



E-ISSN: 2664-6773

P-ISSN: 2664-6765

Impact Factor: RJIF 5.6

IJCBS 2024; 6(1): 118-125

[www.chemicaljournal.org](http://www.chemicaljournal.org)

Received: 28-01-2024

Accepted: 03-03-2024

**Mohammed Adnan Mohammed**  
Basra Education Directorate, Al-Zubair High School for Outstanding Students, Iraq

**Maytham Ayoub Abdul Qader Al-Hamdani**  
Department of Biology, College of Education for Pure Sciences, University of Basra, Basra, Iraq

## Evaluation of the effectiveness of aqueous and methanolic extracts of pomegranate peels against *Acinetobacter baumannii* bacteria isolated from patients hospitalized in intensive care in some hospitals in Basra Governorate

**Mohammed Adnan Mohammed and Maytham Ayoub Abdul Qader Al-Hamdani**

DOI: <https://doi.org/10.33545/26646765.2024.v6.i1b.93>

### Abstract

*Acinetobacter baumannii* is one of the most critical drug-resistant pathogens globally. WHO has highlighted that drug-resistant microorganisms significantly threaten the most vulnerable hospital patients, emphasizing the urgent need for new antibiotic research and development.

*A. baumannii* can survive on dry surfaces for up to a month and employs various drug resistance mechanisms, including efflux pumps, drug-inactivating enzymes, and mutations targeting drugs. Infections caused by these bacteria can lead to severe illnesses such as septicemia, pneumonia, and urinary tract infections. The WHO has classified *A. baumannii* as a critical priority pathogen, underlining the necessity for new antibiotics. This study aimed to identify antibiotics in plant extracts and assess their efficacy. *A. baumannii* was isolated and identified from patients in several hospitals in Basra Governorate using traditional and molecular methods. The study tested the effectiveness of aqueous and alcoholic pomegranate peel extracts against these multi-antibiotic-resistant bacteria, and the results demonstrated the extracts' efficacy.

**Keywords:** *Acinetobacter baumannii*, drug-inactivating enzymes, antibiotic resistance, pomegranate peel extracts, hospital-acquired infections

### Introduction

Over the past three decades, the genus *Acinetobacter* has evolved tremendously, with *Acinetobacter baumannii* becoming a serious global issue for healthcare facilities. Its clinical significance has become particularly prominent in the last fifteen years due to its extraordinary ability to acquire and regulate resistance determinants, making it one of the most formidable threats in the current antibiotic era. *A. baumannii* is recognized as a leading drug-resistant pathogens globally. The World Health Organization recently highlighted that drug-resistant microorganisms, such as *A. baumannii*, predominantly affect the most vulnerable hospital patients, underscoring the urgent need for new antibiotic research and development (WHO, 2017) [9]. These bacteria have several drug resistance mechanisms, such as drug-inactivating enzymes, efflux pumps, and drug-target mutations, and they may live for up to a month on dry surfaces (Jawad A *et al.*, 1998) [10]. Infected patients can suffer from serious diseases such as septicemia, pneumonia, and urinary tract infections (McConnell MJ *et al.*, 2013) [11]. There are dangers associated with bacterial biofilms in hospitals, restaurants, and water treatment plants. Several medicinal herbs are utilized ethnomedically in India to cure infectious diseases (Chmagh, Aalaa A., *et al.*, 2023) [18]. The WHO has classified *A. baumannii* as a critical priority pathogen, highlighting the urgent need for new antibiotics to combat this significant health threat (Davies & Bennett, 2017) [8].

### Materials and Methods

279 samples were collected from patients hospitalized in intensive care and burns at Al-Sadr Teaching Hospital, Al-Fayhaa Teaching Hospital, and Al-Zubair Hospital in the form of (Swabs and blood) for a period from 01/3/2023 to 06/30/2023 using cotton swabs containing carrier medium (Transport media swabs), thus ensuring that the isolates remained active and were transferred to the laboratory as quickly as possible

**Corresponding Author:**  
**Mohammed Adnan Mohammed**  
Basra Education Directorate, Al-Zubair High School for Outstanding Students, Iraq

(The microbiology laboratory at Al-Sadr Teaching Hospital and Al- Zubair Teaching Hospital). All samples were grown on MacConkey agar medium, where the same (Swab) was used, marking the location of the lobby, the source of the isolate, and the date in a planned manner and then passing it on. The petri plates were incubated on the medium at 37 °C for 24 hours. After this, the pure colonies were cultivated on the diagnostic medium Hicrom<sup>TM</sup>

Acinetobacter Agar Base using the loop streaking method. The dishes were then incubated again at 37 °C for 24 hours, after which the results were obtained. Pure colonies with distinctive colors were then phenotypic and microscopic, and diagnosis was performed on the isolates using the Vitek 2 compact device. A confirmatory drug sensitivity test was performed using the Vitek 2 compact device, and molecular diagnosis was performed at the Bayan National Laboratory. The pure isolates were then preserved by growing these isolates on a Nutrient Agar medium by taking a portion of the pure colonies that were similar in appearance and growing them on the Hicrom<sup>TM</sup> Acinetobacter Agar Base medium using the Streaking Method. They were obtained as pure colonies after incubating the isolates at 37 °C for 24 hours.

