molecular weights [4]. One of the most important plants is *Trigonella foenum* (Fenugreek). It is an essential medicinal plant was used in traditional medicine in numerous countries. There are several well-known pharmacological effects of Fenugreek, like treatment of hypoglycemia, hypocholesterolemia, gastroprotective, anti-inflammatory, antioxidant, antipyretic activities and appetite stimulation [5]. *Trigonella foenum* is considered one of the herbaceous and annual plants, so it resembles clover, and is characterized by being self-pollinating and having two cotyledons. Its length ranges between (20-60 cm). Its cultivation season is in the winter, starting from October to December [6]. It has a stem with hollow and dark green color. This is due to the presence of large amounts of anthocyanin. It appears circular to oval in cross-section, with a diameter ranging between 0.5-1 cm, and a length of about 50 cm or more. As Its root is wedge-shaped. Its length is 8-30 cm, and there are many root nodes on it. It is characterized by strength and branches into many secondary branches [7].

It has very small fllower with yellow color and triangular shape it comes out in the form of a cluster. They are complete, as they contain the calyx, corolla, stamens, and pistil. Each flower carries between (10-20) seeds and within (35-40) days the flower sprouts after being sown [8]. Misuse and over exploitation of conventional antibiotics has led to the need for development of novel antibacterial medication. Plants sources with active components have proven to be an effective source for extracting compounds with antibacterial property. The extract of fenugreek seed showed prominent effect as antibacterial agent on many pathogenic bacterial strains [9].

II. MATERIALS AND METHOD

A. Collection and Classification of study stations

In Thi-Qar Governorate, between October and December 2023, ***Trigonella foenum*** seeds were obtained from local markets. The seeds were then transported to the laboratory and cleaned of dust before drying in the shade within the laboratory at room temperature. Then, they were ground with an electric grinder and kept in dark glass vessels ,marked with the name of the plant sample. The collection area and the plant part were used and kept in the refrigerator at a temperature of 4 °C until use.

B. Preparation of ethanol extract

To conduct a qualitative screening of chemical compounds, a mixture of 20 g of seed powder and 250 milliliters of ethanol was subjected to Soxhlet continuous extraction. The resulting solution was filtered using Whatman No.13 filter paper and then concentrated at 50°C

https://doi.org/10.32792/utq/utjsci/v11i2.1248

under reduced pressure with a rotary evaporator. Afterward, it dried at 25°C. The extract was finally collected in sterilized glass tubes [10].

C. Qualitative analysis of some phytochemicals

Detection of tannin compounds in plant extract by using 5% concentration of Hydrous acetate mercury chloride was employed, (1 mL) from reagent was added to (1 mL) from plant extract and if a white precipitate showed that means a positive reaction occurred [11].

Detection of peptides and amine-free group was performed according to Romay *et al.* [12], These groups were identified by using 1% Ninhydrin reagent. where 1 ml of reagent was mixed with 1 ml of the extract. The mixture was heated for 10 minutes in a boiling water bath. The presence of peptides and amino acids is indicated by the violet color.

Detection of alkaloids was done using Drakendorff's reagent by taking a few drops of picric acid (C_6 H_3 N_3 O_7) 0.1 % added to 5 ml from a plant extract in the test tube. The emergence of a yellow color refers to presence of alkaloids [13].

Detection of glycosides was done by adding five ml of plant extract into two ml of acetic acid. Then,1 ml of concentrated sulfuric acid H_2SO_4 was added into a drop of ferric chloride $FeCl_3$. The presence of glycosides was indicated by the formation of a brown ring on the inner surface [13].

Detection of flavonoid was done by adding One ml of potassium hydroxide ethanol with a concentration of 5% was added to 1 ml of the Extract, and the yellow precipitate's appearance indicates a positive reaction [14].

Detection of phenols was done by taken (0.5 ml) from plant extract and put it in the test tube. Then, we added some drops of Ferric chloride solution at a concentration of (0.5%). The appearance of the dark green refers to the presence of phenolic compounds [15].

