International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://theaspd.com/index.php

Investigating The Impact Of Melittin On BAP, Gene Expression In Acinetobacter Baumannii Using Real-Time PCR

Doaa Hussein Kadhim Alwohaili[1],Hiyame Abdul Ridha Al-Awade [2]
[1,2]Department of Biology, College of Education for Pure Science, University of Karbala, Iraq
Corresponding author : [1]doaa.hussein@s.uokerbala.edu.iq

Abstract

Background: *Due to increasing antibiotic resistance to A.baumannii, there is an urgent need to find new antibiotic alternatives or supporters for the treatment of disease-causing pathogens. For the past 30 years, strains of* A. baumannii *have acquired resistance to newly developed antimicrobial drugs.Therefore, melittin was used as an experimental treatment, Melittin, considered to be an antimicrobial, antitumor, and anti-inflammatory peptide.*

Objective: *The objective of this study was to investigation the effectiveness melittin extract from bee venom against A. baumannii through genetic expression of the Bap genes by using a qRT-PCR and detection biofilm formation by quantitative assay via Micro-titer plate method in Karbala city.*

Methods:*Two hundred different Clinical specimens were collected from various sources from patient who admitted to Imam Hussein Medical City, the study beginning from the period January 2024 till the August 2024. The specimens which included sputum, wound, urine, blood and fluid and all specimens was collected as 40 cases.*
Melittin was extracted from local bee venom via HPLC and detection of the standard compound and the isolated compound was performed, along with FTIR spectrum analysis. Detection biofilm formation before and after melittin treatment by quantitative assay via Micro-titer plate method divided into three groups(strong biofilm, moderate biofilm and weak biofilm). Also done estimating the effectiveness of melittin extract as anti-bacterial through genetic expression of the Bap gene by using qRT-PCR.

Results: *The collection specimens from patients included 40(20%) positive specimens distributed as 25 (62.5%) from females, and 15 (37.5%) from males and they were divided into: 17(42.5%) sputum, 10(25%) wound, 7(17.5%) urine,5(12.5%) blood ,1(2.5%) fluid specimens. After cultured on Blood agar and MacConkey agar, the isolates were identified via VITIK 2 compact system (Biomérieux, France). All isolates were tested for their resistance to 18 different antibiotics and the results exhibited that highest level of resistance in A.baumannii isolates to total antibiotics used in this study except Minocycline, Colistin and Tigecycline . Our study showed the Colistin, Minocycline and Tigecycline were sensitive in the rate36 (90%), 35 (87.5)and 30(75%) respectively. After melittin treatment The absorbance value for strong biofilm formation decreased to 52%, absorbance value for moderate biofilm formation was reduced to 55% indicating a substantial decrease in biofilm density and absorbance value for weak biofilm formation was recorded 24% showing a slight reduction compared to the pre-treatment measurements. The result of Bap gene expression exhibited strong biofilm formation 77% ,moderate biofilm formation 57% and weak biofilm formation 74%.*

Conclusion *: The study concluded that the most common cases of infection with A.baumannii bacteria in sputum, and that this bacteria has a high resistance to most antibiotics.*

Keywords*: Acinetobacter baumannii, Clinical Specimens, Biofilm, Melittin, Bap gene, Gene Expression, Real Time-PCR.*

INTRODUCTION

Melittin as a highly potent antibacterial agent may have a good synergistic effect on killing the bacteria and, also, inhibiting biofilm formation (Picoli *et al.*,2017). A series of recent studies indicated that melittin has a wide range of bactericidal activity against susceptible and resistant bacteria(Lima *et al.*,2020). Melittin constitutes approximately 40–60% of the dry weight of the bee venom(BV), It is the main component of BV (Wehbe *et al.*,2019). Melittin consists of 26 amino acid polypeptide with a chemical formula $C_{131}H_{229}N_{39}O_{31}$, the N-terminal region is mainly hydrophobic due to +4 charges while the C-terminal region is hydrophilic because of +2 charges hence the total is +6 charges at physiological pH, molecular weight, 2846.46266 g/mol (Raghuraman *et al.*,2007). Melittin amphipathic nature arises from its hydrophilic carboxyl-terminal region, responsible for its lytic activity, and its hydrophobic N-terminal region, which lacks lytic activity, making it an amphipathic molecule [Dempsey,1990]. This amphipathic

property enables Melittin to disrupt both prokaryotic and eukaryotic cells, as well as natural and synthetic phospholipid bilayers, via the process of pore formation [Wehbe *et al.*,2019]. The emanation of bacteria that are resistant to antibiotics has complicated efforts to treat infectious diseases. In light of this, efforts to find novel sources of antibiotic compounds have increased researchers are therefore scouring the natural world for compounds with potential as anti-infective therapy (Bazaid *et al.*,2022).

A.baumannii One of the public health threats that have recently been considered in the United States, Europe, Asia, and the Middle East is the rapid increase in the antibiotic-resistant isolates of *A. baumannii* (Luo,2015). *A. baumannii* Possessing various virulence factors, like the ability to create a biofilms (slimy layer) and survive in an exsiccated ambience situation, has caused difficulties in the health care setting, particularly for patients in intensive care units of hospitals [Bamunuarachchi,2021] Biofilms are complex bacterial communities attached to surfaces, created by an extracellular matrix produced by bacteria. This matrix comprises polysaccharides, DNA, and proteins (Pakharukova *et al.*,2018). Among the diverse factors effective in biofilm formation, the biofilm-associated protein *Bap*, high-molecular-weight proteins encoded by the bap (Azizi *et al.*,2016). This protein is located on the outer surface of bacteria and consists of a central core of the successive iterations of similar sequences (Brossard and Campagnari,2012)Disruption of the bap gene reduces the thickness and volume of biofilm and interbacterial cell adhesion (Loehfelm *et al.*,2008)The biofilm production capabilities of *A. baumannii*-associated infections are counted among the many important causes of drug resistance, and this biofilm formation is linked to quorum sensing (QS) (Elshaer *et al.*,2022)

Our study aimed to evaluate of melittin against strong biofilm producer by MDR *A.baumannii* isolates and biofilm inhibitory.

2.MATERIALS AND METHODS

2.1. Ethical Approval

Before the specimen was collected, written permission was obtained from each study patients, and all subjects involved in this experiment were informed. The university of Kerbala ,College of Education for Pure Science Ethics Committee gave its approval to this study under No. 3434, in 25/12/ 2023..

2.2. Study Design

A cross-sectional study from 40 cases were collected from different types of specimens obtained from 200 clinical cases as total which Includes wounds ,urine ,sputum, fluid and blood of infections from both male and female in different ages, the study beginning between January 2024 to August 2024.

Melittin was extracted from local bee venom via HPLC and detection of the standard compound and the isolated compound was performed, along with FTIR spectrum analysis. Detection biofilm formation before and after melittin treatment by quantitative assay via Micro-titer plate method divided into three groups(strong biofilm, moderate biofilm and weak biofilm). Also done estimating the effectiveness of melittin extract as anti-bacterial through genetic expression of the *Bap* gene by using qRT-PCR.