### Genomic DNA Isolation from Bacterial Cultures

The DNA extraction was carried out using the Mini gDNA Bacteria Kit protocol. 200 µL of the bacterial suspension was transferred into a 15 mL centrifuge tube, and 0.8 mg of lysozyme was added and vortexed thoroughly to ensure complete dissolution. The mixture was incubated at 37 °C for 30 minutes, with gentle inversion of the tube every 10 minutes. After incubation, the mixture was vortexed again, and 20 µL of proteinase K was added. The tube was then incubated at 60 °C for 10 minutes, with gentle inversion every 3 minutes. For lysis, 200 µL of GB Buffer was added to the sample and mixed by vortexing for 10 seconds. The mixture was incubated at 70 °C for 10 minutes, with inversion of the tube every 3 minutes. Concurrently, 200 µL of each sample was preheated to 70 °C for the DNA elution step. 200 µL of absolute ethanol was then added to the lysate sample and mixed immediately by vigorous shaking. The GD column was placed into a 2 mL collection tube, and the mixture, including any insoluble precipitate, was transferred to the GD column and centrifuged at 14,000 x g for 2 minutes. The flow-through was discarded, and the GD column was placed in a new 2 mL collection tube. For the wash step, 400 µL of W1 Buffer was added to the GD column, which was then centrifuged at 14,000 x g for 30 seconds, and the flow-through was discarded. The GD column was placed back into the 2 mL collection tube, and 600 µL of Washing Buffer was added. The column was centrifuged at 14,000 x g for 30 seconds, and the flow-through was discarded. The GD column was placed back into the 2 mL collection tube and centrifuged again at 14,000 x g for 3 minutes to dry the column matrix. For the elution step, the dried GD column was transferred to a clean 1.5-mL microcentrifuge tube, and 100 µL of pre-warmed eluate was added to the center of the column matrix. The column was allowed to stand for at least 3 minutes, and then it was centrifuged at 14,000 x g for 30 seconds to elute the purified DNA.

### Conventional PCR

Primer	Sequence
Forward Primer	AGAGTTTGATCCTGGCTCAG
Reverse Primer	AAGGAGGTGATCCAGCCGCA

**PCR preparation:** For each sample, the PCR reaction was performed in 0.2 ml tubes with a final volume of 25 µl by adding the following.

Material	Quantity
Master Mix	µl 5.25
Forward Primer	µ 5
Reverse Primer	µl 5
Sample DNA	µl 3
Nuclease free water	µl 725
Final volume	µl.5

**PCR program:** The following program was used for PCR amplification.

	Step	Temperature	Duration	Cycles
1.	Initial Denaturation	95 °C	5 minutes	x1
2.	Denaturation	95 °C	30 minutes	x35
	Annealing	62 °C	30 minutes	
	Extension	72 °C	45 minutes	
3.	Final Extension	72 °C	5 minutes	x1

### Gel electrophoresis

1% agarose gels were prepared by mixing 30 ml of 1x TBE buffer with 0.3 g agarose in a beaker, boiling it in the microwave, and allowing it to cool before adding 0.3 µl ethidium bromide and then pouring it into the solidification mold. 7 µL of the ladder and each sample were injected into corresponding wells within the electrophoresis reservoir. Electrophoresis was performed for 45 minutes by applying a voltage of 80 V. Then, the gel was photographed under UV light.

### Preparation of an alcoholic extract of pomegranate peels

Pomegranate peels were purchased from the herbalist in Basra, ground, weighed 200 grams of the plant, placed in a cellulose thimble, and extracted with a Soxhlet extraction device using 70% methanol as a solvent. An extraction process was conducted for 24 hours, after which the solution was concentrated using a rotary evaporator device, where the solvent separated. Then, dry the extract by placing it in a petri dish.

### Preparation of an aqueous extract of pomegranate peels

5 g of pomegranate peels were thoroughly washed and crushed. They were then combined with 100 ml of deionized distilled water in a 500 ml glass beaker and boiled for 10 minutes. Subsequently, the mixture was filtered through No. 1 to obtain the extract.

### Testing the effectiveness of plant extracts against the bacteria under study

The inhibitory effect of the extracts on tested bacteria under study was assessed using the well agar diffusion method, which has been demonstrated to be efficient and easy to execute (Badi *et al.*, 2016) [5]. Instead of paper discs, wells were created using a sterile cork perforator with a diameter of 6 mm (Muller Hinton). The bacterial suspension, containing 1.5 x 10<sup>8</sup> cells/ml, was spread onto the agar medium using the streaking method. Subsequently, 0.1 ml of each concentration of the plant extract was added into the wells, with distilled water used as a negative control. Following the incubation period, the inhibition zones was measured both vertically and horizontally, and the average of these measurements was calculated. Each experiment was repeated three times. To assess bacterial sensitivity to the plant extracts, nutrient agar

plates were inoculated with 1.0 ml of bacterial suspension and left at room temperature for 15 minutes for the inoculum to absorb. Holes were then made in the nutrient agar using a sterile cork drill with a diameter of 7 mm. Using a fine pipette, 50 microliters of each concentration of the plant extracts were transferred into the holes. Control plates were prepared by adding 50 microliters of sterile distilled water into the holes instead of the plant extract. The plates were subsequently incubated at 37°C for 18-24 hours. Subsequent to the incubation period, the zones of microbial growth inhibition, characterized by the absence of bacterial colonies surrounding the wells, were quantified by measuring their diameters using a calibrated ruler.

