JBS
Research Article
Journal of Biological Studies
eISSN: 2209-2560
V. 9, N. 2: 77-93, 2026
https://doi.org/10.62400/jbs.v9i2.18134

GC-MS analysis and antibacterial activity of *Vitex agnus-castus* extracts against gram-negative bacteria isolated from burns and wounds

Rana Mohammed Salman[1], Ragda Ayad Taha[1], Naseer hafedh ibrahim[1], Shahrazad Ahmed Khalaf [2*]

[1] Department of Biology, College of science, University of Diyala, Diyala. Iraq.
[2] Department of Forensic sciences, College of science, University of Diyala, Diyala. Iraq.
*Correspondence author: shahrazadah.kh@uodiyala.edu.iq or shahrazadah.kh@gmail.com

Received 01 March 2026|Accepted 21 April 2026|Published 03 June 2026

Abstract
Antibiotic resistance is a major global health concern. It has become a major concern for the global scientific community, threatening the effectiveness of treatments and increasing the risk of stubborn infections. This challenge is attributed to the overuse of antibiotics and mismanagement of dosages, which accelerate the development of resistant bacterial strains. As a result, using natural chemicals is no longer just an option, but a necessity to reduce the damage caused by bacterial diseases that can be life-threatening due to their difficulty in treating them. This study aimed to isolate and identify bacteria associated with burn and wound infections. The sample included 50 purulent samples taken from patients at Baqubah Teaching Hospital during the period from the beginning of November 2024 to the end of December 2024. When tested for bacterial growth, 82% of the samples reported back positive, and 18% came back negative. Bacteria were identified from wound and burn swabs after microscopy and biochemical tests. We assessed the antibiotic sensitivity of these isolates using the disk diffusion method in accordance with the McFarland standards. The examination revealed that among the isolates, there were several multidrug-resistant (MDR) bacteria. Moreover, both ethanolic and methanolic extracts of the *Vitex agnus-castus* plant were prepared, and their antibacterial effectiveness was evaluated in a laboratory environment by quantifying the inhibition zone against clinical bacterial strains, including *P. aeruginosa, K. pneumoniae, A. baumannii, E. coli, and P. mirabilis*. Antibiotic sensitivity testing was conducted after the biochemical identification of bacteria isolated from 50 patients with burns and surgical wounds. Results indicated that both ethanolic and methanolic extracts exhibited bactericidal properties to some isolates; however, at concentrations of 50 and 100 mg/ml, the methanolic extract demonstrated greater efficacy. Furthermore, gas chromatography-mass spectrometry (GC-MS) was used to examine the active chemical components of the Vitex agnus-castus extracts.
Keywords: Antibacterial, Antibacterial Activity, Antibiotic Resistance, *Vitex agnus-castus*, Gas Chromatography Mass Spectrometry.

1. Introduction

Wounds and burns are among the most common health problems globally, resulting from various factors such as heat, chemical compounds and electricity. Although many wounds heal naturally through the body's own repair mechanisms, even in the presence of bacteria. However, in some cases, the healing process can be complicated by the presence of some dead tissue, bacteria and uncontrolled inflammation responses, which often impede the natural healing process of burns and wounds. These factors, taken together, can slow down or halt the healing process, thereby increasing the risk of severe complications and adverse health effects (Rose and Chan, 2016).

When the skin is damaged due to wounds and burns, the skin barrier is compromised, making it susceptible to infection and microbial colonization. This is due to the accumulation of exudates, which consist of fluid collections of dead cells (including white blood cells) and infected tissues. When this type of damage occurs, the skin and surrounding tissues become inflamed. The moist, nutrient-rich environment is ideal for microbial growth, which can subsequently lead to infections, and recovery was delayed (Dryden, 2010). Wound infections may arise from many microorganisms that can be transmitted from the patient's microflora, their surroundings, or other healthcare personnel(Farina *et al.*,2013). Due to their impaired immune systems, burn patients are at an increased risk of contracting infections, which can lead to life-threatening outcomes such as sepsis (Ahmed and Nsaif,2021). There are several factors that increase the risk of infection, including immunodeficiency, dermatitis, surgical procedures, and prolonged hospitalization. As a result, improving the results of treatment and preventing infections requires innovative methods of therapy (Pujji *et al.*, 2019).

One of the biggest risks to people's health today is the rise of resistance to antibiotics. Since multidrug-resistant bacteria have grown more difficult to cure as a result of the decreased efficacy of antibiotics caused by their misuse, this problem has worsened(Baran *et al.*, 2023).

The overuse of antibiotics has significantly exacerbated this problem, as multidrug-resistant bacteria have become more difficult to treat due to the decreased effectiveness of these drugs. Bacteria have developed several mechanisms to resist antibiotics, including altering cell membrane permeability, modifying the antibiotic targeting site, inhibiting enzymes, and expelling the antibiotic from the cell. Consequently, it has become crucial to search for effective alternative treatments, such as using natural substances found in plants, microalgae, and fungi. These natural substances can offer new treatment options for diseases caused by antibiotic-resistant bacteria (de Arruda *et al.*, 2023).

Vitex agnus-castus belongs to the family Lamiaceae. This plant is widely distributed in the Mediterranean and Asia. It has a wide variety of beneficial components, including castesin, glycoside, flavonoids, phenolic compounds, and medicinal uses are many and extensive, addressing issues including sedation, antiangiogenic effects, and suppression of tumours in certain organs, among many others. Numerous medical disorders are treated with Vitex agnus-castus due to its numerous beneficial therapeutic properties (Sahib *et al.*, 2014; Niroumand *et al.*, 2018).

Plant extracts are highly valuable due to their abundance of various compounds that serve crucial roles in health care, agriculture, and industry. These compounds, derived from plant secondary metabolism, include phenols, flavonoids, and alkaloids, and exhibit unique biological activities, such as: Antioxidants that protect cells from damage, Antimicrobial that fight infection and Anti-inflammatory properties that reduce inflammation (Al-Karawi *et al.*, 2025) In addition, plant extracts can be used as natural alternatives due to the variety of active chemical compounds they contain, which are biodegradable, reducing harmful environmental impacts. This makes plant extracts a promising option for many applications, including alternative medicine, the food industry, cosmetics, and sustainable agriculture (Tsimogiannis and Oreopoulou, 2019).

Therefore, this study aimed to evaluate the antibacterial activity of methanolic and ethanolic extracts of *Vitex agnus-castus* against multidruge-resistant strains (MDR), isolated from wound and burns patients, and to identify their bioactive compounds using GC–MS.

2. Materials and Methods

Plant Sample Collection: Vitex agnus-castus

It was collected from herbal offices in the markets of Baqubah city in Diyala Governorate. The plant material was air-dried at room temperature (25°C) for one week An electric grinder was used to grind the material into a fine powder, which was then stored in opaque, labelled glass bottles for future use.

Preparation of the Plant Samples

Vitex agnus-castus was thoroughly cleaned with sterile distilled water to remove any impurities. The samples were then air-dried at ambient temperatures (25°C ± 2°C) in a dark setting to maintain the stability of the active compounds and prevent degradation, using recognized protocols for plant sample preparation (Hou *et al.*, 2023).

The samples were accurately measured using a sensitive balance. Fifty grams of dried samples were thereafter placed in a Whatman No. 1 filter paper funnel and firmly sealed. A Soxhlet extractor was employed to extract the chemicals utilizing 400 ml of both ethanol and methanol for a duration of 6 h. After extraction, the extract was cooled and subjected to a rotary evaporator, which concentrated the sample to 50 mL.

