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GC-MS Assay to Aqueous and Alcoholic Extracts of *Ocimum basilicum* L. and Detection Antibacterial Activity

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ABSTRACT

This study aims to evaluate the antimicrobial activity of *Ocimum basilicum* L. leaf extracts due to the increasing prevalence of drug-resistant bacteria. The objective is to explore standardized analytical methods for isolating novel bioactive compounds from medicinal plants, which may provide innovative solutions for combating harmful microorganisms. The study involved the collection and preparation of *O. basilicum* leaves from Nasiriyah, Iraq. The leaves were cleaned, dried, and ground into powder. Twenty grams of the dried powder were mixed with 200 mL of ethanol and aqueous solvents, respectively, and subjected to ultrasonic extraction. The extracts were filtered, concentrated, and stored in sterile conditions. Antimicrobial activity was assessed using varying concentrations (25, 50, 75, and 100 mg/mL) against bacterial strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* using the Kirby-Bauer disk diffusion method. The ethanolic extract exhibited the highest inhibitory effect against *S. aureus*, with a zone of inhibition measuring 15.6 ± 1.52 mm at 100 mg/mL. The aqueous extract showed significant activity against *P. aeruginosa*, with an inhibition zone of 12.1 ± 0.28 mm at 75 mg/mL. Comparative analysis revealed that both extracts outperformed several tested antibiotics in terms of efficacy against the respective bacterial strains. The findings indicate that *O. basilicum* extracts possess significant antimicrobial properties, making them potential alternatives to conventional antibiotics in treating bacterial infections. The study highlights the importance of these extracts in developing herbal formulations for antimicrobial applications, contributing to the field of alternative medicine.

Keywords: *Ocimum basilicum* L., *P. aeruginosa, Enterobacter cloacae, K. pneumonia, S. aureus* GC-MS

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INTRODUCTION

In recent years, there has been a growing trend in using plant extracts to treat various ailments. This is particularly due to the rise in resistance of pathogenic bacteria, which pose a significant health concern for individuals in both developed and developing countries [1]. The presence of this resistance presents a significant peril to the well-being of individuals, irrespective of their residence in either industrialized or developing nations. To counteract these disorders, a variety of antibacterial agents are employed. However, the consistent and indiscriminate long-term use of these medications has led to harmful adverse effects for individuals [2].

In addition, currently available synthetic drugs do not inhibit the action of certain pathogens. The use of synthetic chemicals for the control of pathogenic microorganisms, and the treatment is limited because of their potential carcinogenic effect, acute toxicity and potential hazard to the environment. In this regard, use of extract for the control and suppression of resistant pathogenic microorganisms can be of great benefit in the fight against various diseases [3]. Traditional medicine possesses anthelmintic characteristics and can be used to treat sleeplessness, edema, renal inflammation, and diabetes [4].

Medicinal plants include a range of bioactive substances, including alkaloids, flavonoids, phenolic compounds, steroids, tannins, terpenoids, and other secondary metabolites. These compounds have a significant impact on pathogens. Plant-derived chemicals possess distinctive pharmacological characteristics, including cost-effectiveness, reduced toxicity, diminished side effects, and a lower likelihood of resistance development [5]. According to the World Health Organization (WHO), 80% of the developing continues to get advantages from the utilization of traditional medicines derived from medicinal plants [6]. The utilization of bioactive compounds obtained from plants or their synthetic counterparts in medicine has been enhanced via the advancements in phytochemistry and pharmaceutical chemistry [7].

One of the most significant botanical species *Ocimum basilicum*, formerly known as Basil or sweet basil, is a plant that is typically grown for its leaves and can be either an annual or perennial species. Plants can have a height ranging from 30 cm to 100 cm, depending on their diversity. The leaves of this plant are lush green and have an oval shape. However, they exhibit a wide range of sizes and shapes depending on the specific cultivar. The length of the leaf's ranges from 3 to 11 cm, while the width ranges from 1 to 6 cm. The plant produces small, white blooms that develop from a central inflorescence emerging from the top of the primary stalk [8].

In terms of botany, is an annual spicy herb of the Labiatae family. Due to its many uses in health, cosmetics, pharmaceuticals, and the food industry, basil has earned the title "King of the Herbs," from the Greek "Basileus," which means "Royal" or "King." [9], this tropical plant grows in Africa, South Asia, and India. Now spread throughout the world, O. basilicum is grown commercially in Iraq and many warm and temperate regions, including France, Greece, and southern Europe, as well as North and South America [10,11].