D. Separation of Alkaloid from plant extract

We used the same methodology of Bobby *et al* [16]. we took (20 g) of dried seeds powder added to (200 ml) of hexane to extract the fats for 24 hours, by using a soxhlet extraction device or magnetic stirrer. For the plant powder was dried at room temperature to extract alkaloid from it. The extraction process was repeated using a Soxhlet extractor or magnetic stirrer by adding 250 ml of 10% acetic acid in 95% ethyl alcohol for 24 hours. Then, the solution was concentrated by using the condenser rotary evaporator device at 50°C. After this step, we added the concentrated ammonium hydroxide solution in drops to the acid solution until the PH becomes equal to 9, using pH meter. After that, we filtered the solution and placed it in a separatory funnel. 100ml of chloroform was added to it several times, then it was left to settle and separate into two layers. The lower layer was taken, which represents the layer in which the alkaloids were dissolved. Then, we concentrated the solution using a concentrator to 5 ml or 10 ml and dry it at room temperature.

E. GC-MS Analysis of Extracts GC-MS Conditions

The GC-MS profile was conducted out at GCMS-QP2010 plus device (Shimadzu, Kyoto, Japan) that is equipped with a 5 ms capillary column measuring 30 x 25 mm and a 0.25 μm film thickness, as well as an autoinjector. The carrier gas, helium, has a flow rate of 1.15 milliliters per minute. The 70eV ionized charge system was used to apply mass spectroscopic scanning. After two minutes, the temperature was progressively raised from 80°C to 280°C over the next five minutes at a rate of 10°C per minute. Splitting mode was applied to the injected samples at 250°C. The National Institute of Standards and Technology (NIST14) and the Wiley 10th/NIST 2014 mass spectral library (W10N14) were the two mass spectral databases used to characterize the separated components using mass spectra and retention times. All the analyses were completed at the Industrial Research Directorate of the Ministry of Industry in Baghdad, Iraq [17].

F. Culturing of Samples

For patients suffering from gastrointestinal infections, Imam Hussein Teaching Hospital provided ready to use and diagnosed bacterial samples. They underwent a VITEK test as well as other biochemical testing to validate the diagnosis of bacteria.

G. Antibiotic sensitivity test

Antibiotic sensitivity testing was carried out using the previously described disk diffusion method. Mueller Hinton agar medium included Nitrofurantoin (300 μg), and some of antibiotics were used in this study, including Gentamicin, Amikacin, Ceftriaxone, Cefotaxime, and other antibiotics (Bioanalysis, Turkey). We compared our results with the standard tables that was mentioned in this study to determine the diameter of the inhibition zone 0.22 Micrometer. Concentrations of 1000 ppm were of extract attended [18].

H. Analytical Profile Index

To identify the isolated bacteria, a fully automated system called VITEK, which performs bacterial identification and antibiotic susceptibility testing, was used.

III. RESULTS

A. Quantitative phytochemical screening of Trigonella foenum seeds ethanol extract results

Table 1: Detection of active compounds

Ethanol extract of *T.fonum*	Alkaloids	Amino acid	Glycoside	Tannins	Phenols	Flavonoid
	+	+	+	+	+	+

B. GC-MS analysis of chemical compounds in the alkaloid Extrac of Trigonella foenum

Thirty compounds were extracted from the *Trigonella foenum* seed, and the extraction of the compounds began from the fifth minute to the thirty-one minute. Also,it showed six chemical compounds occupied most of the surface area of the extract. 55.73%, of these compounds were (Palmidrol), (Hexadecanoic acid, methyl ester), (n-

Hexadecanoic acid), (Phenol, 2,5-bis1,1-dimethylethyl), (Pentan-1-one, 1-(2-fluorenyl)-5), (1-Piperidinepropanol,alpha.–cycl) and (n-Pentadecanol) while the other 24 chemical compounds occupied the other remaining area 44.27% *Trigonella foenum.*