Assessment of Vitex agnus-castus Extracts via Chemical Methods

The experiment was carried out on an Agilent Technologies 7890 Gas Chromatography-Mass Spectrometry (GC-MS) system. Separation was performed on a column with a stepwise heating from 40°C to 300°C after a microliter input, and the chemical components in the extracts were identified. The carrier gas, helium, was introduced at a rate of 1 mL/min. Accurate identification of the chemical substances found in the extracts was achieved through compound identification utilizing the NIST database and the instrument's memory list. (Sivakrishnan and Pradeepraj, 2019).

Isolation and Identification of Pathogenic Bacterial Isolates

Bacteria causing burns and wounds have been isolated. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, and *Escherichia coli* were isolated from 50 patients at Baquba Teaching Hospital suffering from burns and wounds. Samples were cultured directly on blood agar and MacConkey agar, then incubated aerobically for 24 hours at 37°C. This procedure allows for bacterial growth and detection, enabling the identification of bacterial species based on phenotypic characteristics of the bacterial isolates, which were examined by culturing them on different media such as Blood agar, Eosin Methylene Blue, MacConkey agar, Urea agar, and Cetrimide agar, in addition to performing some chemical tests such as IMViC test, Oxidase test, and Catalase test (Mahmoud, 2020).

Antibiotic Susceptibility Testing

It was performed using the disk diffusion method, according to CLSI guidelines (CLSI, 2022). This test aims to determine the effectiveness of different antibiotics against isolated bacteria. Cefotaxime was used as a positive control, and its antibiotic activity was compared with that of the antibiotics under investigation.

Determination of Antibacterial Activity of Vitex agnus-castus

The antibacterial activity of the plant extract was evaluated using the agar well diffusion. A bacterial suspension was prepared by adjusting the turbidity to 0.5 McFarland standards and then inoculated onto Mueller-Hinton agar (MHA) using a sterile cotton swab, and it was left to dry. 4 holes were made on the culture medium with a diameter 5mm by a cork drill, and they were filled with 100μL of the serial concentrations of plant extracts (25,50,100) in each hole. The fourth hole was treated with distilled water as a negative control to show that the effect is due to the extract. The plates were incubated at 37°C for 24 h, and the diameter of the inhibition zone around each hole was measured to determine the antibacterial efficacy of the extract (Mahmoud, 2020). To ensure clear and accurate results, the experiment was repeated three times (separate plates), n=3 for each concentration.

Statistical analysis

The study results were interpreted using relevant statistical methods, specifically SPSS, by using numerical values and percentages for the descriptive values of the variables. The results were interpreted using a t-test (p-value < 0.005), while the results comparing the areas of inhibition were interpreted using a one-way ANOVA test (Levesque, 2007).

3. Results

The GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) was used to analyse the chemical composition of Vitex agnus-castus extracts. The results, shown in Tables (1) and (2) and Figures (1) and (2), identified 93 chemical compounds in the methanolic and 123 compounds in the ethanolic extracts. The ten most biologically active compounds were Eugenol, Coumarin, D-Carvone,5-Hydroxymethylfurfural(5-HMF), gamma-Sitosterol, alpha-Amyrin, n-Hexadecanoic acid (Palmitic acid),9-Octadecenoic acid (Oleic acid), and Tetradecanoic acid (Myristic acid) in Methanolic extracts Table (1) and Figures (1). And the seven most biologically active compounds were Eugenol, D-Carvone, 5-Hydroxymethylfurfural (5-HMF), Tetradecanoic acid (Myristic acid), n-Hexadecanoic acid (Palmitic acid),9, -12, -15-Octadecatrienoic acid (Linolenic acid), and γ-Sitosterol in Ethanolic extracts of the plant Table (2) and Figures (2). The peaks were identified by using the National Institute of Standards and Technology (NIST) database and also the device memory list (Sivakrishnan and Pradeepraj, 2019).

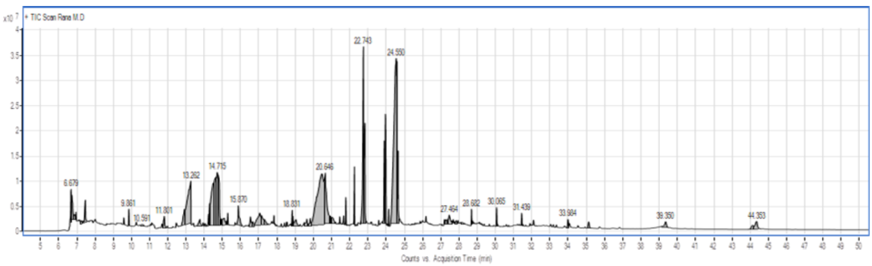

Figure 1. The GC-MS Analysis of the Methanolic Extract of *Vitex agnus-castus*

Table 1. Phytochemical compounds identified in the Methanolic extracts of *Vitex agnus-castus*

Compounds	Chemical Class	Formula	CAS ID	Area	RT	TIC % Area	Library	Biological activity	Ref.
Eugenol	Aromatic Phenols	$C_{10}H_{12}O_{2}$	0-53-97	6099271	15.87	0.352271	NIST 20.L	Antibacterial Antifungal	(Thormar *et al.*, 013)
Coumarin	Ben-zopy-rones	$C_{9}H_{6}O_{2}$	5-64-91	7176358	17.056	0.41448	NIST 20.L	Anticoagulant Anti-inflammatory antitumor	(Simopoul , 2002)
D-Carvone	Terpenoids	$C_{10}H_{14}O$	-22448-16	2189566	14.291	0.126461	NIST 20.L	Antimicrobial Antispasmodic	(Chae *et al.*, 2019)
5-Hydroxymethylfurfural (5-HMF)	Furan Derivatives	$C_{6}H_{6}O_{3}$	0-47-67	2492562	9.2	0.143961	NIST 20.L	Antioxidant	Hou *et*) (*al.*,2019
gamma-Sitosterol	Phytosterols	$C_{29}H_{50}O$	6-47-83	4882259	33.988	0.281981	NIST 20.L	anti-inflammatory anti-tumor	(Santos *et al.*, 2018)
alpha-Amyrin	Triterpenoids	$C_{30}H_{50}O$	-95-6389	3395374	35.128	0.196104	NIST 20.L	Anti-inflammatory anti-ulcer	Wong *et*) (*al.*, 2018
n-Hexadecanoic acid (Palmitic acid)	Satu) rated Fatty Acid)	$C_{17}H_{34}O_{2}$	3-10-57	125135454	22.827	7.227364	NIST 20.L	Antibacterial antifungal	(Gupta *et* (*al.*, 2011
9-Octadecenoic acid (Oleic acid)	Monounsaturated Fatty Acid	$C_{18}H_{34}O_{2}$	-19378-62	136963849	24.65	1.72165	NIST 20.L	Anti-inflammatory	(Shahkoomahally *et al.*, 2021)
9-12-15-Octadec-atrienoic acid (Linolenic acid)	Polyunsaturated Fatty Acid	$C_{18}H_{30}O_{2}$	-40-4631	118479029	24.586	6.842913	NIST 20.L	Anti-cardiovascula	(Tsanaktsidou *et al.*,2020)
Tetradecanoic acid (Myristic acid)	Saturated Fatty Acid	$C_{14}H_{28}O_{2}$	-63-5448	13175493	20.645	0.760968	NIST 20.L	Antiviral, Antiviral herpes virus	Borges) *et al.*, (2005

The diverse chemical composition of the Vitex agnus-castus can be attributed to its unique chemical composition, which includes glycosides, phenolic compounds, essential oils, and flavonoids. The variation in the concentrations of these chemicals may be due to differences in the plant's genetic patterns, resulting in variations in its chemical composition and biological properties (Aumeeruddy-Elalfi *et al.*,2015).