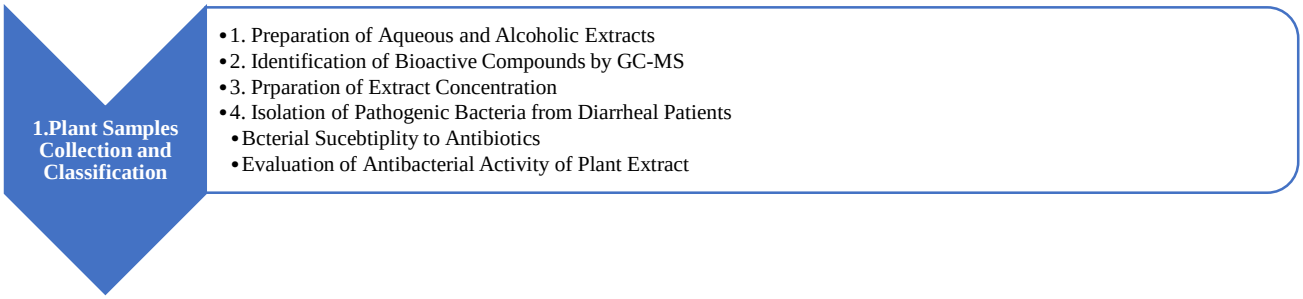
Many studies of basil extract have been demonstrated its significant anti-inflammatory, antioxidant, anti-stress [12,13-18] and antimicrobial activity [19,20]. That's the reason why it is used in medicine, particularly in aromatherapy, and in the treatment of cardiovascular diseases, diabetes, Alzheimer's disease and cancer [21].

In recent years, considerable study has been done on the herb's therapeutic benefits, organic growing, extraction, and effective chemical analysis. Biochemical components, domestication, and breeding properties of herbs are important to study. Basil is a medicinal herb, essential oil, and culinary and perfume ingredient [22].

Therefore, there are numerous plant-based pharmaceutical excipients available in the marketplace. Moreover, the capacity of these compounds to exert diverse effects is contingent upon their inherent properties and molecular weight. The application of antibiotics in animals, whether for growth promotion or infection treatment, has resulted in the development of antibiotic resistance [23, 24]. Due to the increase in drug-resistant bacteria, there is a necessity for standardized contemporary analytical methods to separate novel bioactive chemicals from medicinal plants. Compounds obtained from medicinal plants have the potential to offer a new and innovative method for combating harmful microorganisms. This study investigates the antibacterial properties of *O. basilicum* plant-derived compounds, including their potential modes of action and chemical properties.

MATERIAL AND METHODS

Study Design



Collection and Classification of Study Stations

The plant specimens used in this study were obtained from Nasiriyah, located in the Thi-Qar Province in southern Iraq, during October in the year 2023. Specifically, the leaves of *O. basilicum* L. were collected. Dr. Haider Radhi, a professor at the University of Thi-Qar, College of Science, discovered and identified the plants. The impurities were completely removed from them, and they were transformed into a fine powder using an electric mill. After that, they were kept in sterile glass bottles until they were ready to be used [25].

Preparation of Plant Extract

Preparation of Aqueous and Ethanol Extract

The chemical components were qualitatively screened by subjecting a mixture of 20 grams of plant powder and 200 milliliters of distilled water (Aqueous Extract), as well as 200 milliliters of ethanol (Ethanol Extract), to an ultrasonic bath. The resulting solution was filtered using multiple layers of Whatman 0.22 filter paper and then concentrated at 50 °C under reduced pressure using a rotary evaporator. Afterwards, it was subjected to a drying procedure at a temperature of 25 °C. The extract was ultimately gathered in sterilized glass tubes that are now prepared for utilization [26].

Preparation Concentration

To obtain different concentrations, we dilute the stock solution (100%) with DMSO dissolving solute.

Table 1 Preparation concentration in this study.

Concentration	Extract (mL)	Solvent DMSO (mL)
75	750	250
50	500	500
25	250	750

GC-MS Analysis of Extracts

Gas chromatography-mass spectrometry (GC-MS) conditions the GC-MS analysis was performed using the GCMS-QP2010 plus instrument (Shimadzu, Kyoto, Japan), equipped with an autoinjector and a 5 ms capillary column of 30×0.25 mm with a film thickness of 0.25 µm. The carrier gas used is helium, with a flow rate of 1.15 ml/min. The 70eV ionized charge system was used to do mass spectroscopic scanning. The temperature was initially set at 80 °C for 2 minutes, and then increased steadily at a pace of 10 °C per minute until it reached 280 °C for 5 minutes. The samples that were injected were exposed to splitting mode at a temperature of 250 °C. Two databases contain mass spectral data. The National Institute of Standards and Technology (NIST14) and Wiley 10th/NIST 2014 mass spectral library (W10N14) was utilized to characterize the isolated components based on them

Culturing of Samples and Antibiotic Susceptibility

Bacterial cultures of E. cloacae, S. aureus, P.s aeruginosa, and K. pneumonia were maintained at 4° C in Brain Heart Infusion agar with glycerol and subculture on MacConkey and blood and then on Muller Hinton agar were used Disc diffusion methods to determine the sensitivity of isolates to antibiotics [27].