2.3. Clinical Specimens

All Specimens collection was directly transferred to the laboratory and inoculation onto suitable culture media (5% human blood agar, MacConkey agar and brain heart infusion (BHI) broth(HiMedia, India)by using streaking method ,the samples were cultured aerobically at 37°C for duration of 24 hrs, after incubation period, the growth was examined daily.

2.4. Inclusion and Exclusion criteria

The inclusion criteria filled out by the patients participating in our study and included knowledge of their age, gender, symptoms**,** while the exclusion criteria for persons with Cancer and smokers.

2.5. Identification of bacterial Isolates

The isolates from pure colonies was phenotypically identified based on morphological, cultural, and biochemical properties by using gram negative card (GN) cards (ID) of the VITEK 2 system (Biomérieux, France), Antibacterial sensitivity testing on isolated bacteria was also accomplished using the compact automated system VITEK 2 (Ha *et al.*, 2018).

2.6. Antibiotics susceptibility test

Antibiotics susceptibility testing (AST) for isolates was determines the susceptibility to a group of antibiotics. The cards were laden into the VITEK 2 compact system automatic reader-incubator afterward being inoculated via card (AST). Used turbidity meter to make sure the number and density of microorganisms inoculated into the VITEK 2 cards were right (Bazzi *et al.*, 2017).

2.7. Evaluation of *bap* Gene Expression by Real-time PCR

RNA was extracted from *A. baumannii* isolates to study the expression of the *bap* gene. RNA extraction was performed according to the manufacturer's instructions (Macrogen Co., Korea) .The concentration of used RNA was considered to be about 1 - 2 µg. For this purpose, light absorption was measured at a wavelength of 260 nm. Moreover, light absorption at 280/260 nm was assessed to ensure the lack of protein contamination, and light absorption at 260/230 nm was measured to ensure the lack of salt contamination. The cDNA was synthesized from the extracted RNA after DNase I treatment. DNA gyrase A was used as an internal control to study the expression of the *bap* gene. The sequences of the forward and reverse primer pairs of the two *bap* and 16 SRNA Housekeeping gene are shown in table 1. The Real-Time PCR components and conditions are presented in table 2.

One Step Real-Time PCR Procedure

One-step real-time PCR is a widely used method for quantifying gene expression. It involves two main ways in one step:

1. Reverse Transcription (cDNA Synthesis) – Converts RNA into complementary DNA (cDNA).
2. Quantitative PCR (qPCR) – Amplifies and quantifies the cDNA using a Add AddScript Taq Enzyme.

Reverse Transcription (cDNA Synthesis)

Table (1):The sequences of the forward and reverse primer pairs of the two Housekeeping gene and *bap*

Specific Primers Sequences (5' to 3')			
16 SRNA Housekeeping gene		**Size**	**References**
F	5-CAGCTCGTGTCGTGAGATGT-3	150 bp	(Rusul and Suhad,2022)
R	5-CGTAAGGGCCATGATGACTT-3		
bap **Gene of Interest**		121 bp	(Bahador *et al.*,2015)
F	5-TGCAACTAGTGGAATAGCAGCCCA-3		
R	5-TGCTGACAGTGACGTAGAACCACA-3		

Table (2) : Real-Time PCR Components and Conditions

Target Gene	components	Conditions
Bap	Forward primer 1.5 µl Reverse primer 1.5 µl 2.5x Buffer 8 µl 20x Add Script Enzyme Solution 1 µl Nuclease-Free H20 6 µl cDNA 2 µl	Initial denaturation 95℃, 10 min PCR cycling (40 cycles) 95℃, 15 sec 64℃, 1 min Melting analysis 60℃ → 90℃

2.8 Statistical Analyses

The results were analyzed statistically in SPSS version 22 to observe Chi-square and the Probability levels were less than 0.05 was significant ($p < 0.05$).

RESULTS AND DISCUSSION

Result of Melittin Extracted

1-Active compound identification:

100 microliters of the standard was injected according to the conditions specified in the paragraph analysis conditions HPLC, The results showed a peak with a retention time of (4.10 min) ,Which returns to the standard component Melittin as in the figure (1) :

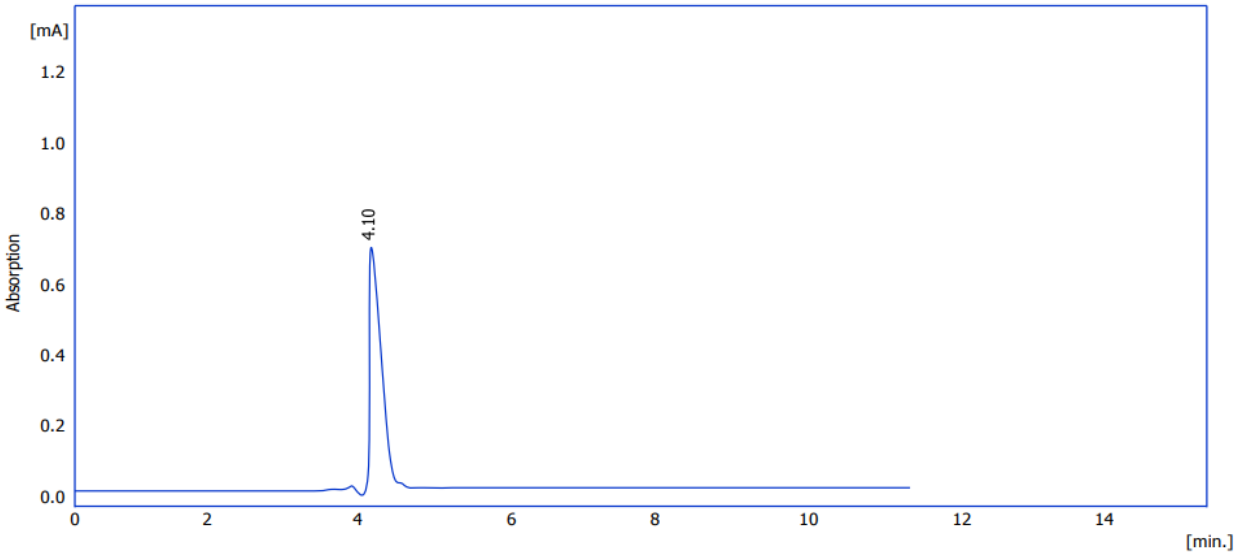

figure (1) The results showed a peak with a retention time (4.10 min) of melittin

Table(1): Result chromatography Table (Uncal - F:\ melittin 5 ppm)

No	Retention Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	Wo5 [min]	Compound Name
1	4.10	1874.98	674.09	100.00	100.00	0.15	Melittin
	Total	1874.98	674.09	100.00	100.00		

100 microliters of the sample BV were injected under the same conditions as the standard compound (Melittin) was injected .The results showed the presence of several peaks. After matching the retention time of the standard compound with the closest time that appeared in the sample, it was found that there was a peak matching the retention time of the standard compound which belongs to the compound Melittin in figure(2).