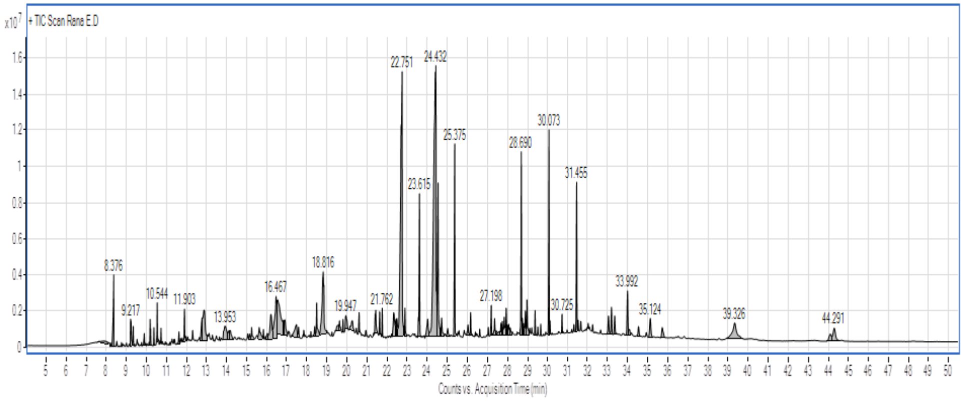

Figure 2. The GC-MS Analysis of the Ethanolic Extract of *Vitex agnus-castus*

Table 2. Phytochemical compounds identified in the Ethanolic extracts of *Vitex agnus-castus*

Compounds	Chemical Class	Molecular Formula	CAS ID	Area	RT	TIC% Area	Library	Biological activity	Ref.
Eugenol	Aromatic phenols	C10H12O2	-970-53	1092811	15.839	0.87609	NIST 20.L	Antibacterial/Gram Positive Antifungal Anti-inflammatory painkiller	(Wong *et al.*,2018)
D-Carvone	Monoterpenoids	C10H14O	-22448-16	2189566	14.12	0.148258	NIST 20.L	Antimicrobial Antifungal	(Chae *et al.*, 2019)
5-Hydroxymethy-lfurfural ((5-HMF))	Furan derivatives	C6H6O3	-670-47	7157513	14.167	0.45912	NIST 20.L	Antioxidant Antimicrobial	Thormar) (*et al.*, 2013)
Tetradecanoic acid (Myristic acid)	Saturated fatty acid	C14H28O2	-5448-63	2587753	20.607	0.444254	NIST 20.L	Antibacterial, Antiviral herpes virus	(Hou *et al.*, 2019)
n-Hexadecanoic acid (Palmitic acid)	Saturated fatty acid	C16H32O2	-573-10	4727330	22.748	12.54016	NIST 20.L	Antibacterial antifungal	(Gupta *et al.*, 2011)
9-12-15-Octadecatrienoic acid (Linolenic acid)	Unsaturated fatty - acid	C18H30O2	-4631-40	34910533	24.432	5.993279	NIST 20.L	Anti-inflammatory Antibacterial, Boosting immunity against viral infections	(Shahkoomahally *et al.*, 2021)
gamma-Sitosterol	Phytosterols	C29H50O	-836-47	7683212	33.99	1.319018	NIST 20.L	Anti-inflammatory antiviral	(Borges *et al.*, 2005)

Isolation of Bacterial isolates

Fifty samples of burns and wounds were collected from patients at Baqubah Teaching Hospital in Diyala Governorate. The samples included both genders and different ages, from the beginning of November 2024 to the end of December 2024. The results showed that 82% of the samples tested positive for bacterial culture, while 18% tested negative. The total number of bacterial isolates was 41 (*K. pneumoniae* 13 (31.7%), *A. baumannii 8* (19.5%), *P. aeruginosa* 8(19.5%), *P. mirabilis* 7(17.1%), *E. coli* 5(12.2%), as shown in Figure 3.

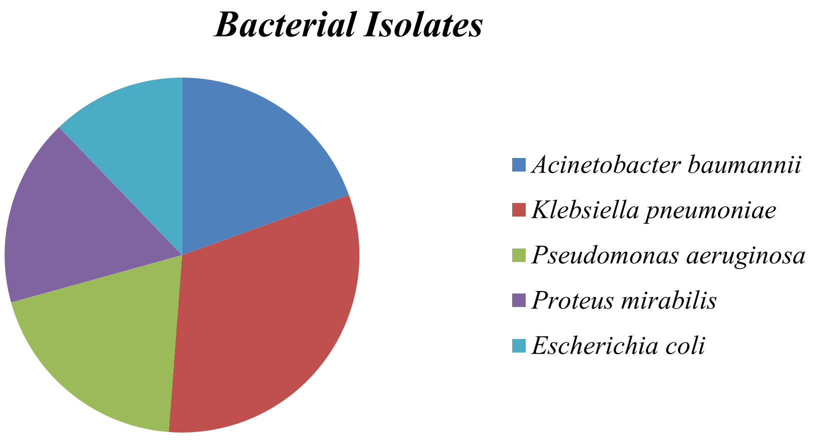

Figure 3. The Percentage of Bacterial Isolates

Identification of Bacterial Isolates

Bacterial isolates were identified by examining the morphology and characteristics of the cultures on various media, EMB agar, Cetrimide agar, Blood agar, MacConkey agar, and Urea agar base. In addition to conducting biochemical tests, including Catalase reagent and Oxidase reagent (Taha, 2018).

Klebsiella pneumoniae was identified by its characteristic growth on culture media. On MacConkey agar, colonies appeared mucoid, round, large, and pink, with the ability to ferment lactose. In contrast, on blood agar, the colonies were mucoid, grayish-white, and non-hemolytic (Qaisar *et al.*,2023). By analyzing its biochemical and physical traits, *P. aeruginosa* was positively identified. Because they couldn't ferment lactose, the colonies on MacConkey agar looked pale. As a result of the pigments pyoverdine and pyocyanin synthesis, colonies on cetrimide agar showed a yellow-green or blue-green hue. The capacity of the colonies to hemolysis on blood agar was another distinguishing feature (Abdelaziz *et al.*,2022). Because of their rod-shaped structure, negative Gram stain, and ability to produce catalase and oxidase, biochemical tests further validated that *P. aeruginosa* was the correct identification (Mahmoud, 2020).

Different culture media showed unique morphological features of *A. baumannii* isolates. Colonies on MacConkey agar were all the same size, smooth, and pale; some of them were even sticky. A lack of hemolysin production was indicated by the grey colonies on blood agar, which did not show hemolysis. The bacterial cells were tiny, round, and typically found in pairs or singly when examined under a microscope.