#### Measure Minimum inhibitory concentration

The inhibitory effectiveness of the plant extracts under study was compared with each standard antibiotic. Serial dilutions of the extracts were prepared to assess their impact on bacterial growth. Starting with an initial concentration of 200 mg/ml, 1 ml of the alcoholic extract was diluted with 1 ml of DMSO to achieve a 100 mg/ml concentration. This process was repeated to obtain a series of decreasing concentrations: 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, and finally 1.5 mg/ml. Each of these concentrations was then tested for their inhibitory effects on the bacterial cultures.

#### Gas chromatography-mass spectrometry (GC-MS)

GC-MS finds widespread applications in the pharmaceutical industry, including research and development, quality control, quality assurance, manufacturing, and pilot plant operations

for active pharmaceutical ingredients (APIs). It plays crucial roles in the development of analytical procedures and methods, as well as in the detection and identification of API impurities. Moreover, various aspects of pharmaceutical analysis, such as stability testing, impurity profiling, pharmacology, process control, biotechnology, and synthetic/analytical characterization of medicinal compounds, heavily rely on GC-MS techniques.

In this study, 20 grams of plant powder was immersed in methanol and subjected to a specific temperature program in an oven. Initially, the sample was heated to a maximum temperature of 300 °C with slow fan speed for 1 minute, followed by heating at 60 °C for 10 minutes. Subsequently, the temperature was reduced to 10 °C for 1 minute, and finally, it was heated again to 280 °C for 3 minutes. The total run time for the process was 35 minutes. The samples were analyzed using GC- MS at the Nahran Omar Center in the northern region of Basra Governorate.

#### Results

279 samples were collected (swabs, blood) from three Basra Governorate hospitals: 91 samples from Al-Zubair Hospital, 76 samples from Al-Fayhaa Hospital, and 112 samples from Al-Sadr Teaching Hospital. The samples were cultured on MacConkey agar medium, and the results of culturing the samples were as follows: 138 (49%) were negative for growth on MacConkey agar, and 141 (51%) were positive for growth on MacConkey agar, including 88 (62%) positive samples for growth on High Chrom Agar.

**Table 1:** Shows the sources of the isolates and the number of samples obtained

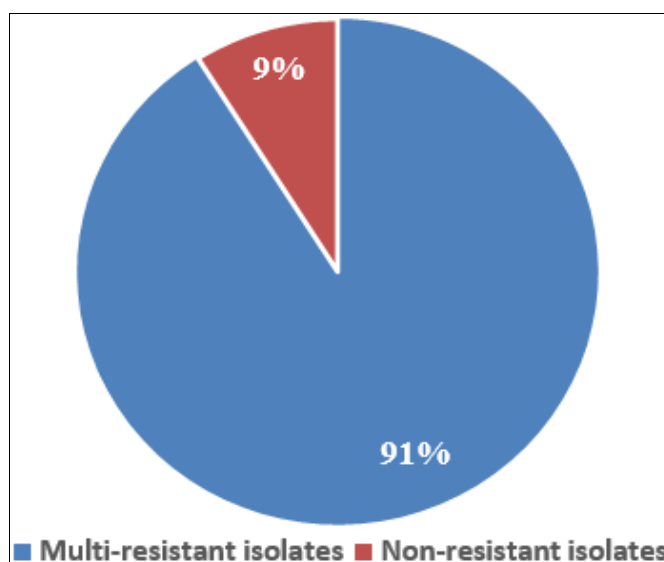
Sample Source	Number of samples	Percentage	Number of isolates	Percentage
Wounds	561	%57	51	%6
Burns	88	%3.	1	%1
Blood	35	%55	5	%3
Total	.79	%511	55	%4

#### Diagnosis of *Acinetobacter baumannii*

##### Microscopic examination (Gram stain)

Gram-negative bacteria appears in different forms, such as single or double coccobacilli. When grown on HiCrom Agar medium, the colonies appear purple and are negative for the oxidase test and positive for the catalase test.

**Diagnosis of bacteria using the Vitik device:** The bacteria diagnosis was confirmed with the Vitek device, as 38 isolates were entered into the device, and the device performed a set of biochemical tests. The results showed variation in bacterial species and species, as 11 isolates were obtained of bacteria under study that are multi-resistant to antibiotics, giving 29% of the total isolates entered to test the device Alfaitak.



**Fig 1:** Shows the percentage of isolates resistant to antibiotics

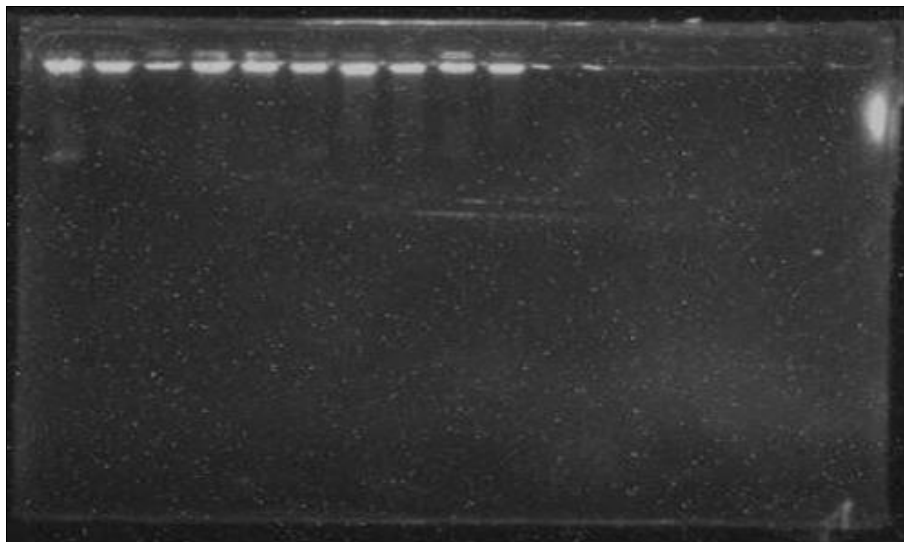
**Table 2:** Shows the results of the Vitek test