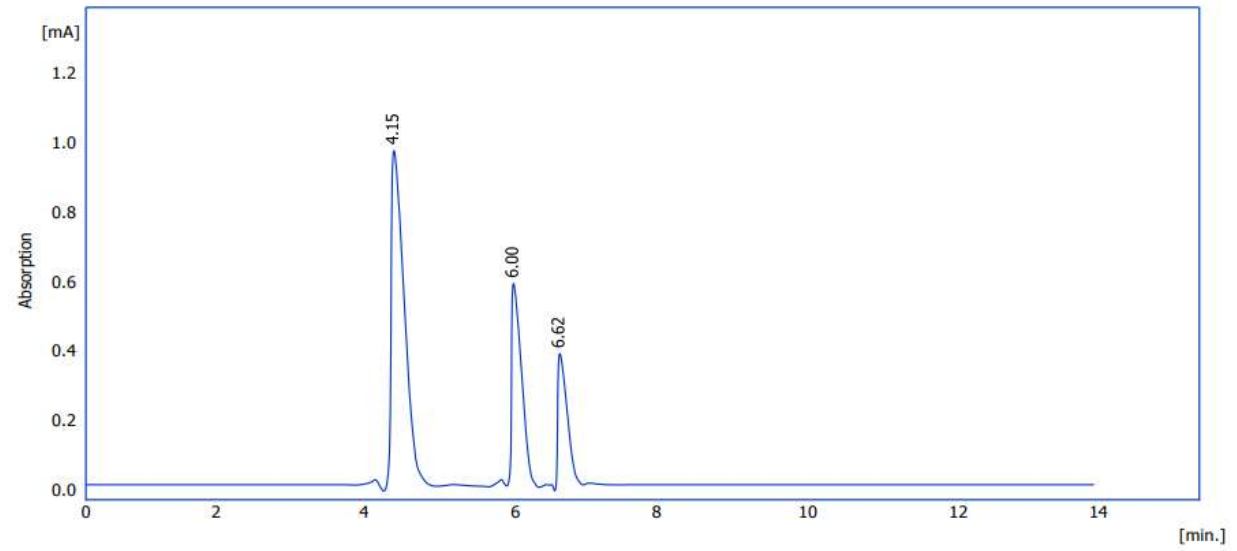

figure (2) peak matching the retention time of the standard compound which belongs to the compound Melittin

Table (3) :Result chromatography Table (Uncal - F:\ sample)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.15	157445.90	985.04	60.00	60.00	0.25	
2	6.00	8541.00	592.11	30.00	30.00	0.15	
3	6.62	2265.09	378.44	10.00	10.00	0.10	
	Total	168521.99	1955.59	100.00	100.00		

After applying the concentration calculation equation, the results showed that the concentration of the compound in the sample is 620 mg / gm.

2-Isolation of Melittin by HPLC :

The Melittin complex was collected using the technique fraction calculator HPLC , After collecting the compound, confirmatory tests were conducted to prove the purity of the compound:

1- The isolated compound was re-injected using HPLC technology and the results showed the presence of a single peak with a retention time 4.17 min, which belongs to the Melittin compound as in figure (3).

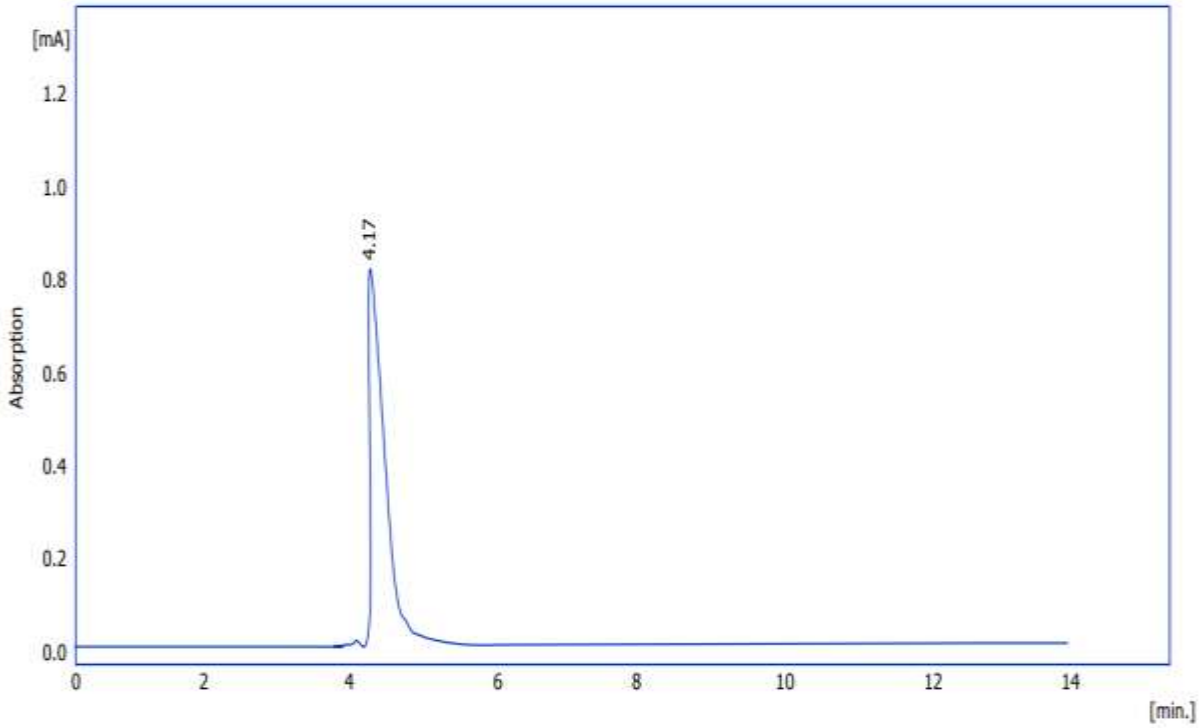

figure (3) : Melittin re-injected using HPLC technology

Table (3) : Result chromatography Table (Uncal - F:\ melittin isolation

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.17	5241.99	800.11	100.00	100.00	0.15	
	Total	5241.99	800.11	100.00	100.00		

2-FTIR spectrum of the standard compound and the isolated compound were performed. The results showed the following figure (4.8):

