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Evaluation of *Rosmarinus officinalis* Leaves Essential Oils Activity Against Vancomycin Intermediate *Staphylococcus aureus* (VISA) Isolated from Baghdad Hospital Patients

Osamah Z. Al-Hayali[1]*, Mohamed. F. AL Marjani[2], Abbas Maleki [3]

[1]Department of Biology, Al-Farabi University College , Baghdad, Iraq

[2]*Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq*

[3]*Clinical Microbiology Research Centre, Ilam University of Medical Sciences, Ilam, Iran*

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Abstract

Rosemary is a well-known aromatic and medicinal plant used to treat various ailments. This study evaluated *Rosmarinus officinalis* essential oil for its phytochemical and antibacterial properties. The essential oil was analysed by using a gas chromatography-mass Spectrometry (GC-MS) that revealed the common chemicals containing verbenone 36.20% and 1,8-cineol (Eucalyptol) 12.14%. Extracted essential oils were tested for antibacterial activity against vancomycin intermediate *Staphylococcus aureus* (VISA), a strain of bacteria obtained locally from bacteremia patients. Three isolates were found to be VISA positive using the E-test (strips) and the population analysis profile method (PAP). VISA showed lower resistance to the essential oils (MIC: Minimum inhibitory concentration, MIC = 1.5 mg/ml). Overall, the genetic influence of VISA was evaluated by looking at the *hld* gene expression after being isolated from blood samples and RNA extraction for qRT-PCR to determine *hld* gene expression. *R. officinalis* gene expression was analysed and calculated by using real-time PCR of RNA which revealed that it had decreased.

Keywords: Essential oil, Antimicrobial activity, GC-MS, Real-time PCR

تقييم نشاط الزيوت الاساسية لاوراق نبات إكليل الجبل ضد المكورات العنقودية الذهبية /متوسطة المقاومة من فانكومايسين

اسامه زهير الحيالي[1]*,محمد فرج المرجاني[2], عباس مالكي[3]

[1]قسم علوم الحياة، كلية الفارابي الجامعة ، بغداد، العراق

[2]قسم علوم الحياة، كلية العلوم، الجامعة المستنصرية، بغداد، العراق

[3] مركز ابحاث علم الاحياء الدقيقة الطبية، جامعة ايلام للعلوم الطبية، ايلام، ايران

*Email: usamazuhair@hotmail.com

الخلاصة

يعد إكليل الجبل من النباتات العطرية والطبية المعروفة والذي يمكن استخدامه لعلاج مجموعة متنوعة من الأمراض. تمت دراسة زيوت نبات اكليل الجبل لخصائصها الكيميائية النباتية والمضادة للبكتيريا، تم إجراء تحليل الزيت العطري باستخدام كروماتوجرافيا الغاز – مطياف الكتلة (GC–MS) والذي كشف عن المواد الكيمياوية الشائعة والتي تحتوي على verbenone حوالي 36.20٪ و 1,8–cineol (Eucalyptol) 12.14٪. تم اختبار الاحتمالية المضادة للبكتريا للزيوت العطرية المستخرجة (MIC: الحد الأدنى من التركيز المثبط) ضد المكورات العنقودية المتوسطة من فانكومايسين (VISA) المعزولة محليًا من مرضى تجرثم الدم. ثلاث عزلات كانت إيجابية VISA باستخدام اختبار E بالاشرطة وطريقة تحليل السكان (PAP). أظهرت العزلات امكانية مقاومة أقل (MIC = 1.5 مجم / مل) للزيوت الأساسية. بشكل عام ، تم استقصاء التأثير الجيني لـ (VISA) من خلال دراسة تعبير الجين *hld* بعد عزله من عينات الدم واستخراج الحمض النووي الريبي لـ qRT–PCR لتحديد التعبير الجيني *hld*. تم تحليل التعبير الجيني لـ *R. officinalis* وحسابه باستخدام PCR في الوقت الحقيقي للـ RNA الذي كشف عن انخفاض ملحوظ.