Organism	Antibiotic	Susceptibility	MIC mg/L
<i>Acinetobacter</i>	Ticarcillin	R	≤5.8
<i>baumannii</i>	Ticarcillin/Clavulanic acid	R	≤5.8
	Piperacillin	R	≤5.8
	Piperacillin/Tazobactam	R	≤5.8
	Ceftazidime	R	≤64
	Cefepime	R	≤64
	Aztreonam	R	≤56
	Imipenem	R	≤56
	Meropenem	R	≤56
	Amikacin	R	≤56
	Gentamicin	R	≤56
	Tobramycin	R	≤4
	Pefloxacin	R	≤4
	Minocycline	S	≥5
	Colistin	S	≥0.5
Trimethoprim/Sulfamethoxazole	R	≤3.	
<i>Acinetobacter</i>	<i>Ampicillin/Sulbactam</i>	R	56
<i>baumannii</i>	<i>Piperacillin/Tazobactam</i>	R	≤5.8
	<i>Cefotaxime</i>	R	≤64
	<i>Ceftazidime</i>	R	3.
	<i>Cefepime</i>	R	≤3.
	<i>Imipenem</i>	R	≤56
	<i>Meropenem</i>	R	≤56
	<i>Amikacin</i>	R	≤64
	<i>Gentamicin</i>	R	≤56
	<i>Ciprofloxacin</i>	R	≤4
	<i>Colistin</i>	S	≥0.5
<i>Trimethoprim/Sulfamethoxazole</i>	R	≤3.	
Organism	Antibiotic	MIC (mg/L)	Susceptibility
<i>Acinetobacter baumannii</i>	Amikacin	≤16	R
	Amikacin	≤64	R
	Ampicillin/Sulbactam	16	R
	Aztreonam	≤16	R
	Cefepime	≤64	R
	Cefepime	≤32	R
	Cefotaxime	≤64	R
	Ceftazidime	≤64	R
	Ceftazidime	32	R
	Ciprofloxacin	≤4	R
	Colistin	≥0.5	S
	Colistin	≥0.5	S
	Gentamicin	≤16	R
	Gentamicin	≤16	R
	Imipenem	≤16	R
	Imipenem	≤16	R
	Minocycline	≥1	S
	Meropenem	≤16	R
	Meropenem	≤16	R
	Pefloxacin	≤4	R
	Piperacillin	≤128	R
	Piperacillin/Tazobactam	≤128	R
	Piperacillin/Tazobactam	≤128	R
	Ticarcillin	≤128	R
	Ticarcillin/Clavulanic acid	≤128	R
	Tobramycin	≤4	R
Trimethoprim/Sulfamethoxazole	≤32	R	
Trimethoprim/Sulfamethoxazole	≤32	R	

**Molecular diagnosis**

**Bacterial diagnosis by the 16S rRNA gene:** The results of amplifying the 16s RNA gene for the isolated samples electrophoresed on an agarose gel at a rate of 100%, and the size of the pb gene was 1500, showed that they possessed this

gene. Using a polymerase chain reaction device, the results showed the appearance of a single band for the bacterial isolates, and the molecular weight of the resulting bands was similar, which was 1500 base pairs about the molecular weight of the 16S gene. RNA, as shown in Figure 3.

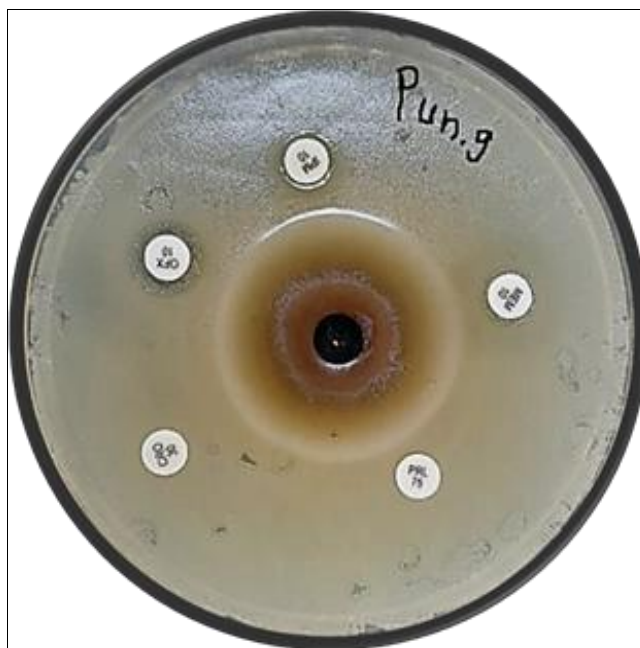


**Fig 2:** Shows the results of electrophoresis

### Results of testing the effectiveness of plant extracts against the bacteria under study

The current study showed the effectiveness of pomegranate peels alcoholic extracts against the bacteria under study, as shown in Figure 4, which shows the zone of inhibition. The maximum zone of inhibition was 28 mm, followed by 27.5, and the next zone was 27 mm. The average zone of inhibition

was  $0.57 + 27.5$  mm. Table No. 3 also shows the results of chemicals detection of the active compounds and groups in the alcoholic extract of pomegranate peels that were detected, namely: 2,5-Furandione, 3-methyl, 5-Hydroxymethylfurfural, 1,3-Propanediol, 2-(Hydroxymethyl)- 2-nitro-, alpha. -Methyl-1-sorboside. The minimum inhibitory concentration was 25%.