The distinctive appearance on culture media allowed for the identification of *P. mirabilis* isolates. Colonies on MacConkey agar were tiny, spherical, convex, and edgeless; they couldn't ferment lactose, and they had that distinct fishy smell. On blood agar, swarming layers of a confluent, sticky layer of bacteria formed. The isolates were further confirmed by their Gram-negative staining, coccobacilli morphology, and positive responses to catalase and urease enzymes, with no oxidase activity (Garrity and Bergey, 2007).

Escherichia coli isolates were identified by their characteristic growth on various culture media. On MacConkey agar, they produced round, translucent, and pink colonies due to lactose fermentation. On blood agar, the colonies had a creamy consistency. When cultured on eosin methylene blue (EMB), the colonies displayed a brown colour with a greenish metallic sheen. Biochemical tests confirmed the bacterial identity, showing a positive catalase reaction, a negative oxidase reaction, specific results in the IMViC tests, and gas production on Kligler iron agar(Mahmoud, 2020).

Antibiotic Sensitivity of Bacterial Isolates

The results of the present study (shown in Figure 4) indicated that all *P. aeruginosa* strains showed many antibiotic resistances: Piperacillin 29%, Cefepime 100%, Ceftazidime 86%, Ticarcillin-clavulanate 71%, Piperacillin /Tazobactam 0%, Aztreonam 57%, Ciprofloxacin 57%, Ofloxacin 57%, Netilmicin, Imipenem and Meropenem 14%. Also, the results of the study that showed in Figure 4 indicated that among all *A. baumannii, Piperacillin* showed many antibiotics resistance, Piperacillin 59%, Cefepime 100%, Ceftazidime 90%, Ticarcillin-clavulanate 81%, Piperacillin /Tazobactam 40%, Aztreonam and Ciprofloxacin 70%, Ofloxacin 60%, Netilmicin 40%, Imipenem, and Meropenem 25%.

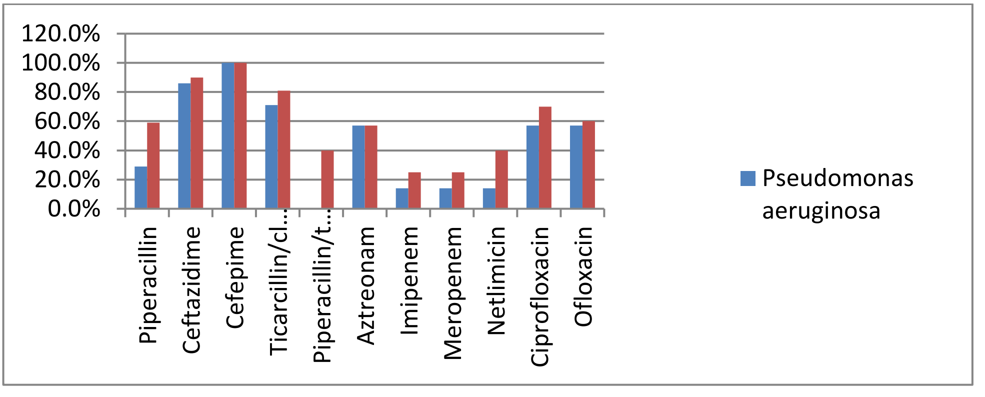

Figure 4. The percentage of antibiotic resistance of *P. aeruginosa and A. baumannii*

The resistance of the antibiotics of *K. pneumoniae* isolates showed variable resistance as shown in Figure 5, which includes: Ciprofloxacin 31%, Tetracycline 38%, Aztreonam 54%, Amoxicillin100%, Amoxicillin-clavulanate 23%, Co-trimazole 15%, Levofloxacin 15%, Gentamicin 23%, Cefotaxime 92%, Amikacin 8%, Imipenem 8% and Cefepime 85%. *Proteus mirabilis* isolates showed a variety of resistance to antimicrobial agents: Ciprofloxacin 33%, Tetracycline 50%, Aztreonam 33%, Amoxacillin 100%, Levofloxacin 17%, Imipenem 17%, Co-trimazole 19%, Amoxicillin-clavulanate 50%, Cefepime 50%, Cefotaxime 67%, Gentamycin and Amikacin was 33%, Figure (5).

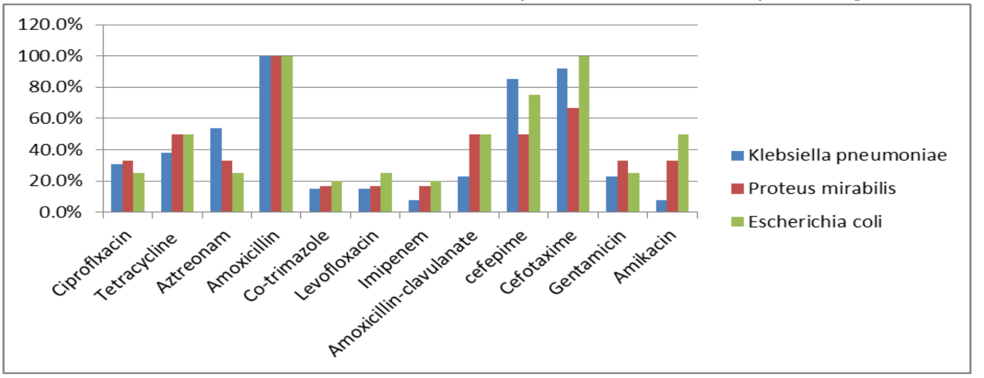

Figure 5. The antibiotic resistance percentages for *K. pneumonia, E. coli* and *P. mirabilis*

The results (also shown in Figure 5) indicated that the *E. coli* showed resistance, including: Ciprofloxacin 25%, Aztreonam 25%, Cefotaxime 100%, Amoxicillin 100%, Cefepime 75%, Amoxicillin-clavulanate 50%, Levofloxacin 25%, Gentamicin 25%, Amikacin 50%, Tetracycline 50%, Imipenem and Co-triazole were resistant 20%.

Antimicrobial activity of Ethanolic and Methanolic extracts of *Vitex agnus-castus*

Determination of the antibacterial Activity of the Methanolic and Ethanolic Extracts of *Vitex agnus-castus* against isolates. In Table), the results of the study showed the inhibitory effect of the methanolic and ethanolic extracts of the *Vitex agnus-castus* against the bacterial isolates isolated from clinical samples in this study, which recorded the highest effect of the methanolic extract against *P. mirabilis* and *K. pneumoniae* bacteria with an inhibition diameter of (26±0.59) mm and (26±0.5)mm at a concentration of 100 mg/ml, while the diameter of the inhibition zone was recorded as (25±0.58)and(23±0.4)mm against *E. coli* and *A. baumannii* isolates at a concentration of 100 mg/ml, and (18±0.35) mm average diameter of inhibition against *P. aeruginosa* isolates at a concentration of 100 mg/ml.

The ethanolic extract exhibited the highest inhibition zone against *P. mirabilis*, which was (23±0.6) against *P. mirabilis* at a concentration of 100mg/ml, while at a concentration of 50 mg/ml, the inhibition zone diameter was (20±0.6)mm. The result showed at a concentration of 100 mg/ml (20±0.6) and (20±0.5)mm, for both *E. coli* and *K. pneumoniae*. Also showed it that at a concentration of 100 mg/ml, an inhibition zone of (19±0.7) and (18±0.35)mm for both *A. baumannii* and *Pseudomonas*, while at a concentration of 50 mg/ml, it gave varying inhibition diameters against bacterial isolates, with significant difference at (P value <0.005), ranging between (20±0.71)mm for *P. mirabils* (19±0.55),(19±0.37)mm for both *E. coli* and *K. pneumoniae*, while the lowest inhibition diameter was (17±0.16)mm for *P. aeruginosa* and (18±0.36)mm for *A. baumannii*.