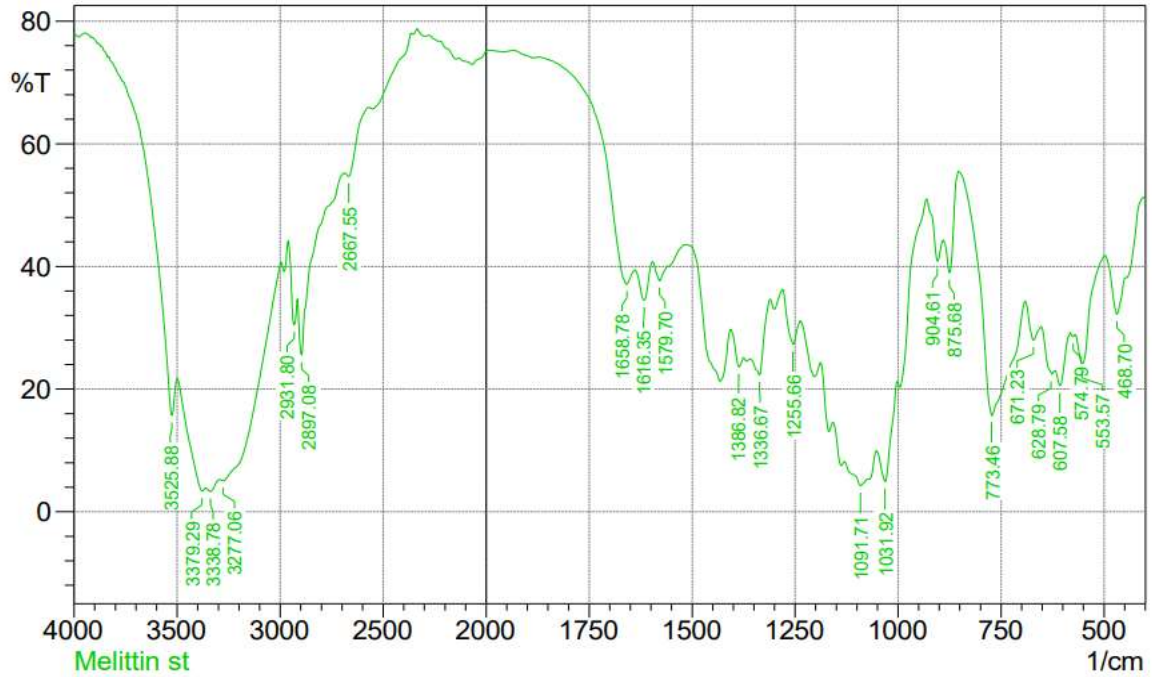

figure (4): FTIR spectrum of the standard compound melittin

Table (4) : FTIR spectrum of the melittin

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	468.7	32.242	7.449	497.63	447.49	22.067	2.035
2	553.57	24.103	7.637	569	499.56	33.945	2.459
3	574.79	28.562	0.439	582.5	570.93	6.259	0.042
4	607.58	20.554	4.508	619.15	584.43	21.76	1.483
5	628.79	22.426	2.34	651.94	621.08	18.776	0.785
6	671.23	27.985	4.12	690.52	653.87	19.231	1.116
7	773.46	15.679	29.253	854.47	692.44	86.144	27.725
8	875.68	38.96	10.292	891.11	856.39	12.156	1.668
9	904.61	40.872	5.655	931.62	891.11	13.884	0.799
10	1031.92	4.924	9.63	1053.13	1001.06	52.699	9.092
11	1091.71	4.251	1.972	1130.29	1074.35	69.902	3.912
12	1255.66	27.296	6.081	1280.73	1236.37	23.032	2.028
13	1336.67	22.286	7.109	1359.82	1309.67	29.007	2.323
14	1386.82	23.621	3.251	1406.11	1375.25	18.104	0.731
15	1579.7	37.664	3.737	1597.06	1519.91	30.377	1.422
16	1616.35	34.487	5.638	1637.56	1597.06	17.401	1.335
17	1658.78	37.113	5.218	1869.02	1639.49	50.982	1.359
18	2667.55	54.679	2.342	2686.84	2571.11	25.532	0.147
19	2897.08	25.543	10.893	2916.37	2688.77	82.194	3.976
20	2931.8	30.483	7.015	2960.73	2918.3	19.403	1.94
21	3277.06	5.086	2.205	3294.42	2993.52	256.63	5.27
22	3338.78	3.297	1.021	3360	3296.35	89.058	3.457
23	3379.29	3.368	2.75	3498.87	3361.93	144.928	4.699
24	3525.88	15.682	9.991	3832.56	3500.8	105.844	4.866

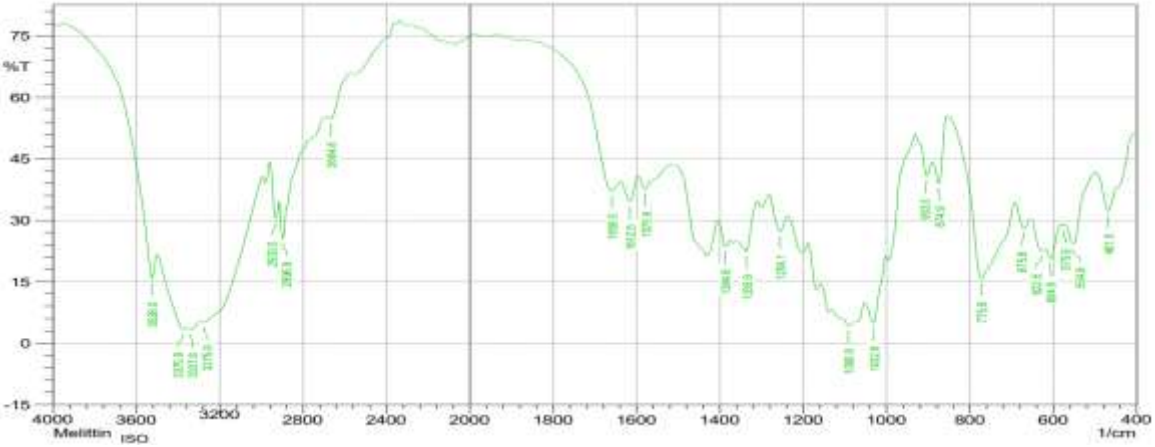

figure (5): FTIR spectrum of the melittin, matching in items of the active groups in the sample (BeeVenom) after comparison with the active groups present in the standard compound (Melittin).

The sample was matching in items of the active groups in the sample (BeeVenom) after comparison with the active groups present in the standard compound (Melittin) aggrement with (Flanjak *et al.*,2021).

Biofilm formation in *Acinetobacter baumannii*

The ability of *A. baumannii* to form biofilms also plays essential role in its pathogenesis, particularly in chronic infections, Biofilms provide a protective environment that shields bacteria from host immune responses and antimicrobial agents, making them highly resistant to eradication (Gedefie *et al.*,2021). *Acinetobacter* infections may be more difficult to treat when forming a biofilm (Pompilio *et al.*,2021)

Biofilm Formation Profiles of *A.baumannii* Isolates before adding Melittin:

The figure (1) depicts the biofilm formation capabilities of the isolated *A.baumannii* samples. Strong biofilm formation was observed in 17(42.5%) isolates. Moderate biofilm formation was classified in 19(47.5%) isolates and Weak biofilm formation was exhibited by only 4(10%) isolates.