1. Introduction

Staphylococcus aureus is a prevalent pathogenic bacteria. Around 40–50% of all *S. aureus* isolates are beta-lactam and antimicrobial drug-resistant, thus necessitating the ongoing development of new effective drugs to combat such infections. This pathogen poses numerous treatment challenges, especially in providing appropriate empiric antimicrobial therapy [1]. Because of its antibiotic resistance and capacity to cause various human illnesses, *S. aureus* is a severe problem in terms of treatment and management [2, 3]. As the prevalence of antibiotic resistance continues to grow, *S. aureus* has become a severe public health concern. Glycopeptide antibiotic vancomycin is used to treat Gram-positive bacterial infections. Beta-lactams, aminoglycosides, macrolides, fluoroquinolones, chloramphenicol and tetracycline are among the drugs that *S. aureus* is resistant to [4]. However, because resistance genes are already present, resistance to these antibiotics develops quickly in *S. aureus.* Antibiotics are pumped out of the bacterial cell by a specific efflux pump before they reach the ribosome [5].

Phenotypes resistance of vancomycin:
1. Vancomycin-intermediate *S. aureus* (VISA): This bacterial strain has a thickening cell wall which is thought to reduce vancomycin ability to permeate the cell septum required for effective vancomycin treatment [6].

2. Vancomycin resistance *S. aureus* (VRSA): Although described, only a few cases of *S. aureus* have a high degree of vancomycin resistance. Vancomycin resistance is caused by the presence of the vanA gene [7].

3. Heterogeneous vancomycin-intermediate *S. aureus* (hVISA): An *S. aureus* strain that generates vancomycin resistance at 106 colonies or higher concentrations [8].

Rosmarinus officinalis is a crucial Mediterranean native and a long-cultivated plant known as rosemary which is widely used in folk medicine, pharmaceuticals and cosmetics. It belongs to the Lamiaceae family [68]. It is a fragrant plant and a herbal remedy with medicinal advantages such as blood pressure control, improved circulation, diuretic, migraine relief, antibacterial and antimycotic properties. Rosemary is used in the food industry to prevent germs from adhering to surfaces and forming biofilms on food contact surfaces and food items. Its antibacterial activity and the effects of this extract on biofilm formation against *S. aureus* and *Escherichia Coli* bacteria have been studied [9, 10]. Rosemary contains the antioxidants carnosic, carnoso, ursolic acid, betulinic acid, ursolic acid, camphor, caffeic acid and rosmarinic acid [11].

2. Materials and Methods

2.1 Sample Collection

From January to May of 2021, 165 clinical specimens were collected from hospital patients in Baghdad, including sputum, ear infections, pus, wounds, burns, blood and urine.

2.2. Staphylococci Isolation

Staphylococci were isolated from various clinical samples using standard laboratory procedures. All samples were spotted on mannitol salt agar to see if bacterial isolates could grow on all plates. All the plates were kept at 37°C for 24 hours in an aerobic environment [12].

2.3. *Staphylococcus aureus* Identification

Biochemical tests such as the catalase, coagulase, and oxidase tests were used to identify the isolates, Bergey's Manual [13]. It was proven by the usage of the vitek2 system.

2.4. Methicillin-resistant *Staphylococcus aureus* (MRSA) Strain Identification

CHROMagar^{TM} MRSA medium was used to identify MRSA strains as a selective MRSA medium. Its colour changed from pink to mauve, indicating positive colonies. The *mec A* gene was discovered via PCR as gene Methicillin resistance gene [14].