Antimicrobial Activity of Plant Extracts Against Bacteria

The researchers employed the Kirby-Bauer disk diffusion susceptibility test to assess the sensitivity and resistance of plant extracts to bacteria recovered from gastrointestinal patients [28]. To accomplish this, they evenly distributed 100 µL of the bacterial inoculum obtained from an 18–24-hour broth culture onto the surface of Muller Hinton agar media plates.

Subsequently, the researchers placed antibiotic discs on the inoculated plates, followed by the alkaloid extract at concentrations of 12.5, 25, 50, 100, and 200 mg/mL. Furthermore, several antibiotics were examined in this investigation. The plates were placed in an incubator at a temperature of 37 °C for a period of 18-24 hours, following a chilling period of 2 hours at 4 °C. The inhibitory zones on each plate were then measured in terms of their diameter.

Table 2 Chemical compounds in the aqueous extract of *O. basilicum.*

Pk	Area%	R. Time	Common Name	Formula
1	5.90	4.290	1,2-Hydrazinedicarboxylic acid, diethyl ester	$C_6H_{12}N_2O_4$
2	2.37	5.207	Acetic acid, hydroxy-, ethyl ester	$C_4H_8O_3$
3	5.07	5.986	Benzoic acid, methyl ester	$C_8H_8O_2$
4	28.08	18.649	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
5	7.93	19.238	Pentadecanoic acid	$C_{15}H_{30}O_2$
6	5.08	20.977	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
7	2.35	21.090	1-Methyl-4-[nitromethyl]-4-piperidinol	$C_7H_{14}N_2O_3$
8	9.76	21.324	Methyl stearate	$C_{19}H_{38}O_2$
9	3.28	21.583	1-Propanamine, N,2-dimethyl	$C_5H_{13}N$
10	2.80	21.877	1,8-Octanediamine, N, N'-dimethyl-	$[-(CH_2)_4NHCH_3]_2$
11	3.37	24.370	Ethene, ethoxy-	$C_5H_8O_2$
12	12.87	25.539	Sarcosine, N-(cyclohexyl carbonyl)-, butyl ester	$C_{11}H_{19}NO_3$
13	4.12	27.642	alpha. -Tocopheryl acetate	$C_{31}H_{52}O_3$
14	3.09	29.589	Scillarenin	$C_{24}H_{32}O_4$
15	3.93	30.230	2-(4,5-Dihydro-3-methyl-5-oxo-1-phenyl-4-pyrazolyl)-5 nitrobenzoic acid	$C_{17}H_{13}N5O_5$
Total		100%		

Analytical Profile Index

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

Statistical Analysis

The data was analyzed by using SPSS (Statistical Package of Socio Science) by using one-way ANOVA for variation and LSD at p. value <0.05.

Statistical Nots

- Each p. value has two stars that indicate a high significant at p. value 0.01, p. value has one star indicate significant at 0.05, while p. value without star indicates a non-significant difference.
- Similar small letters above the means indicate the non-significant differences, while different letters indicate the significant differences.
- The LSD value is used for determining the significant differences between means in the ANOVA test, where we subtract any two means from the table and compare the result of the subtraction with the LSD value. If the value of the subtraction is equal to or higher than the LSD value, it indicates a significant difference, while if it is less, it indicates that there is non-significant difference.