The quantitative biofilm biomass was measured using optical density (O.D.) values. Strong biofilm formers had an average O.D. of 0.298, while moderate biofilm formers had an O.D. of 0.233, and weak biofilm formers had an O.D. of 0.121, The use of an ELISA reader at a wavelength of 650 nm for measuring O.D. is appropriate, as it effectively quantifies the biomass of biofilms. The observed differences in O.D. values highlight the variability among isolates in their biofilm-forming capabilities, suggesting that some strains may pose a greater risk in clinical settings due to their enhanced ability to adhere to surfaces and resist eradication figure (6).

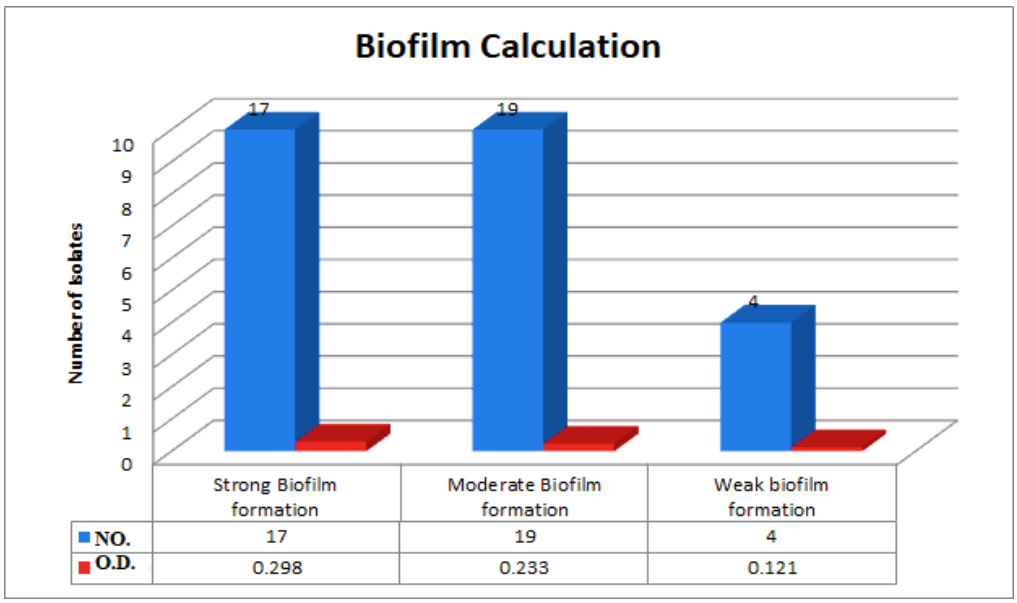

Figure (6) showed Biofilm Formation Profiles of *A.baumannii* Isolates

The results of this study are similar to those of the local study by Al-Mosusawi (2018), but they differ from the findings of Ridha *et al.* (2019), who reported a 94% rate of high-yield biofilm isolates, with a moderate rate of 10%. In a Taiwanese study on clinical isolates of *A. baumannii*, results indicated that among 154 tested isolates, 15.6% were classified as weak biofilm producers, while 32.5% and 45.4% demonstrated moderate and strong biofilm formation abilities, respectively (Yang *et al.*, 2019). These results the importance of biofilm formation in the pathogenicity of *A. baumannii*. The ability to form robust biofilms is associated with increased resistance to antimicrobial agents and enhanced survival in hostile environments, which can complicate treatment efforts (Roy *et al.*, 2022). As a result, infections related to biofilms are more challenging to eliminate and are more likely to relapse.

Biofilm Formation Profiles of *A.baumannii* Isolates after adding Melittin

The biofilm formation capabilities of the *A.baumannii* isolates were assessed prior to and after the addition of the antimicrobial agent melittin. Prior to melittin treatment, strong biofilm formation was observed, with an average optical density of 0.298 ± 0.048. Moderate biofilm formation was classified, exhibiting an average optical density of 0.233 ± 0.02. Weak biofilm formation was also detected, with an average optical density of 0.121 ± 0.011, After the addition of melittin, a component derived from bee venom known for its antimicrobial properties, it would be crucial to evaluate any changes in the optical density measurements. If melittin effectively reduced biofilm formation, this would suggest its potential as a

therapeutic agent against biofilm-related infections. Conversely, the biofilm formation remained high, it may indicate that *A. baumannii* has mechanisms to resist the effects of melittin, highlighting the need for further exploration of its biofilm resilience and potential adaptations (Shi *et al.*, 2022).

Additionally, this study can contribute to understanding the role of melittin in disrupting biofilm structures and its implications for treatment strategies. If melittin proves effective, it could offer a novel approach to managing infections caused by biofilm-forming bacteria, potentially reducing the incidence of treatment failure and relapse.

Following the introduction of melittin, a significant reduction in the biofilm biomass was recorded across all categories of biofilm formation. The optical density of strong biofilm formers was decreased to 0.142 ± 0.048. The optical density of moderate biofilm formers was reduced to 0.103 ± 0.02. The optical density of weak biofilm formers was further decreased to 0.091 ± 0.011.

These results suggest that the antimicrobial compound melittin, derived from bee venom, was effective in disrupting the biofilm formation of *A.baumannii*, regardless of the isolates' inherent biofilm-forming capabilities. This indicates the potential of melittin or similar antimicrobial agents as targeted interventions to mitigate the biofilm-mediated persistence and antimicrobial resistance of this opportunistic pathogen(Lima *and* de Lima.,2023).

Comparative Analysis of Biofilm Formation Before and After Melittin

The results of the absorbance measurements for biofilm formation in *A.baumannii* are presented in table(5), The absorbance values were measured to assess the density of biofilms formed by the bacteria both before and after treatment with melittin, An absorbance value of 0.298 ± 0.048 was observed for strong biofilm formation, indicating a high level of biofilm density. For moderate biofilm formation, an absorbance value of 0.233 ± 0.02 was noted, reflecting a moderate level of biofilm formation. A lower density of biofilm was suggested by an absorbance value of 0.121 ± 0.011 for weak biofilm formation. Following treatment with melittin, a significant reduction in biofilm formation was observed. The absorbance value for strong biofilm formation decreased to 0.142 ± 0.048, representing a reduction of approximately 52%. The absorbance value for moderate biofilm formation was reduced to 0.103 ± 0.02, representing 55% indicating a substantial decrease in biofilm density. The absorbance value for weak biofilm formation was recorded at 0.091 ± 0.011, representing 24% showing a slight reduction compared to the pre-treatment measurements.