2.5. Susceptibility to Antibiotics

Susceptibility testing for *S. aureus* was performed using the vitek2 compact system to determine the MIC values for vancomycin and other antibiotics available in AST GP 76 cards
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2.6. MIC determination of Vancomycin Intermediate *Staphylococcus aureus* (VISA) with E-test

E-test vancomycin strips from BioMerieux, Marcy l'Etoile, France were used. As previously reported [15, 16, 17, 18, 19], the bacteria were grown for 18 hours at 37°C in Mueller Hinton broth. After diluting equal to McFarland no.0.5 tube, inoculums were applied using sterile swabs to Mueller Hinton agar. The antibiotic strips were pushed into the medium and left to incubate overnight at 37°C.

2.7. Population Analysis Profile-area Under the Curve (PAP-AUC)

A small number of colonies were injected into BHI broth and cultivated at 37°C overnight as the PAP method. 10^{-3}, 10^{-6}, and 10^{-8} log dilutions were made. 100 µl of each dilution was lawn grown with vancomycin at dosages ranging from 0 to 8 µg/ml on BHI agar. GraphPad Prism 9.3.0 (GraphPad Software Inc., San Diego, CA, USA) was used to compute the AUC using colony-forming unit (CFU/ml) data. According to the PAP technique, hVISA (ATCC-700698, Mu3), VISA (ATCC-700699, Mu50) and *S. aureus* (ATCC-29213) were used as control strains if the AUC test to AUC of control ratio was between ≥0.9 and <1.3, the isolate was classed as hVISA, and if it was ≥1.3 was classified as VISA (20**).**

2.8. Collection and Preparation, *R. officinalis* Leaves:

The leaves of *R. officinalis* were collected from a garden house in Baghdad, Iraq **(**Figure 1). Mustansiriyah University's Department of Biology, College of Science conducted the identification and authentication procedures. The leaves were grown throughout the winter growing season; they were washed with distilled water and dried at 45°C. In a mortar and pestle, the dried leaves were mashed and stored until required. The ground leaves materials were then transferred to a Clevenger apparatus. In each case, 50 g of the plant material was distilled in 700 ml H_2O in a 1000ml flask for 3 hours. The essential oils were then collected

and stored at 4°C in tightened vials [21].

Figure 1: The leaves of *R. officinalis* from a garden house in Baghdad, Iraq.

2.9. Essential Oil Chemical Analysis

Phytochemical composition of essential oils in *R. officinalis* leaves was investigated using gas chromatography-mass spectrometry (GC-MS), as described by Sandra and Bicchi [22]. Agilent 7820 Gas Chromatography device (Agilent Technologies, Wokingham, United States) was used to analyze the phytochemical composition of the oil extract. Coupled with Agilent 5977 MSD, the GC was equipped with Agilent HP-5MS Ultra Inert column (30m length x 250 µm diameter x 0.25 µm inside diameter). The injection with a split-less method; in the temperatures of the injector and detector at 50-280°C the program arranged the column's oven temperature to be 50°C for 1 min. and then expanded it to 150°C with an 8°C/min; heating ramp. Extracts were separated and identified after a 150°C to 280°C heating ramp at 8°C/min. with a terminal temperature of 280°C per 3 min. The electron energy of the ion source was 70 eV. The oil extract samples were injected into the column in 1µl. Identified the unknown component by comparing its mass spectrum to known components using the NIST11 library database. Later the essential oil was gathered and stored in tightly sealed vials in a refrigerator. Several dilutions of the oils were made with dimethyl sulfoxide for the antimicrobial activity test (DMSO).

2.10. Measuring Antimicrobial Activity using Broth Microdilution.

Broth microdilution technique assessed the minimum inhibitory concentration (MIC) of *R. officinalis* leaves oils. A 96-well microtiter plate was utilized for the test. After dilution in dimethyl sulfoxide (DMSO) to 200 mg/ml, the first row of the well was filled with 50 µl tested extracts stock solution to prepare concentrations ranging 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 mg/ml by serial twofold dilutions. Each test had two control wells: positive control of plant extract with media and negative control of inoculum with media. Each well contained 50 µl of leaf oils and 50 µl of Mueller–Hinton broth. Turbidity standard was prepared for each microorganism of 0.5 McFarland and poured 10 µl into the wells. The plates were incubated for 24 hours at 37°C. After incubation, 50 µl of 0.1 % 2, 3, 5-triphenyl tetrazolium chloride (TTC) solution was poured into each well as an indicator solution, and then incubated the plates for 45-60 minutes at room temperature to determine bacterial growth. MIC plant extract concentration was the lowest. After incubation, the bacterial