Table 3 Chemical compounds in the ethanol extract of *O. basilicum*

Pk	Area%	R. Time	Common Name	Formula
1	0.99	4.835	Hydrazinecarboxamide	CH_5N_3O
2	1.38	5.986	Benzoic acid, methyl ester	$C_8H_8O_2$
3	0.99	15.455	Benzeneethanamine, 4-fluoro-. alpha. -methyl-	$C_{13}H_{16}FN$
4	1.59	16.468	Tetradecanoic acid	$C_{14}H2_8O_2$
5	1.04	16.823	p-Hydroxyamphetamine	$C_9H_{13}NO$
6	4.01	17.411	9-Octadecyne	$C_{18}H_{34}$
7	3.58	17.784	Phthalic acid, isobutyl octyl este	$C_{20}H_{30}O_4$
8	1.56	18.009	3,7,11,15-Tetramethyl-2-hexadecen- 1-ol	$C_{20}H_{40}O$
9	9.93	18.649	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
10	22.76	19.307	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
11	1.01	19.567	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
12	3.37	20.977	Methyl 6,9,12-hexadecatrienoate	$C_{17}H_{28}O_2$
13	7.44	21.133	Phytol	C_6H_5OH
14	3.23	21.324	Methyl stearate	$C_{19}H_{38}O_2$
15	25.66	21.618	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{19}H_{32}O_2$
16	2.84	21.904	Octadecanoic acid	$C_{18}H_{36}O_2$
17	1.08	22.423	3,7,11,15-Tetramethyl-2-hexadecen- 1-ol	$C_{20}H_{40}O$
18	2.07	24.353	Butanal	C_4H_8O
19	0.76	26.967	Benzaldehyde, 2-nitro-,diaminomethylidenhydrazone	$C_8H_9N_5O_2$
20	4.71	29.174	Squalene	$C_{30}H_{50}$
Total		100%		

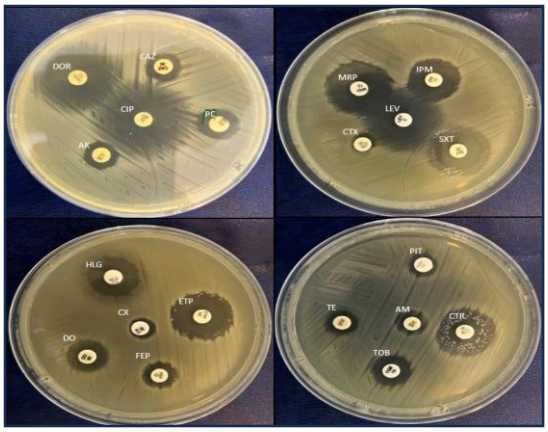


Fig. 1 Antibiotics susceptibility of *E. cloacae*

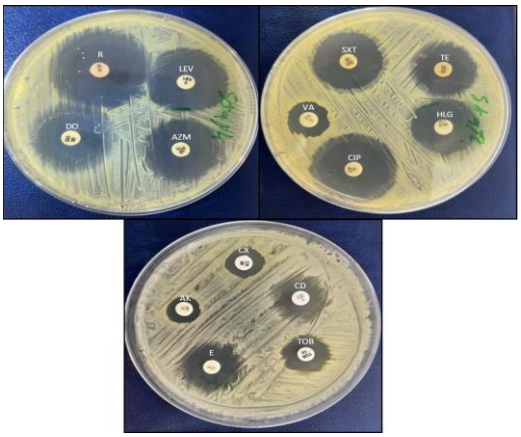


Fig. 2 Antibiotics susceptibility of *S. aureu*

RESULTS

The GC-MS analysis of plant aqueous and alcoholic extracts showed the presence of bioactive chemicals. GC-MS analysis of chemical compounds in the aqueous extract of *O. basilicum*, shows 15 compounds. The Hexadecenoic acid, methyl ester compound is the most abundant with 28.08% of the total area, while the 1-Methyl-4-[nitromethyl]-4-piperidinol compound is the least area with 2.35% (Table 2). Whereas the GC-MS analysis of chemical compounds in the Ethanolic Extract of *O. basilicum*, showed 20 compounds. The 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-compound is the most abundant with 25.66 %, and n-Hexadecanoic acid wth 22.76% of the total area, while the Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone compound is the least area with 0.76 % (Table 3).

Table 4 Antibiotic susceptibility of *E. cloacae* against different antibiotics

Antibiotics	Code	Concentration	Mean ± S. D
Tetracycline	TE	10	11.07 ± 0.90
Ciprofloxacin	CIP	10	33.67 ± 2.52
Ceftriaxone	CTR	30	20.33 ± 1.15
Trimethoprim-sulfamethoxazole	SXT	25	22.50 ± 0.87
Cefotaxime	CTX	10	8.20 ± 0.82
Tobramycin	TOB	10	15.33 ± 1.04
Ampicillin	AM	25	12.50 ± 0.50
Ceftazidime	CAZ	30	14.17 ± 1.26
Meropenem	MRP	10	25.17 ± 1.26
Piperacillin-tazobactam	PIT	100/10	11.17 ± 0.76
Levofloxacin	LEV	5	29.33 ± 0.58
Gentamicin	HLG	120	19.17 ± 0.29
Cefoxitin	CX	30	9.67 ± 1.04
Amikacin	AK	10	11.83 ± 0.29
Cefepime	FEP	10	11.83 ± 0.29
Doxycycline	DO	30	15.33 ± 1.04
Doripenem	DOR	10	31.33 ± 1.53
Imipenem	IMP	10	19.67 ± 1.53
Penicillin	PC	100	11.67 ± 0.58
Ertapenem	ETP	10	22.33 ± 2.08
p. value <0.001**	LSD	1.91	