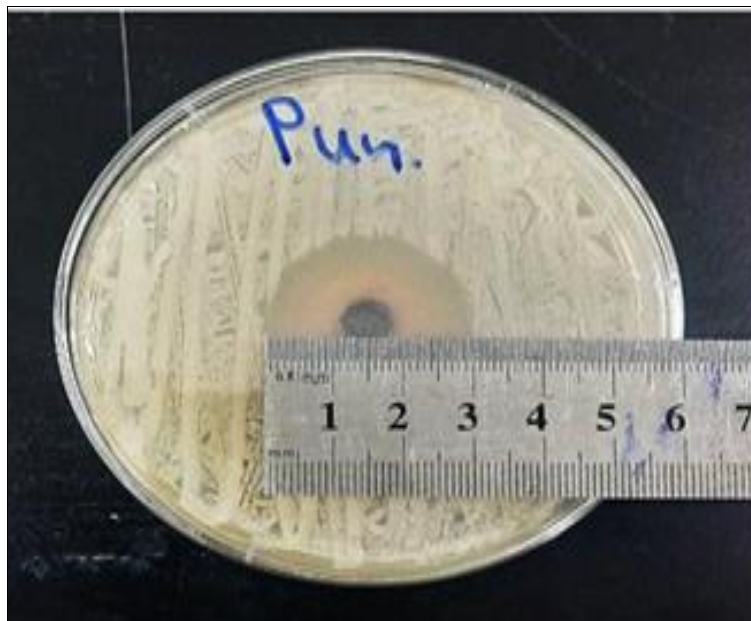
**Fig 3:** Shows the inhibition zone of the of pomegranate peels alcoholic extract

**Table 3:** Active plant substances identified in the of pomegranate peels alcoholic extract

S. No.	RT	Area %	Name of compound	Molecular formula	MW (g/mol)
1.	8.8857	146346	2,5-Furandione, 3-methyl-	C <sub>5</sub> H <sub>4</sub> O <sub>3</sub>	55.21835
2.	14.2035	81199	5-Hydroxymethylfurfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	5.6255
3.	16.8704	408555	1,3-Propanediol, 2-(Hydroxymethyl)-2-nitro-	C <sub>4</sub> H <sub>9</sub> NO <sub>5</sub>	5552558
4.	19.0973	237276	alpha.-Methyl-1-sorboside	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	55152.385

The current study showed the effectiveness of the of pomegranate peels aqueous extract against the bacteria under study, as shown in Figure 5, which shows the zone of inhibition. The maximum zone of inhibition was 30 mm, followed by 29, and the next zone was 29 mm, and the average zone of inhibition was  $29.3 + 0.57$  mm. Table No. 4