Table 3. Antimicrobial activity of Methanolic and Ethanolic Extracts of *Vitex agnus-castus*

Bacterial isolates	Methanolic Extracts Mean±SD			Ethanol Extracts Mean±SD		
	Concentration mg/ml					
	100	50	25	100	50	25
P. aeru-ginosa	18 ±0.35	16 nm±0.77	14 nm±0.15	18 nm±0.15	17 nm±0.16	14 nm±0.3
P. mira-bilis	26 nm±0.59	23 nm±0.6	19 nm±0.34	23 nm±0.21	20 nm±0.71	18 nm±0.73
E. coli	25 nm±0.58	23 nm±0.7	20 nm±0,55	20 nm±0.6	19 nm±0.55	16 nm±0.38
K. pneu-monia	26 nm±0.5	22 nm±0.23	19 nm±0.35	20 nm±0.5	19 nm±0.37	15 nm±0.38
A. bau-mannii	23 nm±0.4	21 nm±0.5	19 nm±0.36	19 nm±0.7	18 nm±0.36	16 nm±0.4

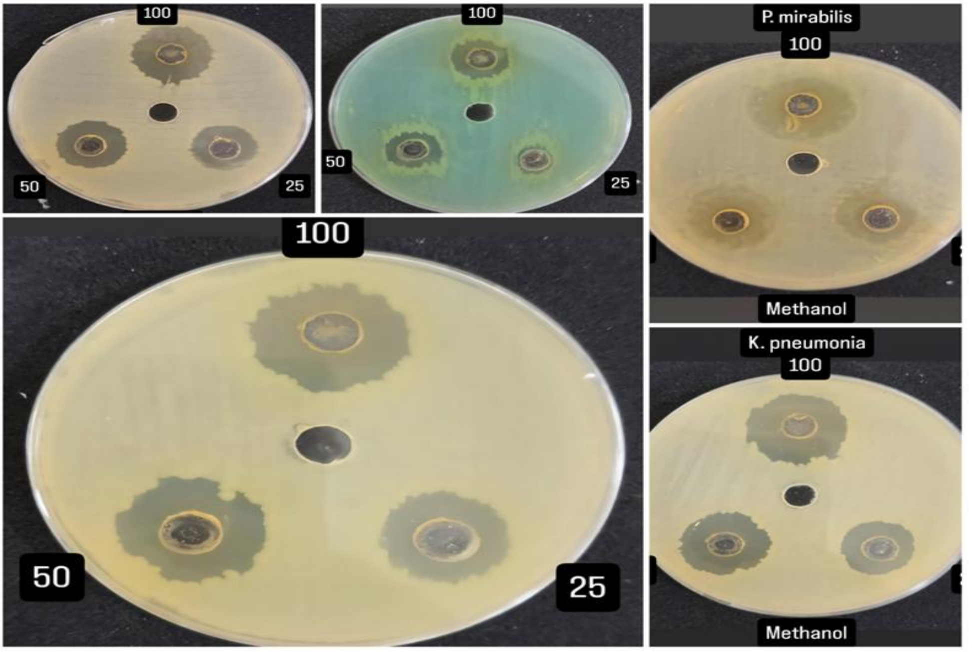

Figure 6. Antimicrobial activity of Methanolic Extract of *Vitex agnus-castus*

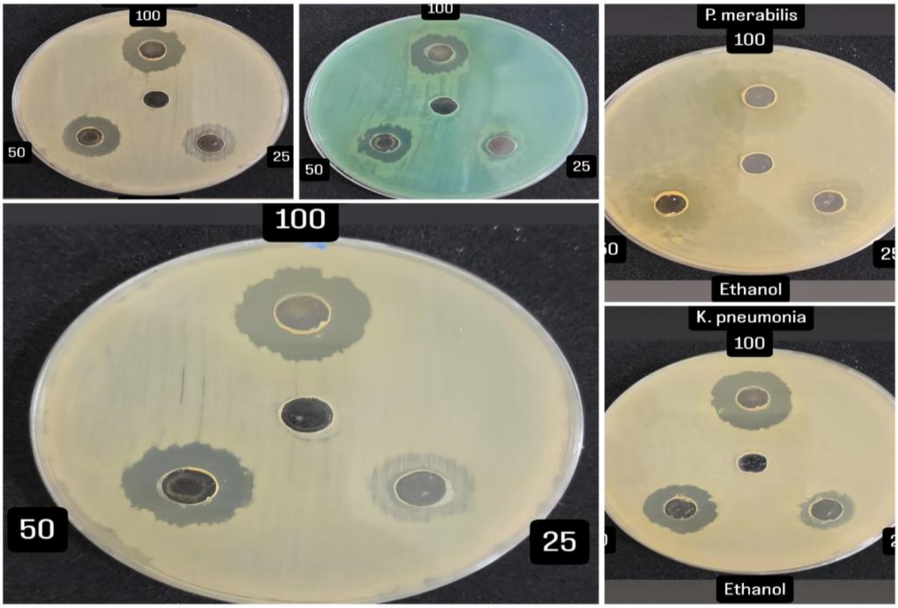

Figure7. Antimicrobial activity of Ethanolic Extract of *Vitex agnus-castus*

The study also yielded the Minimum Inhibitory Concentration (MIC) for alcoholic extracts, ranging from 6 to 38 µg/ml. *A. baumannii* had an MIC of 6 µg/ml, followed by *P. mirabilis* at 9 µg/ml, while *K. pneumoniae* recorded the highest MIC at 38 µg/ml. Minimum Bactericidal Concentration (MBC), results showed MIC ranging from 63 to over 1000 µg/ml.
The results of the current study, which showed MIC and MBC values indicating the type of antibacterial activity against bacterial strains, revealed that if the ratio was less than or equal to 4, the activity was bactericidal; if the ratio was greater than 4, the activity was only inhibitory. The alcoholic extracts of the Vitex agnus-castus were bactericidal against both *E. coli* and *P. mirabilis*, while they were inhibitory against other species.

4. Discussion

Plant secondary metabolites can be considered one of the most important alternatives for controlling human infectious agents with varying resistance to antibiotics. Among the most important of these active antimicrobial compounds are tannins, lavotrides, phenolic compounds, and alkaloids. The antimicrobial activity of medicinal plants may be attributed to these compounds (Berrani *et al.*,2021).

The presence of active compounds with antibacterial activity is one of the most important factors in the resistance of medicinal plants to a wide range of bacteria. Because of this, many researchers felt the need to examine these plants scientifically, which may help many biochemists and pharmacologists discover many therapeutic agents.

Chemical analysis of the Vitex agnus-castus has shown that its alcoholic extracts contain a wide variety of active compounds, including terpenoids, flavonoids, steroids, and carbohydrates. Previous studies have attributed the presence of flavonoids, which possess antimicrobial activity against a broad spectrum of microorganisms, to their ability to form complexes with soluble proteins outside the cell wall. Furthermore, lipophilic flavonoids, which are highly effective at disrupting microbial membranes, exhibit antibacterial activity by inhibiting nucleic acid synthesis through binding to the enzyme DNA gyrase (Nagarsekar *et al.*, 2020).