Table 5 Antibiotic susceptibility of *S. aureus* against different antibiotic

Antibiotics	Code	Concentration	Mean ± S.D
Levofloxacin	LEV	5	28.00 ± 1.00
Rifampin	R	5	34.67 ± 0.58
Azithromycin	AZM	15	27.33 ± 1.15
Doxycycline	DO	30	29.67 ± 0.58
Tobramycin	TOB	10	20.67 ± 0.58
Clindamycin	CD	2	21.00 ± 1.00
Erythromycin	E	10	20.00 ± 1.00
Cefoxitin	CX	30	15.17 ± 1.26
Amikacin	AK	10	13.33 ± 1.53
Tetracycline	TE	10	24.67 ± 0.58
Gentamicin	HLG	120	24.67 ± 0.58
Trimethoprim-sulfamethoxazole	SXT	25	24.67 ± 0.58
Vancomycin	VA	5	15.67 ± 0.58
Ciprofloxacin	CIP	10	29.67 ± 0.58
p. value <0.001**	LSD 1.47		

Table 6 Antibiotic susceptibility of *P. aeruginosa* against different antibiotics

Antibiotics	Code	Concentration	Mean ± S. D
Tetracycline	TE	10	0.00 ± 0.00
Ciprofloxacin	CIP	10	20.50 ± 0.50
Ceftriaxone	CTR	30	0.00 ± 0.00
Trimethoprim-sulfamethoxazole	SXT	25	0.00 ± 0.00
Cefotaxime	CTX	10	0.00 ± 0.00
Ceftazidime	CAZ	30	0.00 ± 0.00
Meropenem	MRP	10	24.00 ± 1.00
Piperacillin-tazobactam	PIT	100/10	0.00 ± 0.00
Levofloxacin	LEV	5	14.17 ± 0.76
Gentamicin	HLG	120	20.00 ± 0.00
Cefoxitin	CX	30	0.00 ± 0.00
Amikacin	AK	10	13.67 ± 0.58
Cefepime	FEP	10	0.00 ± 0.00
Doxycycline	DO	30	7.50 ± 0.50
Doripenem	DOR	10	28.33 ± 1.53
Imipenem	IMP	10	24.00 ± 1.00
Penicillin	PC	100	8.00 ± 0.00
Ertapenem	ETP	10	0.00 ± 0.00
Ampicillin	AMP	10	11.67 ± 0.58
p. value <0.001** LSD 0.93			

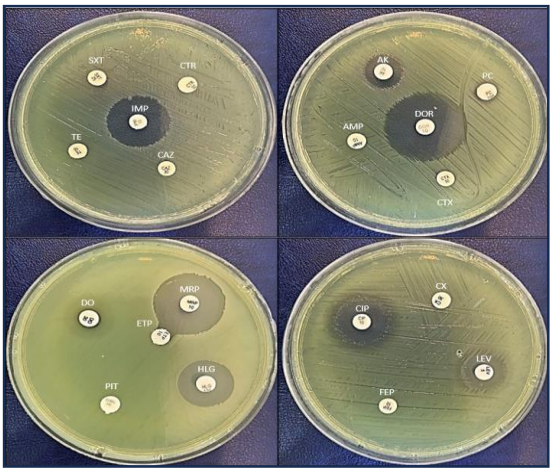


Fig. 3 Antibiotics susceptibility of *P. aeruginosa*

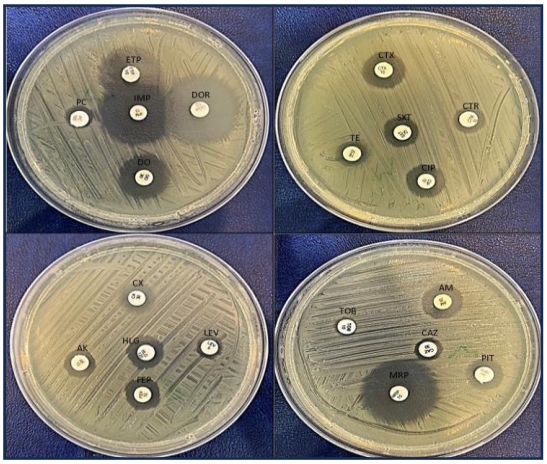


Fig. 4 Antibiotics susceptibility of *K. pneumonia*

Table 7 Antibiotic susceptibility of *K. pneumonia* against different antibiotics