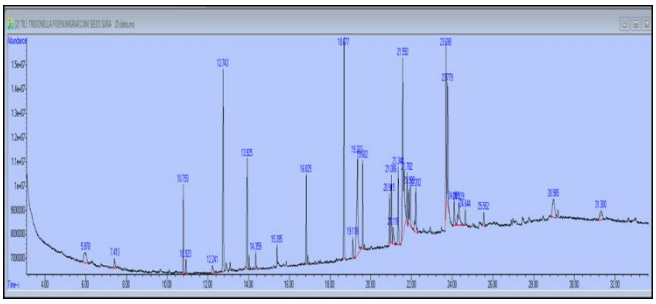


Fig. 1: Retention time for organic compounds extracted from

Table 2: Chemical Compound in in *Trigonella foenum* seed

Peak	R. Time	Common Name	formula	Area%
1	5.975	Benzoic acid, methyl ester	C_8H_8O	1.87
2	7.415	Pyrimidine, 4,6-dimethoxy-5-nitro-	$C_6H_7N_3O_4$	0.75
3	10.790	1-Tetradecanol	$C_{14}H_{30}O$	3.51
4	10.920	Tetradecane	$C_{14}H_{30}$	0.70
5	12.244	cis-Aconitic anhydride	$C_6H_4O_5$	0.67
6	12.746	Phenol, 2,5-bis(1,1-dimethylethyl	$C_{14}H_{22}O$	7.93
7	13.923	n-Heptadecanol-1	$C_{17}H_{36}O$	4.28
8	14.356	4-Butylbenzoic acid, tridecyl este	$C_{24}H_{40}O_2$	0.77
9	15.395	2-Propenoic acid, tridecyl ester	$C17_7H_{32}O_2$	0.72
10	16.823	n-Nonadecanol-1	$C_{19}H_{40}O$	3.41
11	18.675	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	9.40
12	19.117	Dibutyl phthalate	$C_{16}H_{22}O$	0.88
13	19.333	n-Hexadecanoicacid	$C_{16}H_{32}O$	8.29
14	19.601	Behenic alcohol	$C_{22}H_{46}O$	3.89
15	20.917	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O$	2.03
16	21.003	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O$	2.84
17	21.116	Pyrimidine, 4,6-dimethoxy-5-nitro-	$C_6H_7N_3O_4$	1.48
18	21.350	Methyl stearate	$C_{19}H_{38}O_2$	3.38
19	21.592	Palmidrol&Octanamide,N-(2-hydroxyethyl)		13.63
20	21.782	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	1.86
21	21.912	Octadecanoic acid C18H36O & Oleic Acid	$C_{18}H_{34}O_2$	3.24
22	22.207	Pentafluoropropionic acid, pentade cyl este	$C_{18}H_{31}F_5O_2$	2.70
23	23.695	Tridecanedioic acid,dimethylester	$C_{15}H_{28}O_4$	7.25
24	23.782	Octanamide, N-(2-hydroxyethyl)-	$C_{10}H_{21}NO_2$	4.95
25	24.102	Decanamide, N-(2-hydroxyethyl)	$C_{12}H_{25}NO_2$	1.49
26	24.647	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	2.40
27	24.647	cis-Aconitic anhydride	$C_6H_4O_5$	0.76
29	28.984	NON		
30	31.303	Benzaldehyde,2-nitro-,diaminomet hylidenhydrazone	$C_8H_9N_5O$	1.38
Total				100%

C. Antimicrobial Activity of alkaloid extract for Trigonella foenum seeds

This study has been designed for determain the antibacterial activity of Trigonella foenum seed alkaloid extracts against various pathogenic bacteria, including both Gram-positive (S. aureus) and Gram-negative (P. aeruginosa, K. pneumoniae, and E. coli) Table (3). The stock solution of the plant extract was prepared in different concentrations of 12.5, 25, 50, 100, and 200 mg ml^1 and tested against these four types of bacteria. The antibacterial activity of the extract was ascertained by measuring the bacteria growth inhibition zone, which was clearly visible. The maximum activity of the alkaloid extract was observed against *K. pneumonia* at 200 mg ml^1 concentration, with an inhibition zone of (21.0 ± 0.63) followed by *P. aeruginosa* at 200 mg ml^1 concentration with an inhibition zone of (20.6 ± 0.51). On the other hand, the minimum activity was observed in 25 mg ml^1 concentration against *P. aeruginosa* with inhibition zone (6.16 ± 0.40) Additionally, there was no biological activity observed against *E. coli*, *S. aureus* and *P. aeruginosa* in 12.5 mg ml^1 concentration and *S. aureus* in 25 mg ml^1 concentration. This study also included the use of antibiotics such as (Imipenem, Meropenem, Amikacin, Levofloxacin, and Ciprofloxacin).