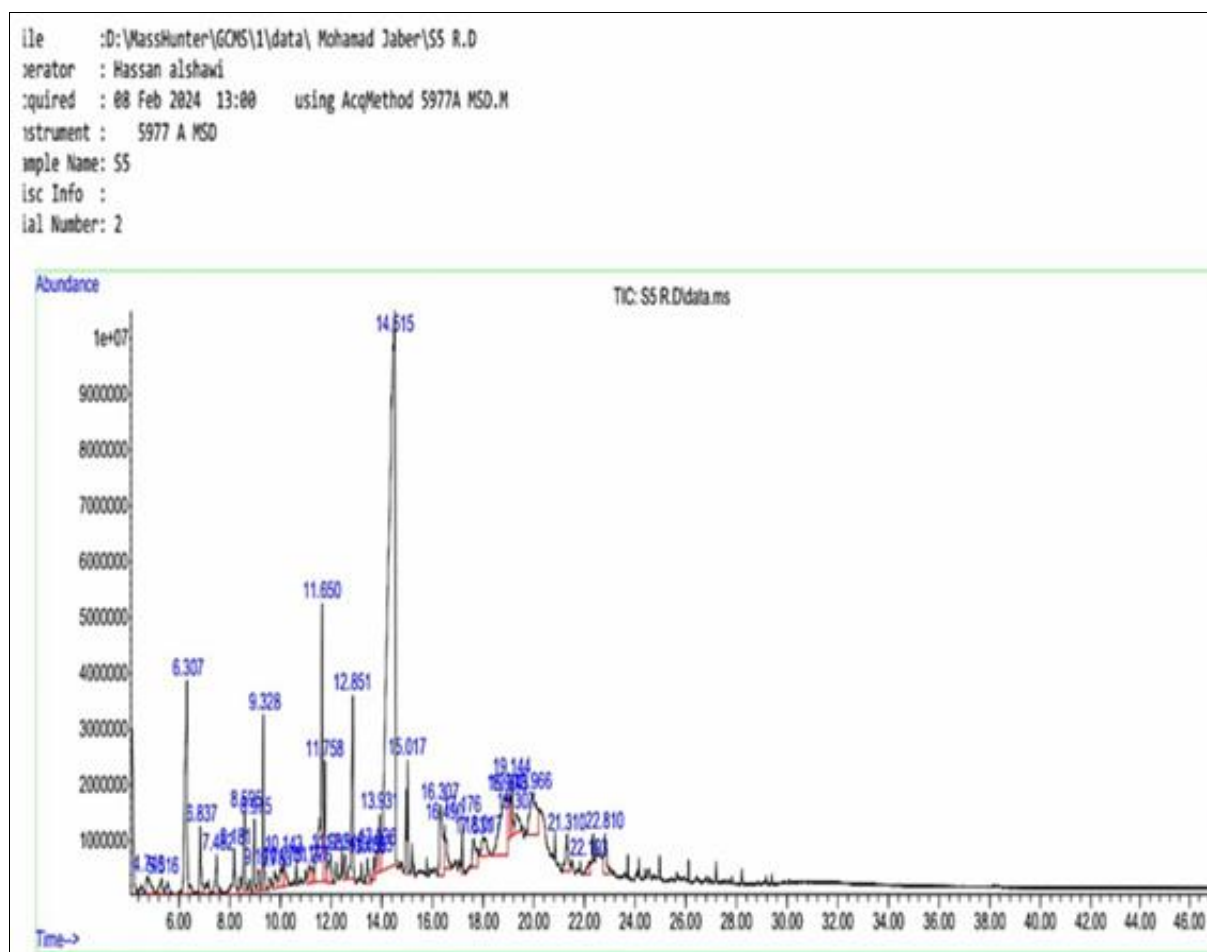
also shows the results of chemical detection of the active compounds and groups in the alcoholic extract of pomegranate peels that were detected, namely 5-Hydroxymethylfurfural, Furfural, 2,5-Furandicarboxaldehyde, and 2-Butyn-1-al diethyl acetal. The minimum inhibitory concentration was 12.5%.



**Fig 4:** Shows the diameter of inhibition of the of pomegranate peels aqueous extract

**Table 4:** Shows the active compounds of the of pomegranate peels aqueous extract

S. No	RT	Area %	Name of compound	Molecular formela	WM (g/mol)
1.	14.5141	106513623	5-Hydroxymethylfurfural	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126.11
2.	6.3109	17050398	Furfural	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	96.08
3.	11.6489	17254960	2,5-Furandicarboxaldehyde	C <sub>6</sub> H <sub>4</sub> O <sub>3</sub>	124.09
4.	14.3811	63122862	2-Butyn-1-al diethyl acetal	C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>	142.2