Antibiotics	Code	Concentration	Mean ± S. D
Tetracycline	TE	10	12.67 ± 1.15
Ciprofloxacin	CIP	10	14.17 ± 0.76
Ceftriaxone	CTR	30	15.07 ± 0.60
Trimethoprim-sulfamethoxazole	SXT	25	12.83 ± 0.29
Cefotaxime	CTX	10	13.67 ± 1.53
Tobramycin	TOB	10	0.00 ± 0.00
Ampicillin	AM	25	13.83 ± 0.76
Ceftazidime	CAZ	30	9.83 ± 1.26
Meropenem	MRP	10	26.17 ± 0.76
Piperacillin-tazobactam	PIT	100/10	13.00 ± 1.00
Levofloxacin	LEV	5	9.17 ± 1.04
Gentamicin	HLG	120	14.17 ± 0.76
Cefoxitin	CX	30	14.33 ± 0.76
Amikacin	AK	10	14.50 ± 0.50
Cefepime	FEP	10	16.00 ± 0.50
Doxycycline	DO	30	21.17 ± 1.04
Doripenem	DOR	10	31.33 ± 1.53
Imipenem	IMP	10	29.17 ± 1.04
Penicillin	PC	100	11.17 ± 1.04
Ertapenem	ETP	10	22.17 ± 1.04
p. value < 0.001** LSD 1.55			

Antibiotic Susceptibility Test

The results were read by observing the inhibiting zones formed by the disk and explained that the bacteria, sensitive, media, or resistant according to standard specifications. the result for *E. cloacae* shows in table (4) and figure (1), *S. aureus* in table (5) and

figure (2), *P. aeruginosa* in table (6) and figure (3) and *K. pneumonia* in table (7) and figure (4).

Antimicrobial Activity of the Ethanolic and Aqueous Extract of *O. basilicum*

The efficacy of the ethanolic extract of *O. basilicum* as an antibacterial agent against specific human pathogens, including Gram-positive (Staphylococcus aureus) and Gram-negative (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*) bacteria, is demonstrated in Table (8) and figure (5 and 6). These four species of bacteria were evaluated using stock solutions of the plant extract that were generated at quantities of 25, 50, 75, and 100 µg/µL. By measuring the inhibition zone of bacterial growth, the antibacterial activity of the extract was evaluated. This zone was clearly visible at almost all concentrations. The ethanolic extract exhibited the highest level of activity against *S. aureus* when used at a concentration of 100%. This resulted in an inhibition zone of (15.6 ± 1.52 mm). The next highest level of activity was recorded against *S. aureus* at a concentration of 75%, resulting in an inhibition zone of (14.8 ± 1.04) mm. In contrast, the lowest level of activity was reported in the 75% concentration against *P. aeruginosa*. In addition, no biological activity was detected against E. cloacae bacteria at a concentration of 50, 75, and 100%.

Table 8 Activity of the ethanolic Extract of *O. basilicum* (mm).

Con.	Cases No.	*E. cloacae*	*S. aureus*	*P. aeruginosa*	*K. pneumonia*
		O. basilicum Ethanolic Extract Mean ± S. D			
25%	3	11.2 ± 0.26 a	13.0 ± 1.00 ab	11.7 ± 0.57b	14.0 ± 0.50 a
50%	3	00.0 ± 0.00 b	12.8 ± 0.76 b	11.6 ± 0.76 b	12.3 ± 0.28 ab
75%	3	00.0 ± 0.00 b	14.8 ± 1.04 ab	10.1 ± 0.76 c	11.8 ± 1.25 b
100%	3	00.0 ± 0.00 b	15.6 ± 1.52 a	14.6 ± 0.57 a	11.5 ± 1.32 b
Control		0.0	0.0	0.0	0.0
p. value		< 0.001 **	0.037 *	< 0.001 **	0.041 *
LSD		0.24	2.10	1.27	1.80

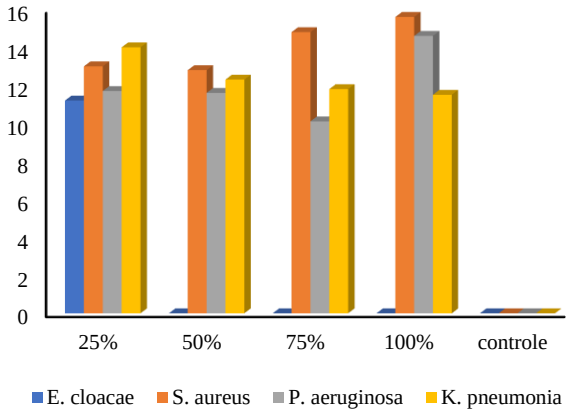


Fig. 5 Antibacterial activity of *O. basilicum* Ethanolic extract against four strains of bacteria.