Table(5): Comparative analysis of biofilm formation before and after melittin

Types of Biofilm formation	Absorbance of Biofilm Before adding Melittin Mean ±SD	Absorbance of Biofilm after adding Melittin Mean ±SD
Strong Biofilm formation	**0.298± 0.048**	**0.142± 0.048**
Moderate Biofilm formation	**0.233 ± 0.02**	**0.103 ± 0.02**
Weak biofilm formation	**0.121 ± 0.011**	**0.091 ± 0.011**

The results indicated a significant reduction in biofilm formation in *A.baumannii* after treatment with melittin. The strong biofilm formation showed an absorbance of 0.298 ± 0.048 before treatment, which decreased to 0.142 ± 0.048 afterward. This finding is consistent with the author (Picoli *et al.*,2017) who was found that melittin effectively destroyed Staphylococcus aureus biofilms at concentrations lower than the minimum inhibitory concentration (MIC) Similarly, the moderate biofilm formation exhibited a decrease from 0.233 ± 0.02 to 0.103 ± 0.02, aligning with the observations that melittin disrupts biofilms of varying strengths. Even in cases of weak biofilm formation, the absorbance values dropped from 0.121 ± 0.011 to 0.091 ± 0.011, reflecting the capacity of melittin to target biofilms regardless of their initial strength (Liu *et al.*, 2024). As well as The mechanisms underlying melittin's action likely involve its interaction with bacterial membranes, which has been reported in other studies. This interaction may lead to increased permeability and subsequent cell lysis as pores in bacterial cell membranes and causes cytoplasm leakage, further supporting the findings on its anti-biofilm activity across different bacterial strains (Sun *et al.*, 2022).

Gene expression level of the *Bap* gene in *A.baumanni* before and after adding Melittin

The gene expression level of the *Bap* gene in *A.baumannii* was found to be high before Melittin treatment, particularly in the strong biofilm formation category figure (7). After Melittin treatment, a gradual decrease in the *Bap* gene expression was observed across the different biofilm formation categories. For the strong biofilm formation, the *Bap* gene expression level was reduced significantly ($P< 0.05$) following Melittin treatment. In the moderate biofilm formation, the *Bap* gene expression was also decreased after Melittin treatment, but the reduction was less pronounced compared to the strong biofilm category. The smallest decrease in *Bap* gene expression was noted in the weak biofilm formation category after Melittin treatment. These results suggest that Melittin, as an anti-biofilm agent, effectively suppressed the expression of the *Bap* gene in *A.baumannii*, with the strongest effect observed on the robust, strong biofilm formations. The gradual decrease in *Bap* gene expression across the biofilm formation categories indicates that Melittin can disrupt the regulatory mechanisms underlying biofilm development in *A.baumannii* (figure 2).

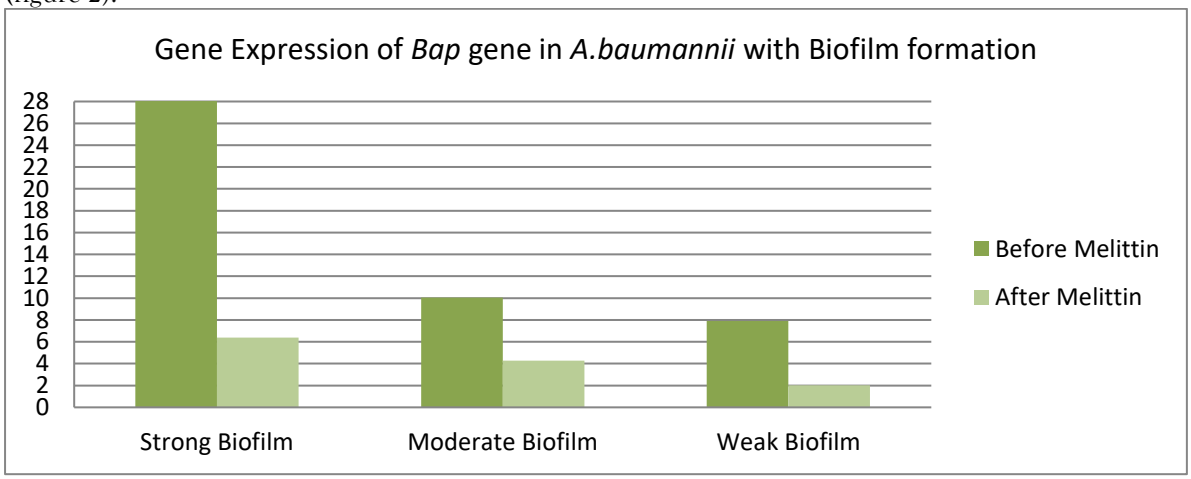

Figure (7): Gene Expression of *Bap* gene in *A.baumannii* with Biofilm formation" before and after treatment with Melittin

After treatment with melittin, a notable decrease in *Bap* gene expression was observed across different biofilm formation categories. Specifically, in the strong biofilm formation category, *Bap* gene expression levels were significantly reduced ($P < 0.05$) following melittin treatment. In the moderate biofilm formation category, while there was also a decrease in *Bap* gene expression, the reduction was less pronounced compared to the strong biofilm category, These findings can be compared to the study (Mohamad *et al.*, 2023) that investigated the effects of Glycyrrhiza glabra on *A.baumannii*, where similar assessments of biofilm production and quorum sensing were conducted. In that study, the active components of Glycyrrhiza glabra were evaluated for their ability to reduce biofilm formation in acenitobacter baumani from patient with various hospitals in Erbil, Northern part of Iraq, including cerebrospinal fluid (CSF), blood, pus, sputum, and wound swabs.

In comparing the findings with the study (Abd-alkader & Al-dragh, 2023) who was found the rblB and plcN genes, it is evident that there is a strong association between the presence of these genes and biofilm formation in *A. baumannii* and the results showing a gradual decrease in *Bap* gene expression following melittin treatment align with the findings of downregulation of both plcN and bap genes in the presence of antibiotics. This suggests that melittin may similarly influence gene expression related to biofilm formation and he found chalcone led to the overexpression of these genes, indicating that different compounds can have varied effects on gene regulation linked to biofilm production.

The graph showed the amplification plot for the *Bap* gene expression in *A.baumannii*. Multiple amplification curves are shown, each representing a different experimental condition or sample. Variation is observed in the Ct values, which indicate the cycle number at which the gene of interest is first detected, across the different curves. The separation and patterns of the amplification curves suggest that the *Bap* gene expression levels differ among the experimental conditions or samples. The Ct value corresponds to the cycle number at which the amplification curve crosses a defined threshold line, indicating the point at which the *Bap* gene is first detected in figure (8).