Table 3: Activity of *Trigonella foenum* against isolated bacteria

Con mg ml^1	Cases No.	*E. coli*	*S. aureus*	*P. aeruginosa*	*K. pneumonia*
		Trigonella foenum Mean ± S. D (Inhibition zone dimeter)			
12.5	6	0.00 ± 0.00^e	0.00 ± 0.00^d	0.00 ± 0.00^e	10.0 ± 0.00^e
25	6	9.83 ± 0.40^d	0.00 ± 0.00^d	6.16 ± 0.40^d	10.8 ± 0.40^d
50	6	10.6 ± 0.51^c	7.83 ± 1.16^c	7.16 ± 0.41^c	11.6 ± 0.81^c
100	6	14.6 ± 0.51^b	13.1 ± 0.75^b	16.5 ± 0.83^b	15.6 ± 0.81^b
200	6	16.0 ± 0.63^a	16.0 ± 0.89^a	20.6 ± 0.51^a	21.0 ± 0.63^a
p. value		< 0.001	< 0.001	< 0.001	< 0.001
LSD		0.55	0.87	0.60	0.73

Table 4: Antibiotic susceptibility of *K. pneumonia against different antibiotics*

Antibiotics	*K. pneumonia* Inhibition Zone Mean ± S. D
Imipenem	36.3 ± 2.30^a
Meropenem	37.6 ± 0.57^a
Amikacin	21.6 ± 0.57^c
Levofloxacin	29.6 ± 0.57^b
Ciprofloxacin	31.0 ± 1.00^b
Gentamycin	31.0 ± 1.00^b
Ceftriaxone	17.6 ± 2.08^d
Cefotaxime	13.6 ± 1.15^e
Cefoxitin	21.0 ± 1.00^c
p. value < 0.001 LSD= 1.88	

Table 5: Antibiotic susceptibility of ***P. aeruginosa*** against different antibiotics

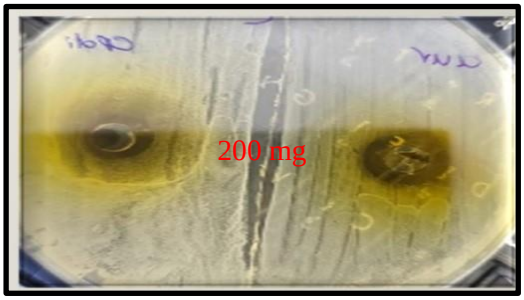
Antibiotics	P. aeruginosa Inhibition Zone Mean ± S. D
Imipenem	31.0 ± 3.60b
Meropenem	40.3 ± 0.57a
Amikacin	20.6 ± 2.30d
Levofloxacin	28.0 ± 1.00c
Ciprofloxacin	33.6 ± 2.08b
Gentamycin	22.0 ± 1.00d
Ceftriaxone	8.33 ± 1.15f
Cefotaxime	4.33 ± 1.52g
Piperacillin	18.0 ± 2.00e
p. value < 0.001 LSD= 2.78	

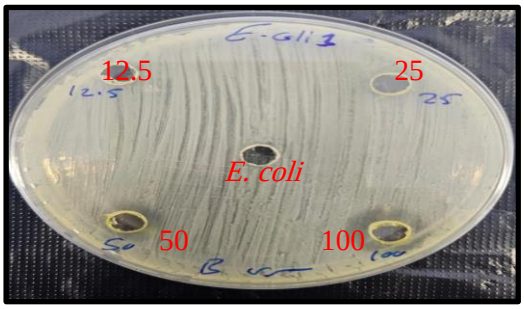
Table 6: Antibiotic susceptibility of *E. coli* against different antibiotics

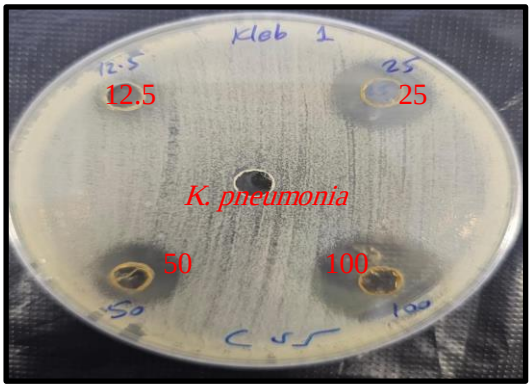
Antibiotics	E. coli Inhibition Zone Mean ± S. D	
Imipenem	18.3 ± 1.52a	p. value < 0.001 LSD= 2.21
Meropenem	11.0 ± 1.00c	
Amikacin	16.6 ± 1.52ab	
Levofloxacin	2.33 ± 0.57d	
Ciprofloxacin	3.33 ± 0.57d	
Gentamycin	14.0 ± 2.64b	
Ceftriaxone	0.00 ± 0.00e	
Cefotaxime	0.00 ± 0.00e	