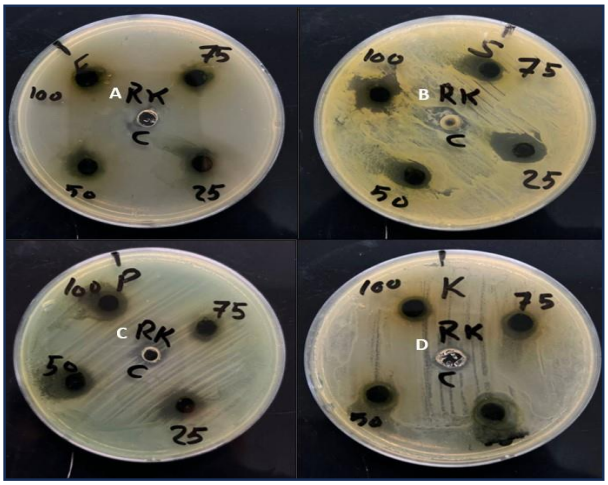


Fig. 6 Activity of *O. basilicum* ethanolic extract with different concentrations against growth of (A: *E. cloacae*, B: *S. aureus*, C: *P. aeruginosa*, D: *K. pneumonia*) *C = control

Table 9 Activity of *O. basilicum* aquatic extract with different concentrations against bacteria (mm).

Conce	Cases No.	*E. cloacae*	*S. aureus*	*P. aeruginosa*	*K. pneumonia*
		O. basilicum Aquatic Extract Mean ± S. D			
25%	3	00.0 ± 0.00 c	00.0 ± 0.00 a	11.5 ± 0.50 ab	8.33 ± 0.57 a
50%	3	8.50 ± 0.50 b	00.0 ± 0.00 a	11.1 ± 0.28 bc	8.16 ± 0.28 a
75%	3	10.6 ± 0.57 a	00.0 ± 0.00 a	12.1 ± 0.28 a	8.83 ± 0.28 a
100%	3	11.8 ± 1.04 a	00.0 ± 0.00 a	10.3 ± 0.57 c	8.50 ± 0.50 a
Control		0.0	0.0	0.0	0.0
p. value		< 0.001 **	1.000	0.006 **	0.341
LSD		1.21	Non-sig	0.81	Non-sig

The antibacterial activity of the aqueous extract of *O. basilicum* was evaluated by measuring the inhibition zone of bacterial growth, which was consistently present across almost all concentrations. Table (9) figure (7 and 8) shows the results of this assessment. At a concentration of 75%, the aqueous extract showed the greatest effectiveness against *P. aeruginosa*. This resulted in an inhibition zone of (12.1 ± 0.28) mm. The next highest level of activity was recorded against *E. cloacae* at a concentration of 100%, resulting in an inhibition zone of (11.8 ± 1.04) mm. In contrast, the lowest level of activity was reported in the 50% concentration against *K. pneumonia*. In addition, no biological activity was detected against *S. aureus* bacteria at all concentration.

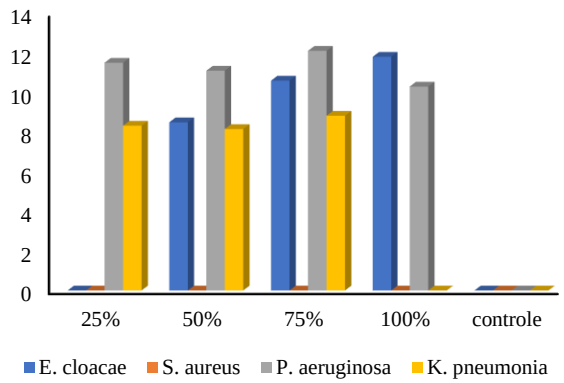


Fig. 7 Antibacterial activity of *O. basilicum* aqueous extract against four strains of bacteria.

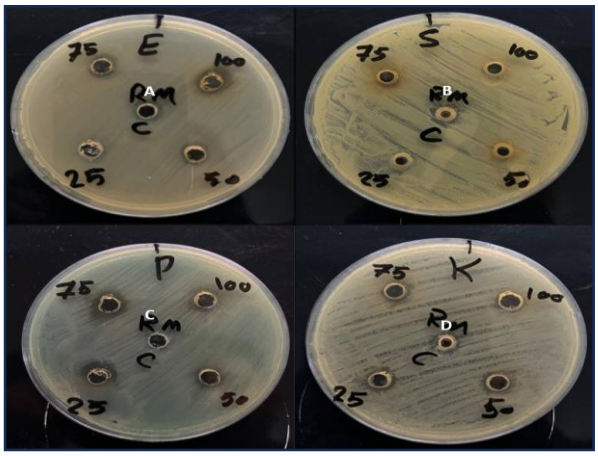


Fig. 8 Activity of *O. basilicum* aqueous extract with different concentrations against growth of (A: *E. cloacae*, B: *S. aureus*, C: *P. aeruginosa*, D: *K. pneumonia*).