growth should result in the reduction of tetrazolium dye which turns into red/pink, while the growth inhibition is inferred when the color of the solution remains intact inside the well growth following TTC incubation [23, 24, 25]

2.11. Gene Expression

Total RNA was extracted from treated and untreated bacteria using the TRIzol™ Reagent (Thermo Scientific, USA) [26], and checked the purity and concentration using a Quantus Fluorometer (Promega, USA). We used the SYBER green dye's, one-step quantitative RT-PCR, GoTaq® Kit 1-Step RT-qPCR System, nuclease-free water, $MgCL_2$ and Quantifluor RNA System (Promega, USA). The expression of the *hld* genes was measured using mic qPCR Cycler (BioMolecular System, Australia) and then amplified the total amount per reaction. The expression of the *hld* gene was evaluated using a real-time PCR method with the primers as follows. 5`ATTTGTTCACTGTGTCGATAATC-3`(F) 5`-GGAGTGATTTCAATGGCACAAG-3`(R) [27]. Housekeeping gene was used as a reference gene with the following primers sequence (16srRNA): FP 5`-CTGCTGCCTCCCGTAG-3`(F) 5`-CCGACCTGAGAGGGTGA-3`(R) [28]. Each one had a final volume of 10µl. These amplifications included (0.5 µl forward primer, 5 µl qPCR master mix, 2.5 µl nuclease-free water, 0.25 RT mix, 0.25 $Mgcl_2$, 0.5 µl reverse primer and 1 µl RNA).

3. Result and Discussion

3.1. *Staphylococcus aureus* and *Staphylococcus* (MRSA) that is Resistant to Methicillin Identification

The results showed 89 (53.93 %) isolates of *S. aureus* fermented mannitol salt agar, positive for coagulase and catalase, oxidase test negative. Blood agar revealed a β-hemolysis pattern. 30 (33.70%) of the *S. aureus* isolates were methicillin-resistant *S. aureu*s (MRSA) by cultivating isolates on CHROMagar™ MRSA media which inhibited all MSSA isolates while allowing MRSA isolates to grow. 30 *S. aureus* isolates developed rose to mauve color and were recognized as MRSA. In this study, MRSA with chromogenic agar made it easier to isolate from primary isolation plates for up to 24 hours after enrichment, without additional biochemical tests [29].

This procedure is less expensive, takes less time and delivers similar results to the PCR [30]. On the other hand, PCR-based methods are the most reliable for detecting MRSA [31]. Detection of the *mecA* gene discovered that 30 (33.70%) of the 89 *S. aureus* isolates were MRSA, 19 (63.3%) and 11 (36.7%) coming from in and outpatients respectively. This study agreed with an Iraqi survey done by Shaima'a [32] who discovered that acquired 44% of MRSA isolates were in hospitals and 34% in the community.

3.2. Phenotypes of Vancomycin Resistance

According to Table 1, 13 (43.3 %) of MRSA isolates were vancomycin resistant (VRSA) (MIC ≥32 µg/ml), 12 (40.0 %) were vancomycin sensitive (VSSA) (MIC ≤ 0.5, 1, and 2 µg/ml) and 5 (16.7 %) of MRSA isolates had intermediate resistance to vancomycin (VISA) (MIC 4 µg/ml) with heterogeneous VISA phenotype (hetero-VISA).

Table 1: Shows the different phenotypes of vancomycin resistance in the isolates of *S. aureus*.