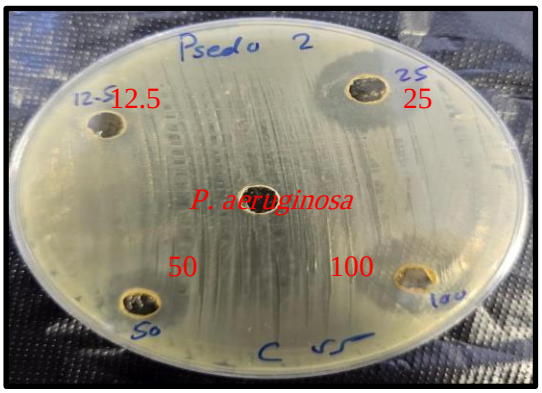
Table 7: Antibiotic susceptibility of *S. aureus* against different antibiotic

Antibiotics	*S. aureus* Inhibition Zone Mean ± S. D	
Imipenem	41.6 ± 1.52[a]	
Meropenem	22.6 ± 1.52[b]	
Amikacin	18.6 ± 1.15[c]	p. value < 0.001
Levofloxacin	4.33 ± 0.57[f]	
Ciprofloxacin	14.6 ± 2.08[d]	LSD= 1.98
Gentamycin	15.0 ± 1.00[d]	
Ceftriaxone	12.6 ± 0.57[e]	
Cefotaxime	0.00 ± 0.00[g]	
Ofloxacin	0.00 ± 0.00[g]	









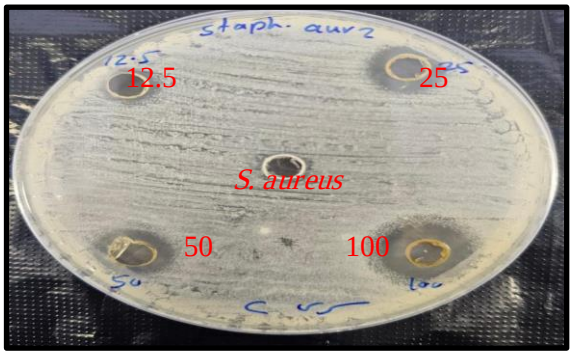


Fig. 2: The antimicrobial activity of *Trigonella fonum* seeds extract at the concentrations (200,100, 50, 25, and 12.5) mgml

IV. Discussion

The ability of a substance to either slow down or inhibit the growth of microorganisms such as bacteria, fungus, viruses, and parasites is known as antimicrobial activity. Through the extraction of antimicrobial chemicals, medicinal plants have been utilized for ages to treat a wide range of ailments. An important public health concern in recent times is the rise in antibiotic- and other antimicrobial drug-resistant bacteria [19]. Antimicrobial resistance is one of the top 10 global public health issues affecting humanity, according to the WHO [20]. In this situation, finding noveland potent antibacterial substances are essential. Because medicinal plants produce a wide range of secondary metabolites, many of them have been shown antibacterial activities, and they represent a promising source of these chemical [21].

Medicinal plants contain compounds that can fight microorganisms by disrupting their cell membranes or walls, or by interfering with their metabolic processes. Identifying and studying these compounds is important as it presents an opportunity to discover new and effective treatments for infectious diseases. The results of this study explain that the *Trigonella foenum* seeds have antibacterial activity against all bacteria strains were used in this study. we can note that in Table (2) that the extract has the maximum inhibitory effects on *Klebsiella pneumoniae* with inhibition zone(21.0 ± 0.63) mm at 200 concentration . When we compared these results with the antibiotics that were used in this study, we found out that the extract has better inhibitory activity than the following antibiotics (Ceftriaxone,Cefotaxime) whose inhibitory diameters were (17.6 ± 2.08 , 13.6 ± 1.15) respectively, In addition, the antibacterial activity of alkaloid extract approximately equals to (Amikacin and Cefoxitin) .Therefore , it is preferable to use the extract instead of the antibiotics followed by *Pseudomonas auroginosa* with an inhibition zone (20.6 ± 0.51) at 200 g/ml concentration. In comparison with antibiotics were used in this study, the plant extract has better inhibitory activity than the following antibiotics: (Cefotaxime , Ceftriaxone, Piperacillin) with inhibitory effectiveness (4.33 ± 1.52 , 8.33 ± 1.15 , 18.0 ± 2.00) respectively.