DISCUSSION

When a chemical has antimicrobial activity, it means it can kill or inhibit the growth of microorganisms like bacteria, fungi, parasites, and viruses. Traditional medicine practitioners have long relied on the antibacterial compounds found in medicinal plants to alleviate a wide variety of symptoms, from serious infections to common colds. A major concern for public health has been the recent uptick in the prevalence of germs that are resistant to antibiotics. One of the top ten global public health threats that humanity faces is antimicrobial resistance, according to the World Health Organization [29]. It is of the utmost importance in this setting to find novel antibacterial chemicals. The wide variety of secondary metabolites present in medicinal plants makes them an attractive candidate for this type of molecule, with many of these metabolites exhibiting antibacterial effects [30]. The chemicals found in medicinal plants can inhibit the metabolic activities of microbes or damage their cell membranes or walls. The possibility of finding novel and efficient treatments for infectious diseases makes the identification and research of these chemicals crucial. Antimicrobial resistance is on the rise, which is a major concern for public health. Therefore, finding new treatments is of the utmost importance [31]. Medicinal plants' antibacterial capabilities may be compromised because of climate change's effects on their development, dispersion, and chemical composition [32].

According to the results, *Ocimum basilicum* leaves had antibacterial effects against some of the bacteria that were tested. The high activity of O. basilicum aquatic extract against P. aeruginosa (12.1 ± 0.28 mm) in 75 mg/ml concentration, then against E. cloacae (11.8 ± 1.04 mm) in 100 mg/ml concentration. In contrast the lowest activity in both 25 and 50 mg/ml concentrations against K. pneumonia (8.33 ± 0.57 and 8.16 ± 0.28mm), was showed the high activity of O. basilicum ethanolic extract against S. aureus (15.6 ± 1.52 mm and 14.8 ± 1.04) and in both 100 and 75 mg/ml concentrations, then against *P. aeruginosa* (14.6 ± 0.57 mm) in 100 mg/mL concentration. In contrast the lowest activity in 25 mg/mL concentration against E. cloacae (11.2 ± 0.26 mm) and 100 mg/mL concentration against K. pneumonia (11.5 ± 1.32 mm), furthermore a non-biological activity against E. cloacae in 50, 75, and 100 mg/mL concentrations. The effectiveness of the plant can be attributed to its content of active compounds such as hexadecanoic acid methyl ester has Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, Alphareductase inhibitor [33]. in addition, 9,12,15-Octadecatrienoic acid (Z, Z, Z) has Antibacterial, anticandidal, anti-inflammatory, hypocholesterolemia, cancer preventive, hepatoprotective, nematicide, insectifuge antihistaminic, antiarthritic, ant coronary, antieczemic, antiacne, 5-Alpha reductase inhibitor and antiandrogenic activities. furthermore [34] squalene has Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase inhibitor, Pesticide [35] and phytol has Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal against S. typhi, resistant gonorrhea, joint dislocation, headache, hernia, stimulant and antimalarial [36]

CONCLUSION

Given the numerous side effects associated with most antibiotics employed in the medical profession, our current study aimed to investigate the effects of aqueous and alcoholic extracts of *Ocimum basilicum* L. on pathogenic bacteria, demonstrating superior inhibition compared to some medicines. The aqueous and alcoholic extracts exhibit antimicrobial properties due to their chemical composition, which includes compounds such as hexadecenoic acid methyl ester, 9,12,15-octadecatrienoic acid (Z, Z, Z), phytol, squalene, benzoic acid, and methyl ester, positioning them as potential alternative medicinal treatments.

Practical Relevance

The current study identified natural compounds with both ant-bacteria properties, it can be applied in the medical field.

Research Limitations

No limitation in this study

Prospects for Further Research

Applying the current extract to other types of bacteria and extracting other compounds from the same plant and proving their effectiveness.

Conflict of Interest

The writers affirm that they are free from any financial, personal, authorship, or other conflicts of interest that may influence the study or its findings.

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None

Data Availability

Requests for data will be promptly addressed.

Use of Artificial Intelligence

The authors affirm that they did not employ any AI tools in the development of this piece.

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