Additionally, quercetin, a phenolic compound, weakens metabolic processes by inhibiting nucleic acid synthesis and plays a role in inhibiting the activity of efflux pumps, thereby preventing or halting the development of antibiotic resistance. The active efficacy of medicinal plants is also attributed to their content of terpenoid compounds, which are one of the common classes of secondary metabolites. They possess antimicrobial activity, as results have shown that these compounds inhibit oxygenation and oxidative phosphorylation, which are among the most important metabolic processes necessary for the survival of bacteria. Monoterpenes such as carvacrol, geraniol, thymol, and menthol have shown antibacterial activity against Gram-negative and Gram-positive bacteria. The efficacy of geraniol lies in inhibiting the efflux pumps that release antibodies outside the cell, which contributes to increasing the sensitivity of aerobic *Enterococci aergenes* (Nagarsekar *et al.*, 2020). Both thymol and menthol have also shown activity against *Staph. aureus* and *E. coli.* Carvacrol's antimicrobial activity is mediated by inhibiting biofilm formation in *Salmonella typhimurium* and *S. aureus.*

Another derivative that has shown anti-inflammatory activity is oleanic acid, a tritrapenoid compound, which has been shown to have a synergistic effect when mixed with soniazide, ethambutol and rifampicin, thus reducing the MIC of those antibiotics (Ababutain and Alghamdi,2021). Most of the secondary metabolites of the Vitex agnus-castus are terpenoids and phenolic compounds. This aligns with the findings of numerous previous published studies that focused on the biological activity of these compounds, whose efficacy is attributed to their antibacterial activity. Many studies have focused on alcoholic (methanolic and ethanolic) extracts of the plant, indicating that compounds found in its leaves are primarily responsible for its antimicrobial activity. These compounds exhibit high polarity, as ethanol and methanol are highly polar solvents with a strong affinity for polar compounds. It is well-known that chaste tree species contain very high levels of diterpenoids in their structure, and this may explain the antimicrobial activity of these compounds. However, further experiments and studies are needed to better understand and characterize the activity of these compounds (Zhelev *et al.*, 2022).

Multiple factors, such as environmental variables, testing procedures, and the source of samples, contribute to the variance in antibiotic resistance among *P. aeruginosa* isolates. Many mechanisms, which include changes to the permeability of membranes, efflux pumps, production of beta-lactamase, biofilm formation, and the incorporation of resistant plasmids transporting different resistance genes, are implicated in the development of antibiotic resistance in bacteria, according to studies. The capacity of bacteria to modify themselves and withstand antibiotics is explained by these mechanisms (Hong *et al.*,2016).

An antibiotic-resistant strain of *A. baumannii* may have evolved through the production of aminoglycoside enzymes, including phosphotransferase, nucleotide-transferease, and broad-spectrum beta-lactamase. Furthermore, bacteria may increase their resistance to antibiotics by decreasing the concentration of these drugs within their cells through the reduction of their outer membrane channels. Diseases resulting from these bacteria are notoriously difficult to treat due to their numerous processes (Zahn *et al.*, 2016; Nirwati *et al.*, 2019).

As a public health issue*, K. pneumoniae* poses serious challenges in the clinic. When it comes to the transmission of genes that make bacteria resistant to certain antibiotics, this bacterium is pivotal. A Gram-negative, multidrug-resistant bacterium, it poses a serious threat to infection control measures because of its association with healthcare-associated infections (Girlich *et al.*,2025).

One of the main causes for resistant to antibiotics was the formation of enzymes called extended-spectrum beta-lactamase (ESB). These enzymes break down medicines, making them useless. Furthermore, it is believed that unchecked antibiotic usage helps bring about and spread antibiotic resistance, particularly in healthcare facilities where resistant strains are passed from one patient to another.

P. mirabilis strains that are resistant to many drugs, such as carbapenems, ESBLs, and AmpC enzyme, are becoming more common. Despite this, fluoroquinolones can occasionally kill these bacteria. The effects of clavulanic acid can be inhibited or resisted by different species of *Proteus*. Although these species can break down narrow-spectrum penicillin, they often can't handle carbapenems or second or third-generation cephalosporins (Manolitsis *et al.*, 2023).

The Enterobacteriaceae family includes the highly antibiotic-resistant *P. mirabilis* and *K. pneumoniae*, which are able to manufacture beta-lactamase enzymes and employ various resistance mechanisms. One of these methods was lowering the antibiotics' permeability to the cell. Another is lowering their binding to the penicillin-binding protein. Beta-lactamase hydrolyzes them. The results of this study show that resistance to antibiotics was mostly caused by their misuse in hospitals and other healthcare facilities (Lledo *et a*l., 2009).

The synthesis of beta-lactamase enzymes was a key mechanism by which *E. coli* displays resistance to beta-lactamase drugs. Encoding these enzymes is a trait shared by many Gram-negative bacteria, including *E. coli*. In addition, *E. coli* has many ways to resist antibiotics, such as by changing the target sites of antibiotics, forming biofilms, and using efflux pumps (Lledo *et a*l., 2009).

Vitex agnus-extracts castus contain a wide variety of biochemical components, including polyphenols, fatty acids, terpenes, and terpenoids, as shown in this study. Some compounds, such as benzoic acid, isobutyl 4-hydroxybenzoate, and 4-5-dichloro-1,3-dioxolane-2-one, were most abundant in ethanol and methanol. Several of the components of *Vitex agnus-castus* demonstrated anti-Candida effectiveness, suggesting that the plant may be useful as a medicinal agent in the treatment of fungal diseases. The findings of this study can guide future investigations into the possible medical uses of these compounds (Garvey *et al.*, 2011). Natural inhibitors compound in Vitex agnus-castus extract can inhibit efflux pumps or operate in many ways, improving the effectiveness of antibiotics (Garvey *et al.*, 2011). The synergistic interactions between antibiotics and plant extracts have been recently explained by a number of mechanisms, such as increased permeability, suppression of drug efflux, and beta-lactamase suppression. Consistent with previous studies, our findings demonstrate that natural compounds can enhance the effectiveness of antimicrobial drugs through synergistic effects. Such combinations may provide a promising new approach to treating infections caused by bacteria that have developed resistance to conventional antibiotics (Kaur *et al.*, 2016).

In a study conducted by Ahmed *et al.* (2016), the results showed that crude extracts of the plant Vitex agnus-castus exhibited significant efficacy in inhibiting the growth of methicillin-resistant *Staphylococci aureus* (MRSA). This indicates the considerable potential of using this plant as a primary source for isolating active ingredients of significant medical importance. These findings provide a foundation for subsequent studies to identify the plant's active compounds and evaluate their high efficacy and safety in therapeutic applications.

Another study found that the vitex extracts and antibiotics worked together to kill *P. aeruginosa* more effectively. The inhibitory zones were significantly wider than expected. According to the researcher, the combination of vitex extract and meropenem resulted in a maximum rise of 330%, these results suggest that the combination of vitex extract with antibiotics may affect the treatment of drug-resistant bacteria, especially *P. aeruginosa*. This methodology has the potential to aid in the development of new therapeutic methods to fight drug-resistant bacteria (Khames and Ahmed,2025).