Susceptibility Pattern	No. of Isolates	Percentage (%)
(VRSA) Resistance	13	43.3
(VSSA) Sensitive	12	40.0
(VISA) or (hVISA) Intermediate	5	16.7
Total	30	100

hVISA= heterointermediate resistance *Staphylococcus aureus,* VISA= Vancomycin intermediate resistance *Staphylococcus aureus,* VSSA= Vancomycin sensitive *Staphylococcus aureus,* VRSA= Vancomycin resistance *Staphylococcus aureus.*
Vancomycin treatment has been associated with a prolonged duration of MSSA bacteremia and a delayed clinical response compared to β-lactam treatment and more common complications in individuals with endocarditis [33]. For MRSA treatment, vancomycin is considered the first-line drug [34].

3.3. E-test and Population Analysis Profile Method (PAP)

E-test technique was used to determine the MIC and the strips contained elliptical inhibition zones, as seen in Figure 2. Vancomycin strips and MIC values of (≥8 µg/ml) were obtained for MRSA isolates. The MIC strip is a quick and accurate way to determine a bacteria's antimicrobial sensitivity to antibiotics. All 30 MRSA isolates were subjected to E-tests. Five isolates had a vancomycin MIC of (≥8 µg/ml)

hVISA because the test isolates to Mu3 ratio was 0.9 or higher but less than 1.3 compared to the Mu3 reference strain. When tested using the PAP-AUC technique, three isolates, in particular, had an AUC of the test isolate to a Mu3 strain ratio of 1.3 or higher (Figure 3). These isolates were categorized as VISA after additional testing.

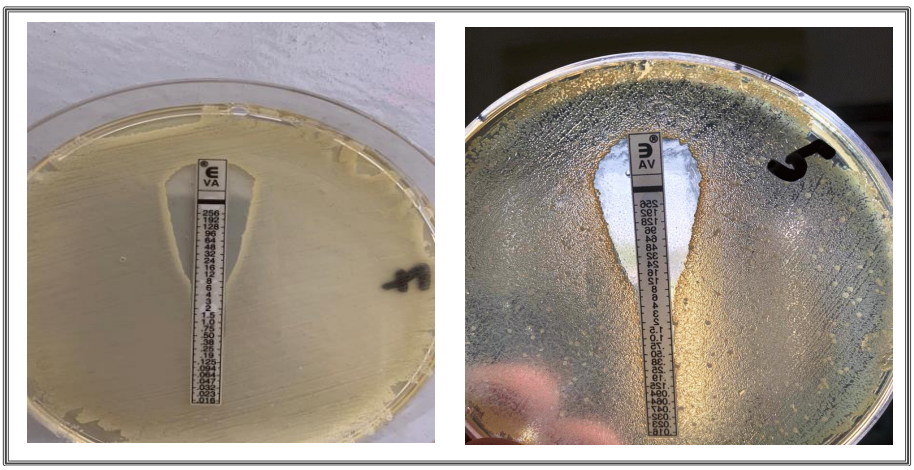

Figure 2: Vancomycin E-test strip to determine MIC for *S. aureus* MRSA.

Our studies revealed an alarming VISA prevalence rate of 10% which was significantly higher than the global average of 3% [19, 35, 36, 37, 38]. While differences in enrolment criteria or screening processes may be to blame, it's also possible that the pathogen evolved to become more resistant to vancomycin. The increased incidence of hVISA and VISA and the decreased vancomycin susceptibility of MRSA isolates should be cause for concern.