**Fig 5:** Shows the results of GC-MS of pomegranate peels aqueous extract

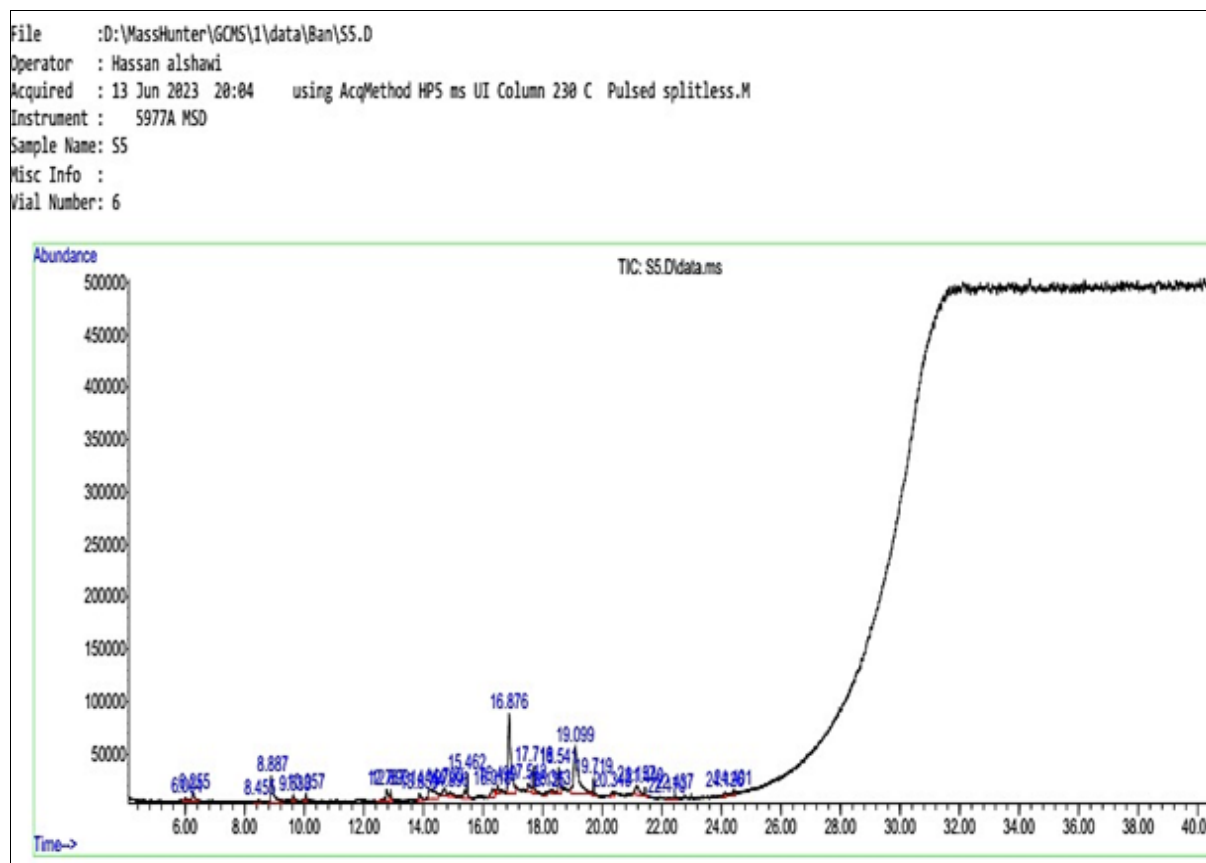


Fig 6: Shows the compounds found in the pomegranate peels alcoholic extract

### Results of statistical analysis

The T-test program statistical analysis show a significant difference in inhibition rate between the aqueous and alcoholic extracts of pomegranate peels. This significance was observed at a significance level of  $p \geq 0.5$ , with a calculated P value of 0.1.

### Discussion

Plant extracts are the active chemical substances found in plants. They are mostly secondary metabolic products and may be in the form of aqueous or alcoholic extracts, oils, powders, or any other form from which the active substance can be utilized. The preparation process for the extraction process is the first and most important stage in obtaining the highest concentration of the active substance. Natural plants have been an important source of many pharmaceutical substances since ancient times and to this day. People use them to treat many diseases. This is because it contains a large number of bioactive compounds. Pomegranate, the fruit of the pomegranate tree *Punica granatum*, is one of those plants of medicinal importance. Its original homeland is southwest Asia, or Carthage, and its cultivation is widespread commercially in most western countries, northwest India, and even North Africa. It has become clear that the pomegranate plant, *Punica granatum* L. (Pomegranate), which belongs to the pomegranate family, Punicaceae, has many medicinal benefits and therapeutic properties, whether the fruit as a whole or its seeds and even its peels. It has been shown that peels treat internal diseases such as diarrhea because they contain tanning substances for the stomach. The bodies of the fruit and seeds are also considered to strengthen the heart and stomach. As for the juice of this plant, it is very rich in vitamins and minerals necessary for the body. During the research, it became clear that the active substances in the pomegranate plant are alkaloids, the most important of which

are gall tannin, pelerine granatin, and a bitter substance called punitive. The benefits of pomegranate peels do not stop there. It has only been shown that they are one of the most powerful drugs that kill worms and treat amoebic dysentery. It has been noted that the outer peel of the pomegranate fruit contains tannic acid, an astringent, and its powder is an excellent antidote to treat diarrhea. Boiling peels work as repellents for worms, especially tapeworms, because they contain many alkaloids, including pelletierine. The substance contained various alkaloids, including N-ethyl, methylisopellelerine, ethylpelleticrin, and isopellefierine pseudopelleticrin, alongside other compounds. Numerous studies have highlighted pomegranate fruit's lethal and inhibitory effects on the growth of Gram-negative and Gram-positive microbes. For instance, a study by Baraka *et al.* (2023) [6] focused on determining the antibacterial properties of *Punica granatum* peels, which are rich in biologically active compounds such as polyphenols, tannins, flavonoids, and anthocyanins (Cyanidin and delphinidin), as supported by prior research. The peel extracts exhibit antibacterial activity against pathogens like *Klebsiella pneumoniae* and *E. coli*. Interestingly, it was observed in the current study that the effectiveness of the alcoholic extract is comparatively lower than that of the aqueous extract, consistent with findings from other researchers (Piyawan *et al.*, 2005; Thankare, M., 2004) [14, 15]. This difference in effectiveness is attributed to the higher solubility of active substances in water compared to alcohol, thus enhancing the efficacy of the aqueous extract. The inhibitory efficacy of pomegranate peel extract is attributed to the presence of flavones, which are known to disrupt cell membranes by forming complexes with extracellular proteins. Phenols also enhance the activation of enzymes responsible for essential metabolic reactions through their interaction with proteins, ultimately leading to their denaturation and halting bacterial growth. Additionally, alkaloids interfere with

bacterial DNA, further inhibiting their growth. These findings align with previous studies, such as by Cruz-Valenzuela *et al.* (2022) <sup>[7]</sup>, which demonstrated the effectiveness of pomegranate peels aqueous extract against microbial growth. Such results suggest the effectiveness of pomegranate peel extract in developing new, safe, broad-spectrum natural antimicrobials and antioxidants for food applications. Furthermore, studies by Alexandre *et al.* (2019) <sup>[3]</sup> and Abdel-Aziz *et al.* (2021) <sup>[1]</sup> showed selective antimicrobial activity of pomegranate peel extracts against pathogenic bacteria without affecting beneficial bacteria. These high-pressure extracted extracts can be used as a source of bioactive compounds for applications, including antioxidants and antimicrobials. Moreover, studies like the one by Al-Defiery *et al.* (2021) <sup>[2]</sup> indicate the antimicrobial potential of garlic extracts and pomegranate peels against disease-causing bacteria. While garlic extract exhibits more potent antibacterial activity than pomegranate peel extract, the effectiveness of the extract depends on the plant type and the solvent used for extraction. Most previous studies have utilized alcoholic extracts of pomegranates, which can extract a wider range of compounds than aqueous extracts. This could explain why alcoholic extracts are generally more effective than aqueous extracts (AlHumndu & Farj, 2010) <sup>[4]</sup>.