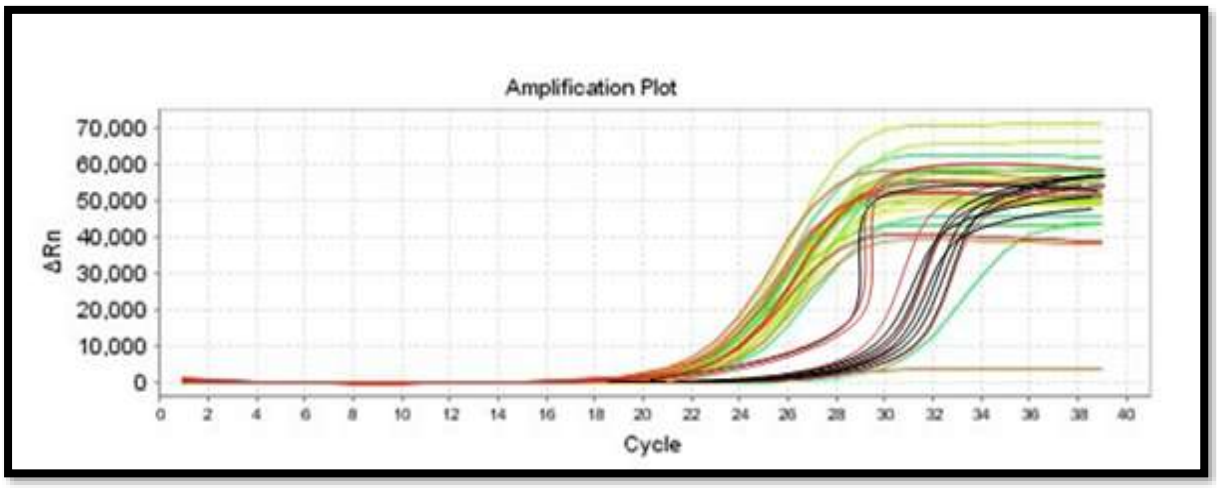

Figure (8): showed the amplification curves for the expression of the *Bap* gene in *A.baumannii*

The importance of the *Bap* protein in the development of biofilm in *A. baumannii* has been well described, and one of the reference studies showed that the ~200 kDa Bap protein is localized on the surface of the cell and is directly linked to biofilm formation. This conclusion is consistent with the observed correlation between *Bap* gene expression levels and biofilm strength during the current study, which showed that strong biofilm-forming strains had higher levels of bap gene expression (2.5-fold) than their moderate and weak biofilm-forming counterparts (Loehfelm *et al.*, 2008).

The down regulation of the bap gene by melittin was noted in all categories of biofilm-forming strain types as follows: decomposition for strong biofilm-forming strains reduced from 2.5-fold to 2.1-fold, moderate biofilm-forming strains decreased from 1.3-fold to 0.8-fold, and weak biofilm-forming strains reduced from 0.4-fold to 0.1-fold. These data imply that melittin tries to target the bap gene, which results in diminished biofilm formation. Instead of inhibiting biofilm formation with Bap-specific antibodies as done in the reference study, the current study novel demonstrates that melittin can do so by down regulating the bap gene. This paints melittin in a new light as a potential therapeutic for biofilm associated *A.baumannii* infections. More work will be needed to understand how melittin inhibits the *bap* gene (Gaddy and Actis, 2009 ; Memariani *et al.*, 2020).

Melittin, a major component of bee venom, has been widely studied for its antimicrobial and anti-biofilm properties. While direct studies linking melittin to the regulation of the *Bap* gene in *A.baumannii* are limited, the mechanisms of melittin's action can be inferred from its known effects on bacterial cells and biofilms. Below is an explanation of how melittin might influence bap gene expression and biofilm formation (Maitip *et al.*, 2021).

It is a cationic amphipathic peptide that interacts with bacterial cell membranes, leading to pore formation and membrane disruption. This activity can compromise the structural integrity of bacterial cells, including *A.baumannii*, and may indirectly affect the expression of genes involved in biofilm formation, such as the bap gene. The destabilization of the cell membrane could interfere with signaling pathways or regulatory systems that control *Bap* gene expression (Issam *et al.*, 2015).

It was shown the Melittin has been shown to inhibit biofilm formation in various bacterial species by disrupting the extracellular matrix and preventing bacterial adhesion. In *A.baumannii*, the *Bap* protein is a critical component of biofilm formation, as it facilitates cell-surface attachment and intercellular interactions. By down regulating the *Bap* gene, melittin may reduce the production of the Bap protein, thereby weakening biofilm structure and stability (Memariani *et al.*, 2019). As well as, the down regulation of the *Bap* gene by melittin aligns with its broader role in reducing bacterial virulence. By targeting genes involved in biofilm formation, melittin can diminish the ability of *A.baumannii* to establish infections and resist host immune responses. This effect is particularly significant in clinical settings, where biofilms contribute to the persistence of *A.baumannii* infections and antibiotic resistance (Ait Abderrahim *et al.*, 2015).