The antibacterial properties of *Trigonella foenum* were due to the presence of many active chemical compositions. According to the results of GC-MS analysis, they varied in numbers and percentages. The highest percentage of (palmidrol) was recorded about13.63, and this

active compound was Non-Steroidal, analgesic, anti-Inflammatory, antibacteria antihypertensive, neuroprotective, antiviral,anticonvulsant and anti-oxidative properties [22]. Then, (Hexadecanoic acid, methyl ester) recorded 9.40. This compound has antioxidant, also it has antifungal, antitumor and antibacterial properties, nematicide, hypocholesterolemic, pesticide, lubricant, antiandrogenic, flavor, and hemolytic activity [23], exhibit both hepatoprotective and anticancer properties [24]. Additionally, it has been claimed to have anti-Inflammatory, anti-cancerous, antioxidant, and hypercholesterolemia properties [25]. Then, Hexadecanoic acid recorded 8.29. This compound is responsible for the antioxidant, antifungal and antimicrobial properties [26]. Additionally, many other compounds have bioactive properties. Climate change can have significant impacts on the growth, distribution, and chemical composition of medicinal plants, which can affect their antimicrobial and antioxidant properties [27]. In general, Alkaloids are among the most effective therapeutic substances, as they have a physiological effect, even if they are in small concentrations, and this agrees with the researcher [28].

V. CONCLUSION

The active components of the *Trigonella foenum*, alkaloid seed, were identified in the previous investigation. the results of this research aids in the production and development of contemporary medications. GC-MS analysis is the initial stage in identifying the active components in the plant extract. The biologically active compounds in the extracts, together with some plant chemical components can manage diseases and make them a natural source of treatment for a variety of ailments due to their antibacterial and antioxidant qualities.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

[1] R. A. A. L. Askary, "Effectiveness study of Artemisia herba-alba and Borage officinalis leaf extract against bacteria Staphylococcus aureus," Univ. Thi-Qar J. vol. 8, no. 1, pp. 113-117, 2021

[2] H. Archana and V. G. Bose, "Evaluation of phytoconstituents from selected medicinal plants and its synergistic antimicrobial activity," *Chemosphere*, Vol. 8, no. 1. Pp. 113-117, 2022.

[3] S. Wang, X.-Q. Liu, O. Kang, and D. Kwon, "Combination of Sanguisorbigenin and Conventional Antibiotic Therapy for Methicillin-Resistant Staphylococcus aureus: Inhibition of Biofilm Formation and Alteration of Cell Membrane Permeability," *International Journal of Molecular Science*, Vol. 23, no. 8. Pp. 4220-4232, 2022.

[4] H. A. H. Alsaidy and A. A. Ali, "Reviews on Plants Gum and Mucilage I: The Composition and Pharmacological Significance of Turmeric (Curcuma longa) Rhizomes Polysaccharides and Mucilage/their Aqueous Extracts Commercial Products," *Univ. Thi-Qar J. Sci.*, vol. 8, no. 1, pp. 14–25, 2021.

[5] H. Huang *et al.*, "The Effects of Fenugreek Extract on Growth Performance, Serum Biochemical Indexes, Immunity and NF-κB Signaling Pathway in Broiler," *Frontiers in Veterinary Science*, Vol. 9, no. 6. Pp. 882754, 2022.

[6] M. S. Akhtar, M. K. Swamy, and U. R. Sinniah, "Natural bio-active compounds," *Natural Bio-Active Compounds*, vol. 1, pp. 1-608, 2019.

[7] R. Prasad, S. Acharya, and J. Thomas*, "Identification of High Seed Yielding And Stable Fenugreek Mutants" by | Perlego," 6. 01, 2014.*

[8] B. Bin-Hafeez, R. Haque, S. Parvez, S. Pandey, I. Sayeed, and S. Raisuddin, "Immunomodulatory effects of fenugreek (Trigonella foenum graecum L.) extract in mice," *International Immunopharmacology*, Vol. 12, no. 4. Pp, 257-265, 2003.

[9] L. a N. Al-Timimi, "Antibacterial and Anticancer Activities of Fenugreek Seed Extract," *Asian Pacific Journal of Cancer Prevention*, Vol. 20, no. 12. Pp, 3771, 2019.

[10] T. H. Desai and S. V. Joshi, "Anticancer activity of saponin isolated from Albizia lebbeck using various in vitro models," *Journal of Ethnopharmacology*, Vol. 231, no. 6. Pp, 494-502, 2019.