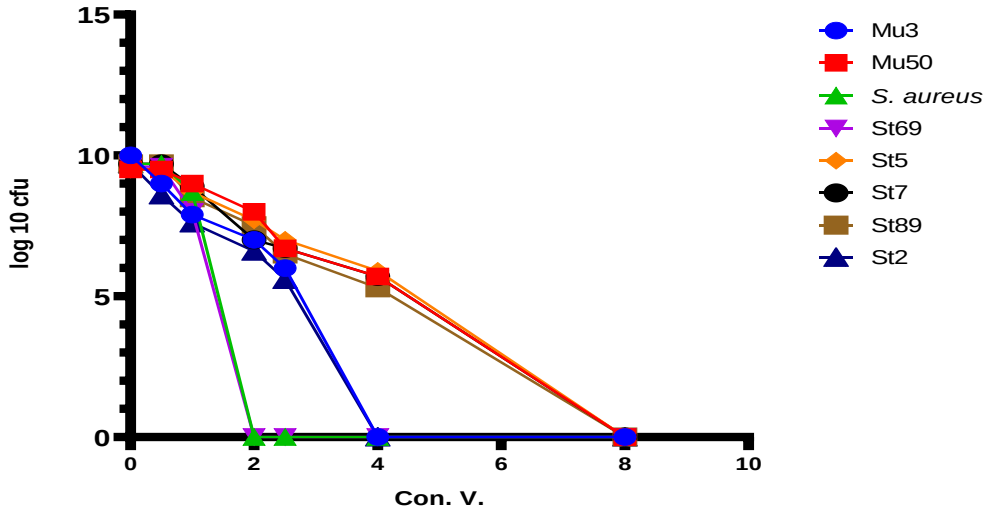

Figure 3: Shows population analysis of control strains ATCC 25923 (VSSA), ATCC700698 (Mu3-hVISA), ATCC700699 (Mu50-VISA) and representative test strains (St69, St2, St5, St7, and St89). Colonies were enumerated after 48 hours of incubation on BHI agar with different concentrations of vancomycin. The positive control strains Mu50, and Mu3 test strains St2 St5, St7, and St89 show reduced vancomycin susceptibility to (<4 µg/mL), and the negative control strain ATCC 25923 shows increased vancomycin susceptibility to (≥4 µg/mL).

3.4. **Chemical Composition *R. officinalis* Leaves**

The gas chromatography analysis of *R. officinalis* leaves extract showed the presence of active chemical compounds. Major compounds, their formulae and molecular weights are shown in Table 2. *R. officinalis* leaves essential oil revealed the presence of 11 prominent peaks on the GC-MS chromatogram (Figure 4). Components belonging to these peaks are listed in Table 2. Major peaks were identified as Verbenone (RT: 9.776); Eucalyptol (RT: 6.245); Benz(a)acridine, 9,10,12-trimethyl (RT: 27.113).