### Conclusion

This study demonstrates the promising antibacterial efficacy of aqueous and methanolic extracts of pomegranate peels against multidrug-resistant *A. baumannii* strains isolated from intensive care patients in hospitals in Basra Governorate, Iraq. The aqueous extract exhibited superior activity to the methanolic extract, with maximum inhibition zones of 30 mm and 28 mm, respectively. The MICs of the aqueous and methanolic extracts were 12.5% and 25%, respectively. GC-MS analysis show the presence of various phytochemicals, such as furfural, 5-hydroxymethylfurfural, 2, 5-furandicarboxaldehyde, and 2, 5-furandione, 3-methyl-, which may contribute to the antibacterial activity. These findings suggest the effectiveness of pomegranate peel extracts as natural antimicrobial agents for combating multidrug-resistant *A. baumannii* infections. However, further research is necessary to elucidate the mechanisms of action, identify the particular bioactive compounds responsible for the antibacterial activity, and evaluate the therapeutic potential of these extracts in clinical settings.

### References

- Abdel-Aziz SH, El-Esawi MA, Hazaa MM, Abdel-Aziz HY, Hassan MG. Antibacterial potential of pomegranate peel extracts on *Escherichia coli* isolated from Benha hospital in Egypt. *Benha. J Appl. Sci.* 2021;6(3):61-64.
- Al-Defiery ME, Al-Muttairi AK, Saeed HH, Hadi RK. Antimicrobial activity of garlic and pomegranate peel extracts against some pathogenic bacteria. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing. 2021;722(1):012017.
- Alexandre EM, Silva S, Santos SA, Silvestre AJ, Duarte MF, Saraiva JA, *et al.* Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction. *Food Res Int.* 2019;115:167-176.
- Al-Humndu MM, Farj DN. The effect of aqueous and alcoholic extracts of *Punica granatum* L. pericarp on hemolysin production of several bacterial species. *Baghdad Sci. J.* 2010;7(1):309-316.
- Badi JM, Resen HM, Majeed AM. Antimicrobial activity of *Populus euphratica* leaves extract on growth of some gram negative bacteria. *Eng. Technol. J.* 2016, 34(5).
- Baraka DM, Hassan MG, Farouk HS, El Awady ME, Khedr M. Antibacterial activity of pomegranate peel against multidrug resistant bacteria. *Egypt Acad. J Biol. Sci. G Microbiol.* 2023;15(1):217-226.
- Cruz-Valenzuela MR, Ayala-Soto RE, Ayala-Zavala JF, Espinoza-Silva BA, González-Aguilar GA, Martín-Belloso O, *et al.* Pomegranate (*Punica granatum* L.) peel extracts as antimicrobial and antioxidant additives used in alfalfa sprouts. *Foods.* 2022;11(17):2588.
- Davies OL, Bennett S. WHO publishes list of bacteria for which new antibiotics are urgently needed. *WHO Newsletters*; c2017.
- World Health Organization (WHO). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva: WHO; c2017. Available from: [http://www.who.int/medicines/publications/WHO-PPL-Short\\_Summary\\_25FebET\\_NM\\_WHO.pdf](http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25FebET_NM_WHO.pdf). [Last accessed on 2017 Feb 25].
- Jawad A, Seifert H, Snelling AM, Heritage J, Hawkey PM. Survival of *Acinetobacter baumannii* on dry surfaces: comparison of outbreak and sporadic isolates. *J Clin Microbiol.* 1998;36:1938-1941.
- McConnell MJ, Actis L, Pachón J. *Acinetobacter baumannii*: human infections, factors contributing to pathogenesis and animal models. *FEMS Microbiol. Rev.* 2013;37:130-155.
- Peršurić Ž, Saftić Martinović L, Malenica M, Gobin I, Pedisić S, Dragović-Uzelac V, *et al.* Assessment of the biological activity and phenolic composition of ethanol extracts of pomegranate (*Punica granatum* L.) peels. *Molecules.* 2020;25(24):5916.
- Phillipson JD, O'Neill MJ. New leads to treatment of protozoal infection based on natural product molecules. *Acta Pharm Nord.* 1987;1:131-144.
- Piyawan VS, Treechada S, Surasak L, Thanomjit S, Tetsuya I, Takeshi H, *et al.* Inhibitory effects of active compounds from *Punica granatum* pericarp on verocytotoxin production by enterohemorrhagic *Escherichia coli* O157: H7. *J Health Sci.* 2005;51(5):590-596.
- Thankare M. Pharmacological screening of some medicinal plant as antimicrobial and feed additives. [Master's thesis]. Virginia: Virginia Polytechnic Institute and State University; c2004.
- Tuschia H, Stato M, Linum M, Yokoyama J, Ohyama M, Tanaka T, *et al.* Inhibition of the growth of carcinogenic bacteria *in vitro* by plant flavones. *Exp. Med. Phytochem.* 1994;50:846-849. *Medicine. Phytochemistry.* 30:3864-3871.
- World Health Organization (WHO). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics.
- Achmagh AA, *et al.* Anti-biofilm activity of *Nigella sativa* extracts against pathogenic bacteria. *HIV Nurs.* 2023;23(2):804-809.