[11] G. K. Devi, K. Manivannan, G. Thirumaran, F. A. A. Rajathi, and P. Anantharaman, "In vitro antioxidant activities of selected seaweeds from Southeast coast of India," *Asian Pacific Journal of Tropical Medicine*, Vol. 4, no. 3. Pp, 205-211, 2011.

[12] Ch. Romay, R. Gonzalez, N. Ledon, D. Remirez, and V. Rimbau, "C-Phycocyanin: A Biliprotein with Antioxidant, Anti-Inflammatory and Neuroprotective Effects," *Current Protein and Peptide Science/Current Protein & Peptide Science*, Vol. 4, no. 3. Pp, 207-216, 2003.

[13] M. Nafees, S. Ullah, Barkatullah, A. Javaid, and Shumaila, "PHARMACOGNOSTIC AND PHYTOCHEMICAL SCREENING OF COCCULUS PENDULUS Diels. *STEM AND ROOT*," Vol. 30, no. 5. Pp, 114-126, 2020.

[14] R. Yadav and M. Agarwala, "Phytochemical *analysis of some medicinal plants*," Vol. 3, no. 12. Pp, 10-14, 2011.

[15] A. A. S. Abdul, A. O. Abbas, and B. N. Abdulhadi, "Comparative study for antibiotic susceptibility against Pseudomonas aeruginosa isolated from otitis media through several years in Thi-Qar Province/Iraq." Journal of Univesity of Thi-Qar | EBSCOhost," *openurl.ebsco.com*, Vol. 16, no. 3. Pp, 33-43,2021.

[16] N. Bobby, E. Wesely, and M. Johnson, "High performance thin layer chromatography profile studies on the alkaloids of Albizia lebbeck," Vol. 2, no. 1. Pp, 1-6, 2012.

[17] "Mass Spectrometry Data Center, NIST." https://chemdata.nist.gov/

[18] "Phytochemical Methods," *SpringerLink*. https://link.springer.com/book/10.1007/978-94-009-5570-7

[19] E. Mezal, Z. K. Hanan, and M. Saleh, *"IDENTIFICATION, ANTIMICROBIAL*

RESISTANCE OF SALMONELLA ENTERICA ISOLATED FROM DIARRHEAL CHILDREN IN THI-QAR PROVINCE DURING 2015, Thesis" 2016.
[20] "WHO model list of essential medicines, 20th list (March 2017, amended August 2017)," 2017. https://iris.who.int/handle/10665/273826
[21] C. Jain, S. Khatana, and R,Vijayvergia, "Bioactivity of secondary metabolites of various plants: A review," *Int. J. Pharm. Sci. Res*., vol. 10, no. 2, pp. 494–504, 2019.
[22] Indriati *et al.*, "GC-MS and Antioxidant Capacity Analysis in Propanol Extract of Carthamus tinctorious L," *Journal Peneitan*, vol. 8, no. 1, pp. 67–73, 2021.
[23] T. Tyagi and M. Agarwal, "Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms," *www.phytojournal.com*, vol. 6, no. 1, pp. 195–206, vol. 10, no. 1, pp. 17–26, 2017.
[24] L. C. Pal, S. Agrawal, A. Gautam, J. K. Chauhan, and C. V. Rao, "Hepatoprotective and Antioxidant Potential of Phenolics-Enriched Fraction of Anogeissus acuminata Leaf against Alcohol-Induced Hepatotoxicity in Rats," *Medical Sciences*, vol. 10, no. 1, pp. 17–31, 2022.
[25] A. A. Adenike, P. Adegbola, and O. S. Fadahunsi, "Antioxidant property and GCMS profile of oil extracted from Cocos nucifera using a fermentation method," *BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology*, vol. 100, 2019.
[26] G. E. Egbung, C. Anosike, A. B. Utu-Baku, I. Ogar, and V. U. Nna, "Phytochemical evaluation and GC-MS analysis of Hyptis verticillata cultivated in Calabar Cross River State, Nigeria," *International Journal of Biological and Chemical Sciences*, vol. 11, pp. 2548-2559, 2017.
[27] V. Kumar and Y. Sharma, "Effects of environment on the chemical constituents and biological characteristics of some medicinal plants," *Apple Academic Press eBooks*, Dec. 12, pp. 279–292, 2018.
[28] L. Salerno *et al.*, "Antioxidant Activity and Phenolic Content of Microwave-AssistedSolanum melongenaExtracts," *the Scientific World Journal/TheScientificWorldjournal*, vol. 20, no. 8, pp. 1–6, 2014