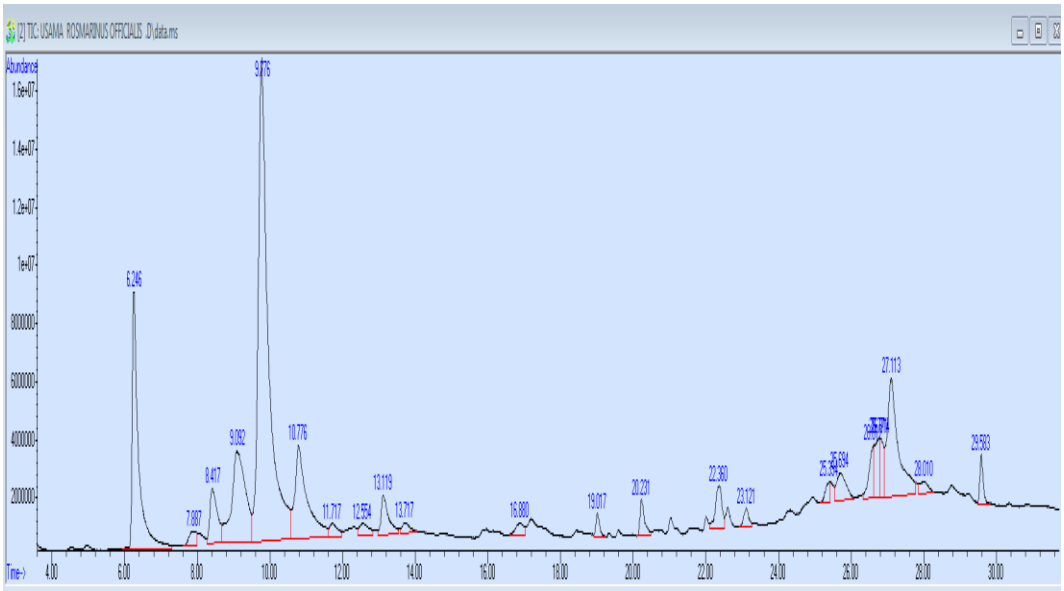

Figure 4: Gas Chromotography-MS profile of *R. officinalis* leaves showed the presence of major peaks.

Table 2: GC-MS analysis of compounds found in *R. officinalis* leaves extract

Retention Time	Constituents	Chemical Formula	Molecular Weight	Area %
6.245	1,8-cineol (Eucalyptol)	C10H18O	154.25	12.14
7.887	Linalool	C10H18O	154.25	0.77
8.417	Camphor	C10H16O	152.23	3.07
9.092	Borneol	C10H18O	154.25	**9.39**
9.776	Verbenone	C10H14O	150.22	**36.20**
10.776	Ethanol, 2-(3,3-dimethylcyclohexylidene)-, (Z)-	C10H18O	154.25	8.63
11.717	Farnesyl acetate	C17H28O2	264.4	0.84
12.554	6-Methylspiro[4.5]decan-6-ol	C11H20O	168.28	0.84
13.119	beta-Caryophyllene	C15H24	204.35	2.14
13.717	4-Isopropyl-cis-bicyclo[4.3.0]-2-nonen-8-one, (4R,S)-	C12H18O	178.27	0.65
16.880	Decahydro-1-naphthol	C10H18O	154.25	0.71
19.017	cis-Pinane	C10H18	138.25	0.65
20.231	Hexadecanoic acid, methyl ester			1.26
22.360	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	C17H32O	252.4	2.09
23.121	1-Monolinolenoyl-rac-glycerol	C21H36O4	352.5	0.72
25.394	1-Hexanesulfonanilide, 4'-(3-acetamido-9-acridinylamino)-	C27H30N4O3S	490.6	0.74
25.694	1,3,5-Tris (trimethylsiloxy) benzene	C15H30O3Si3	342.65	1.97
26.617	1-(4-Methyl-[1,1':4',1"]terphenyl-4"-yl) ethan one	$C_{15}H_{14}O$	210.27	1.82
26.771	5-Androstene, 4,4-dimethyl-	$C_{21}H_{34}$	286.49	2.22
26.814	Ferruginol	C20H30O	286.5	1.51
27.113	Benz(a)acridine, 9,10,12-trimethyl	C20H17N	271.4	**9.68**
28.010	Dehydroabietinol	C20H30O	286.45	0.75
29.583	Squalene	C30H50	410.7	1.20

The bulk of components in the essential oil are represented by bold values.

As a result, verbenone was the main constituent found in our analysis which accounted for 36.20% of the total. Furthermore, the majority of prior investigations in Iran suggested that verbenone was the primary component of *R. officinalis* essential oil component [39], Italy [40] and Spain [41]. However, it was not detected in other studies in Iran [42], Tunisia [43], Morocco [44, 45] and Serbia [46]. 1,8-cineole (Eucalyptol) was the second principal constituent with 12.14% of *R. officinalis* essential oil which this chemical composition is a significant constituent of the previously reported rosemary essential oil [47, 48, 49]. According to a literature review, multiple prior investigations on *R. officinalis* essential oil have been conducted worldwide. Climate, seasons, geography and genetic variations affect the essential oil structure [50, 51, 52].