REFERENCES

1. Abd_alkader, N. N., & Al-dragh, W. A. (2023). Gene Expression of biofilm formation genes (plcN, bap) of Acinetobacter baumannii from wounds and burns patients in Baghdad, Iraq. HIV Nursing, 23(1), 456-462.
2. Ait Abderrahim, L., Abdellah, F., & Boukraâ, L. (2015). The importance of botanical origin for api-products as antibiotics. Anti-Infective Agents, 13(1), 28-35.
3. AL-Mousawi, H. T., AL-Taee, M. I. N., & AL-Hajjar, Q. N. (2018). Evaluation of biofilm formation capacity of Acinetobacter baumannii isolated from clinical samples in Baghdad hospitals using phenotypic methods. *Iraqi journal of biotechnology*, *17*(3).
4. Azizi O, Shahcheraghi F, Salimizand H, Modarresi F, Shakibaie MR, Mansouri S, et al. Molecular Analysis and Expression of bap Gene in Biofilm-Forming Multi-Drug-Resistant Acinetobacter baumannii. Rep Biochem Mol Biol. 2016;5(1):62–72.
5. Bahador A, Saghii H, Ataee R, Esmaeili D.(2015) The Study of Inhibition Effects Satureja khuzestaniea Essence against Gene Expression bap Acinetobacter baumannii with Real time PCR Technique. Iran J Med Microb. ,9(1):42–9.
6. Bamunuarachchi NI, Khan F, Kim Y-M. Inhibition of virulence factors and biofilm formation of Acinetobacter baumannii by naturally-derived and synthetic drugs. Curr Drug Targets 2021;22(7):734-59.
7. Bazaid AS, Barnawi H, Qanash H, Alsaif G, Aldarhami A, Gattan H, et al. Bacterial coinfection and antibiotic resistance profiles among hospitalised COVID-19 patients. Microorganisms 2022;10(3):495.
8. Brossard KA, Campagnari AA. The Acinetobacter baumannii biofilmassociated protein plays a role in adherence to human epithelial cells. Infect Immun. 2012;80(1):228–33.
9. Dempsey, C.E. The Actions of Melittin on Membranes. *Biochim. Biophys. Acta* 1990, *1031*, 143–161.
10. Elshaer SL, Shaldam MA, Shaaban MI. Ketoprofen, piroxicam and indomethacin-suppressed quorum sensing and virulence factors in Acinetobacter baumannii. J Appl Microbiol 2022;133(4):2182-97.
11. Flanjak, I., Primorac, L., Stokanović, M. C., Puškadija, Z., Rajs, B. B., & Kovačić, M. (2021). Melittin stability in honey bee venom under different storage conditions measured with RP-HPLC-PDA method. : 467-471.
12. Gaddy, J. A., & Actis, L. A. (2009). Regulation of Acinetobacter baumannii biofilm formation. Future Microbiology, 4(3), 273–278.
13. Gedefie, A., Demsis, W., Ashagrie, M., Kassa, Y., Tesfaye, M., Tilahun, M., Bisetegn, H., & Sahle, Z. (2021). *Acinetobacter baumannii* Biofilm Formation and Its Role in Disease Pathogenesis: A Review. *Infection and drug resistance*, *14*, 3711–3719.
14. Issam, A. A., Zimmermann, S., Reichling, J., & Wink, M. (2015). Pharmacological synergism of bee venom and melittin with antibiotics and plant secondary metabolites against multi-drug resistant microbial pathogens. Phytomedicine, 22(2), 245-255.
15. Lima, W. G., & de Lima, M. E. (2023). Therapeutic prospection of animal venoms-derived antimicrobial peptides against infections by multidrug-resistant *Acinetobacter baumannii*: A systematic review of pre-clinical studies. *Toxins*, *15*(4), 268.
16. Lima, W. G., de Brito, J. C. M., Cardoso, V. N., & Fernandes, S. O. A. (2020). In-depth characterization of antibacterial activity of melittin against *Staphylococcus aureus* and use in a model of non-surgical MRSA-infected skin wounds. *European Journal of Pharmaceutical Sciences*, *156*, 105592.
17. Liu, X., Qin, P., Wen, H., Wang, W., & Zhao, J. (2024). Seasonal meropenem resistance in Acinetobacter baumannii and influence of temperature-driven adaptation. BMC microbiology, 24(1), 149.
18. Liu, X., Shi, D., Cheng, S., Chen, X., Ma, C., Jiang, Y., ... & Zhou, M. (2024). Modification and synergistic studies of a novel frog antimicrobial peptide against Pseudomonas aeruginosa biofilms. Antibiotics, 13(7), 574.
19. Loehfelm, T. W., Luke, N. R., & Campagnari, A. A. (2008). Identification and characterization of an *Acinetobacter baumannii* biofilm-associated protein. *Journal of bacteriology*, *190*(3), 1036-1044.
20. Luo LM, Wu LJ, Xiao YL, Zhao D, Chen ZX, Kang M, et al. Enhancing pili assembly and biofilm formation in Acinetobacter baumannii ATCC19606 using non-native acyl-homoserine lactones. BMC Microbiol. 2015;15:62.
21. Maitip, J., Mookhploy, W., Khorndork, S., & Chantawannakul, P. (2021). Comparative study of antimicrobial properties of bee venom extracts and melittins of honey bees. Antibiotics, 10(12), 1503.
22. Memariani, H., Memariani, M., & Moravvej, H. (2020). Melittin: A potent antimicrobial peptide with anti-biofilm properties. Current Protein and Peptide Science, 21(4), 362–371.
23. Memariani, H., Memariani, M., Shahidi-Dadras, M., Nasiri, S., Akhavan, M. M., & Moravvej, H. (2019). Melittin: from honeybees to superbugs. Applied microbiology and biotechnology, 103, 3265-3276.
24. Mohamad, T. S., Rahman, J. K., Ahmed, A. A., & Ganjo, A. R. (2023). Down-regulation of abaI, abaR, Bap and OmpA genes in Acinetobacter baumannii by ethanol extract of Glycyrrhiza glabra after toxicity assessment. Cellular and Molecular Biology, 69(12), 194-200.
25. Mohamad, T. S., Rahman, J. K., Ahmed, A. A., & Ganjo, A. R. (2023). Down-regulation of abaI, abaR, Bap and OmpA genes in Acinetobacter baumannii by ethanol extract of Glycyrrhiza glabra after toxicity assessment. Cellular and Molecular Biology, 69(12), 194-200.
26. Pakharukova N, Tuittila M, Paavilainen S, Malmi H, Parilova O, Teneberg S, et al. Structural basis for Acinetobacter baumannii biofilm formation. Proc Natl Acad Sci U S A. 2018;115(21):5558– 63. [PubMed ID: 29735695].
27. Picoli, T., Peter, C. M., Zani, J. L., Waller, S. B., Lopes, M. G., Boesche, K. N., ... & Fischer, G. (2017). Melittin and its potential in the destruction and inhibition of the biofilm formation by Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa isolated from bovine milk. Microbial pathogenesis, 112, 57-62.
28. Picoli, T., Peter, C. M., Zani, J. L., Waller, S. B., Lopes, M. G., Boesche, K. N., ... & Fischer, G. (2017). Melittin and its potential in the destruction and inhibition of the biofilm formation by Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa isolated from bovine milk. Microbial pathogenesis, 112, 57-62.
29. Pompilio A, Scribano D, Sarshar M, Di Bonaventura G, Palamara AT, Ambrosi C. Gram-negative bacteria holding together in a biofilm: the *Acinetobacter baumannii* way. *Microorganisms.* (2021) 9:1353.
30. Raghuraman, H., & Chattopadhyay, A. (2007). Melittin: A membrane-active peptide with diverse functions. Bioscience Reports, 27, 189–223.

31. Ridha, D. J., Ali, M. R., & Jassim, K. A. (2019). Occurrence of Metallo-β-lactamase Genes among Acinetobacter baumannii Isolated from Different Clinical Samples. *Journal of Pure & Applied Microbiology*, *13*(2).
32. Roy, S., Chowdhury, G., Mukhopadhyay, A. K., Dutta, S., & Basu, S. (2022). Convergence of biofilm formation and antibiotic resistance in *Acinetobacter baumannii* infection. *Frontiers in medicine*, *9*, 793615.
33. Rusul, H. S., & Suhad, S. M. (2022). First Report In Iraq: amino acid substitution in pmrcab genes and there corellation with colistin resistance among A. Baumannii isolates. The Iraqi Journal of Agricultural Science, *53*(2), 237-251.
34. Shi, P., Xie, S., Yang, J., Zhang, Y., Han, S., Su, S., & Yao, H. (2022). Pharmacological effects and mechanisms of bee venom and its main components: Recent progress and perspective. *Frontiers in pharmacology*, *13*, 1001553.
35. Sun, L., Wang, S., Tian, F., Zhu, H., & Dai, L. (2022). Organizations of melittin peptides after spontaneous penetration into cell membranes. Biophysical Journal, 121(22), 4368-4381.
36. Sun, L., Wang, S., Tian, F., Zhu, H., & Dai, L. (2022). Organizations of melittin peptides after spontaneous penetration into cell membranes. Biophysical Journal, 121(22), 4368-4381.
37. Wehbe, R.; Frangieh, J.; Rima, M.; Obeid, D.E.; Sabatier, J.M.; Fajloun, Z. Bee Venom: Overview of Main Compounds and Bioactivities for Therapeutic Interests. *Molecules* 2019, *24*, 2997.
38. Yang, C. H., Su, P. W., Moi, S. H., & Chuang, L. Y. (2019). Biofilm formation in *Acinetobacter baumannii*: Genotype-phenotype correlation. Molecules, 24(10), 1.