In a study conducted by Bramki *et al* (2025), alcoholic extracts of the leaves of the Vitex agnus-castus plant were used to inhibit the growth of *Staph. aureus* and *Bacillus subtilis* strains with inhibition diameters of (22.33±0.47) mm and (18.33±0.47) mm, respectively. It was noted that the greatest activity was against *Bacillus subtilis*, which is due to the presence of effective secondary compounds that were extracted, such as Flavonols, flavonoids, and total phenolic compounds. The study showed the possibility of using Vitex agnus-castus leaves in treatment, which contributes to encouraging researchers to conduct more experiments on large plants to contribute to the development of plant-based drug production.

The results of this investigation demonstrated that methanol extracts had larger inhibition zones than ethanol extracts when it came to preventing the growth of the isolates of bacteria that were studied. This may be due to methanol's high ability to extract active compounds. This is due to its high polarity, which makes it a better solvent for polar compounds such as alkaloids and glyceroides (natural antibiotics), phenols, and organic acids (strong antibacterial agents) (Patel *et al.*, 2021). Ethanol may interact with some phenolic compounds and reduce their effectiveness, while methanol maintains their stability and better disintegrates bacterial cell walls (Alam *et al.*, 2019). Methanol also has the ability to penetrate bacterial membranes more easily due to its small molecular size (smaller than ethanol) and its ability to break hydrogen bonds in bacterial proteins (Kumar *et al.*, 2020). In a comparative study conducted by researchers Ibrahim *et al* (2018) on methanolic and ethanolic extracts of Allium sativum, the results showed that the methanolic extract achieved an inhibition diameter of 18 mm against *E. coli*, while the ethanolic extract achieved only 12 mm.

The previous studies by researchers Goncalves *et al* (2017) showed that the essential oils of the chaste tree were used against strains of bacteria that cause tooth decay, ranging from 15.6 to 200 μg/ml, such as *Lactobactos casi* (MIC 15.6 μg/ml), *Streptococcus mitis* (MIC 31 μg/ml), and *Streptococcus mutans* (MIC 15.6 μg/ml). The extract showed remarkable anti-causing activity against these pathogens. Another study conducted by Islam *et al* (2024) on the plant Vitex confirmed that the results of the study showed the antibacterial activity of secondary metabolites extracted from this plant against many strains of bacteria, such as *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *Staph. aureus*. The results also showed its activity against types of fungi such as *Aspergillus niger* and *Candida albicans*, as the MIC of *Staph. aureus* was 12.5 μg/ml.

One of the biggest challenges facing the pharmaceutical industry is developing new drugs with antimicrobial efficacy. This is due to the specific mechanistic pathways that can be followed for antibacterial, antiviral, and antifungal activities. These microorganisms possess high and inherent resistance, conferring immunity against these pathways. Moreover, starting from scratch to develop new drugs is extremely costly and time-consuming, and the transition from theory to clinical trial may not even occur. This is due to numerous constraints, such as synthesis throughput, bioavailability, compatibility of formulations, and many other technical aspects. Because of these limitations, natural plant-based products with antibacterial activity can be used to overcome them. Also, pharmaceutical engineering processes can develop new and different therapeutic options of clinical importance using molecular structures of secondary metabolites. For example, the broad-spectrum antibiotic amikacin belongs to the class of semi-synthetic aminoglycosides and is effective against aerobic Gram-negative bacteria and *P. aeruginosa*. This antibiotic was derived from kanamycin A, which was isolated from Streptomycin kanamyceticui and is a natural product (Mrugała *et al.*, 2021).

5. Conclusion

Plant extracts and their derivatives have proven effective in treating multidrug-resistant strains, making them an alternative therapeutic option, representing a promising source of chemical compounds in the pharmaceutical industry, especially in light of the increasing global antibiotic resistance. Researchers found that *Vitex agnus-castus* ethanolic and methanolic extracts effectively killed bacterial strains found in burn and wound surgery. This effect is attributed to the extracts' content of active compounds such as phenols, terpenoids, and alkaloids, in addition to fatty acids and lipids. These chemicals were identified by GC-MS. These results indicate that plant extracts could be a promising source for the discovery of new pharmaceutical compounds to combat antibiotic-resistant bacteria.

Acknowledgments

We appreciate the anonymous reviewers' insightful comments and suggestions for improving the quality of the paper.

Conflict of interests

The authors declare that they have no conflicts of interest that could have influenced the conduct, analysis or presentation of the research.

Funding

None

Author contribution

All authors contributed to the study conception and design, data collection, analysis and interpretation of results, and draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study confirms its adherence to ethical standards, as ethical approval was obtained from the Diyala Health Directorate under code number 46727 on November 13, 2024. Participants and healthcare workers were informed of the study objectives, and patients were informed of the benefits and the absence of health concerns associated with the study. Participants participated voluntarily with the right to withdraw at any time without adverse effects on their treatment or health. Precautions were taken to maintain data confidentiality through coding. Participants were informed that the data would be used only for study purposes, and verbal consent was obtained from them.

References

Ababutain, I. M., & Alghamdi, A. I. (2021). In vitro anticandidal activity and gas chromatography–mass spectrometry (GC–MS) screening of *Vitex agnus-castus* leaf extracts. *PeerJ, 9*, e10561.

Abdelaziz, A. A., Kamer, A. M. A., Al-Monofy, K. B., & Al-Madboly, L. A. (2022). A purified and lyophilized *Pseudomonas aeruginosa*-derived pyocyanin induces promising apoptotic and necrotic activities against MCF-7 human breast adenocarcinoma. *Microbial Cell Factories, 21*(1), 262.

Ahmed, A., & Nsaif, M. (2021). *Molecular detection of some virulence genes in E. coli isolated from women with urinary tract infections* (Master's thesis). College of Science, University of Diyala.

Ahmad, B., Hafeez, N., Ara, G., Azam, S., Bashir, S., & Khan, I. (2016). Antibacterial activity of crude methanolic extract and various fractions of *Vitex agnus-castus* and *Myrsine africana* against clinical isolates of methicillin-resistant *Staphylococcus aureus*. *Pakistan Journal of Pharmaceutical Sciences, 29*(6).

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2019). Methanol extracts of *Ocimum sanctum* disrupt bacterial cell wall integrity. *Microbial Pathogenesis, 128*, 153–159.

Al-Karawi, A. A., Al-Bayati, N. Y., & Shafeeq, M. A. (2025). Comparative analysis of vegetable chemical compounds from orange peel extract and *Thuja* plants using gas chromatography–mass spectrometry technique. In *Proceedings of the International Conference on Applied Innovation in IT* (Vol. 13, No. 2, pp. 615–622). Anhalt University of Applied Sciences.

Al-Muqdadi, B. M. J., & Al-Saadi, B. Q. H. (2020). Detection of *armA* gene, KPC enzyme, and molecular typing of *Klebsiella pneumoniae* clinical isolates from public hospitals in Baghdad, Iraq. *Biochemical and Cellular Archives, 20*, 2101–2105.

Aumeeruddy-Elalfi, Z., Gurib-Fakim, A., & Mahomoodally, F. (2015). Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. *Industrial Crops and Products, 71*, 197–204.

Baran, A., Kwiatkowska, A., & Potocki, L. (2023). Antibiotics and bacterial resistance—A short story of an endless arms race. *International Journal of Molecular Sciences, 24*(6), 5777.

Berrani, A., Marmouzi, I., Bouyahya, A., Kharbach, M., El Hamdani, M., El Jemli, M., Lrhorfi, A., Zouarhi, M., Faouzi, M. E. A., & Bengueddour, R. (2021). Phenolic compound analysis and pharmacological screening of *Vitex agnus-castus* functional parts. *BioMed Research International, 2021*, 6695311.

Borges, F., Roleira, F., Milhazes, N., Santana, L., & Uriarte, E. (2005). Simple coumarins and analogues in medicinal chemistry: Occurrence, synthesis and biological activity. *Current Medicinal Chemistry, 12*(8), 887–916.

Bramki, A., Barboucha, G., Benslama, O., Andolfi, A., Smati, M., Benouchenne, D., Moussaoui, A., Bensouici, C., Zorrilla, J. G., Salvatore, M. M., & Masi, M. (2025). Bioactive potential and chemical composition of *Vitex agnus-castus* leaf extracts collected in Algeria: A combined in vitro and in silico approach. *Molecules, 30*, 749.

Chae, H. K., Kim, W., & Kim, S. K. (2019). Phytochemicals of *Cinnamomi cortex*: Cinnamic acid, but not cinnamaldehyde, attenuates oxaliplatin-induced cold and mechanical hypersensitivity in rats. *Nutrients, 11*(2), 432.

Clinical and Laboratory Standards Institute (CLSI). (2022). *Performance standards for antimicrobial susceptibility testing*.

de Arruda, M. C. S., da Silva, M. R. O. B., Cavalcanti, V. L. R., Brandão, R. M. P. C., Marques, D. A. V., & de Lima, L. R. A., et al. (2023). Antitumor lectins from algae: A systematic review. *Algal Research, 70*, 102962.

Dryden, M. S. (2010). Complicated skin and soft tissue infection. *Journal of Antimicrobial Chemotherapy, 65*(Suppl. 3), iii35–iii44.

Farina, J. A., Rosique, M. J., & Rosique, R. G. (2013). Curbing inflammation in burn patients. *International Journal of Inflammation, 2013*, 715645.

Garrity, G. (2007). *Bergey's manual of systematic bacteriology* (Vol. 2). Springer.

Garvey, M. I., Rahman, M. M., Gibbons, S., & Piddock, L. J. (2011). Medicinal plant extracts with efflux inhibitory activity against Gram-negative bacteria. *International Journal of Antimicrobial Agents, 37*(2), 145–151.

Girlich, D., Dortet, L., Poirel, L., & Nordmann, P. (2015). Integration of the *blaNDM-1* carbapenemase gene into *Proteus* genomic island 1 in a clinical isolate. *Journal of Antimicrobial Chemotherapy, 70*(1), 98–102.

Gonçalves, R., Ayres, V. F., Carvalho, C. E., Souza, M. G., Guimarães, A. C., Corrêa, G. M., Martins, C. H., Takeara, R., Silva, E. O., & Crotti, A. E. (2017). Chemical composition and antibacterial activity of the essential oil of *Vitex agnus-castus*. *Anais da Academia Brasileira de Ciências, 89*, 2825–2832.

Gupta, R., Sharma, A. K., Dobhal, M., Sharma, M., & Gupta, R. (2011). Antidiabetic and antioxidant potential of β-sitosterol. *Journal of Diabetes, 3*(1), 29–37.

Hong, J. S., Yoon, E. J., Lee, H., Jeong, S. H., & Lee, K. (2016). Clonal dissemination of *Pseudomonas aeruginosa* sequence type 235 isolates carrying *blaIMP-6*. *Antimicrobial Agents and Chemotherapy, 60*(12), 7216–7223.

Hou, X., Li, S., Luo, Q., Shen, G., Wu, H., & Li, M. (2019). Discovery and identification of antimicrobial peptides in *Zanthoxylum bungeanum*. *Applied Microbiology and Biotechnology, 103*(5), 2217–2228.

Ibrahim, H., Eldemerdash, A., & El-Din, M. (2018). Antibacterial efficacy of garlic extracts. *BMC Complementary and Alternative Medicine, 18*(1), 56.

Islam, Z., Caldeira, G., Caniça, M., Islam, N., & Silva, O. (2024). *Vitex* genus as a source of antimicrobial agents. *Plants, 13*, 401.

Kaur, M., Aggarwal, N., & Dhiman, R. (2016). Antimicrobial activity of *Parthenium hysterophorus*. *Research Journal of Medicinal Plants, 10*(1), 106–112.

Khames, S. A.-S. K., & Ahmed, S. T. (2025). Evaluation of antibacterial and synergistic effects of *Vitex agnus-castus* extracts. *Iraqi Journal of Biotechnology, 24*(SI).

Kyriakidis, I., Vasileiou, E., Pana, Z. D., & Tragiannidis, A. (2021). *Acinetobacter baumannii* antibiotic resistance mechanisms. *Pathogens, 10*(3), 373.

Levesque, R. (2007). *SPSS programming and data management* (4th ed.). SPSS Inc.

Mahmoud, A. H. (2020). *Biosynthesis and characterization of nanoparticles using plant extracts* (Master's thesis). University of Diyala.

Manolitsis, I., Feretzakis, G., Katsimperis, S., Angelopoulos, P., Loupelis, E., & Skarmoutsou, N. (2023). Antibiotic resistance patterns in a urology department. *Journal of Clinical Medicine, 12*(9), 3180.

Niroumand, M. C., Heydarpour, F., & Farzaei, M. H. (2018). Pharmacological and therapeutic effects of *Vitex agnus-castus*: A review. *Pharmacognosy Reviews, 12*(23).

Patel, R., Singh, A., Sharma, M., & Pandey, S. (2021). Solvent effects on flavonoid antibacterial activity. *Phytochemical Analysis, 32*(4), 567–575.

Pujji, O. J. S., Nakarmi, K. K., Shrestha, B., Rai, S. M., & Jeffery, S. L. A. (2019). Bacteriological profile of burn wound infections. *Journal of Burn Care & Research, 40*(6), 838–845.

Qaisar, M. U., Aslam, M. A., Ullah, K., Kanwar, R., Ali, S., & Farzand, I. (2023). Occurrence of *Klebsiella pneumoniae* in burn patients. *Pakistan Journal of Medical & Health Sciences, 17*(2), 137.

Rose, L. F., & Chan, R. K. (2016). The burn wound microenvironment. *Advances in Wound Care, 5*(3), 106–118.

Simopoulos, A. P. (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition, 21*(6), 495–505.

Sivakrishnan, S., & Pradeepraj, D. (2019). GC–MS analysis of *Cordia obliqua*. *Asian Journal of Pharmaceutical and Clinical Research, 12*(6), 110–112.

Wong, S. K., Chin, K. Y., Suhaimi, F. H., Ahmad, F., Jamil, N. A., & Ima-Nirwana, S. (2018). Osteoporosis and metabolic syndrome. *Biomedicine & Pharmacotherapy, 98*, 191–200.

Zahn, M., Bhamidimarri, S. P., Baslé, A., Winterhalter, M., & Van den Berg, B. (2016). Structural insights into outer membrane permeability. *Structure, 24*(2), 221–231.

Zhelev, I., Petkova, Z., Kostova, I., Damyanova, S., Stoyanova, A., Dimitrova-Dyulgerova, I., Antova, G., Almeer, R., & Sayed, A. (2022). Chemical composition and antimicrobial activity of *Vitex agnus-castus*. *Plants, 11*, 896.