3.5. Antibacterial Studies Determining *R. officinalis* Leaves Essential Oils MIC

Determining the efficacy of the minimum inhibitory concentration (MIC) value is critical. Low MIC values could indicate high efficiency or that the microorganism will not develop resistance to the bioactive composite. This work revealed that the antibacterial activity of essential oils isolated from *R. officinalis* leaves may be studied *in vitro* using the micro broth dilution technique. According to Jarrar *et al*. [53], the essential oils of *R. officinalis* showed

antibacterial activity against the tested microbiological isolate with *an in vitro* MIC of 0.39–3.13 mg/ml.

Our findings showed that *R. officinal* essential oil had the lowest MIC value (1.5 mg/ml) against Vancomycin Intermediate *Staphylococcus aureus* (VISA) (Table 3). The results showed that bicyclic monoterpenes like verbenone and borneol could inhibit bacterial growth with 1.5 mg/ml MIC values and that their antibacterial properties are also well known [54]. Presence of antibacterial compounds, such as 1,8-cineole (eucalyptol) has considerable antimicrobial action against various ailments [55, 56], thus explaining the essential oil's higher antimicrobial activity.

Table 3: Inhibitory concentration (MIC) *R. officinalis* essential oil

MIC (mg/ml) Bacteria	200	100	50	25	12.5	6.25	3.12	1.5	0.7	0.3
hVISA ST1	-	-	-	-	-	-	-	-	+	+

- : Inhibition; + : Growth

The high prevalence of drug-resistant pathogens is well-known as a problem [57]. As a result, alternative therapies that could help alleviate problems associated with microbial drug resistance are researched [58]. Another study reported *R. officinalis* been used in ethnomedicine[59]. From the 1990s until 2014, rosemary essential oil had the highest antimicrobial activity, accounting for 65% of anti-infectious activity studies. The antibacterial activity of the essential oil outperformed that of the separated components 1,8-cineole and α-pinene [60].

3.6. The *R. officinalis* Influence on *hld* Gene Expression.

RNA isolation was used to investigate *hld* genes expression. The presence of the housekeeping gene (H.K) has been used as a calibration and calculated by fold changes [61], demonstrating that VISA had lower *hld* gene expression than the control group (Figure 5). In these isolates, *hld* expression was reduced after treatment with *R. officinalis* leaves extract (Figure 6). The *hld* gene is a part of the regulatory system for synthesizing several virulence factors [62]. Reduction in *hld* gene expression was observed in 3 isolates of VISA at the concentrations of MIC value (1.5 mg/ml) which means that the *R. officinalis* essential oils play role in inhibiting mRNA transcription and expression. *R. officinalis* has been demonstrated to influence gene transcription, and up and downregulation of several genes [63].

Wright *et al.* [64] discovered that the *hld* gene code for the delta-hemolysin protein is a virulence factor secreted and controlled by *agr* expressed by the *hld* gene located inside the *agr* locus. It is produced from RNAIII, the *agr* effector molecule. As a result, in a VISA strain with intact *hld*, delta-hemolysin expression was employed as an *agr* function marker. Furthermore, other studies have suggested that *agr* dysfunction is not caused by point mutations. It may reduce vancomycin efficacy in MRSA infections and be linked to persistent bacteremia [65, 66]. There have been few studies on the *hld* gene. However, previously no study has been conducted in Iraq on the molecular detection and expression of the *hld* gene in VISA. Our findings are consistent with those of other studies worldwide, which have linked the Howden *et al.* [6] that the *agr* locus is a primary worldwide regulator of virulence factor expression.

Another study conducted in 2012 looked into the involvement of the *hld* gene in *agr* virulence expression, and its promoter which are found to be present both in Mu3 and Mu50, thus the loss of *agr* function most likely caused the absence of delta-hemolysin expression [67] which corresponded perfectly with the hemolytic phenotype observed [6]. After treatment with *R. officinalis*, the expression of *hld* genes was dramatically reduced, showing a gradual downregulation of this gene for VISA.

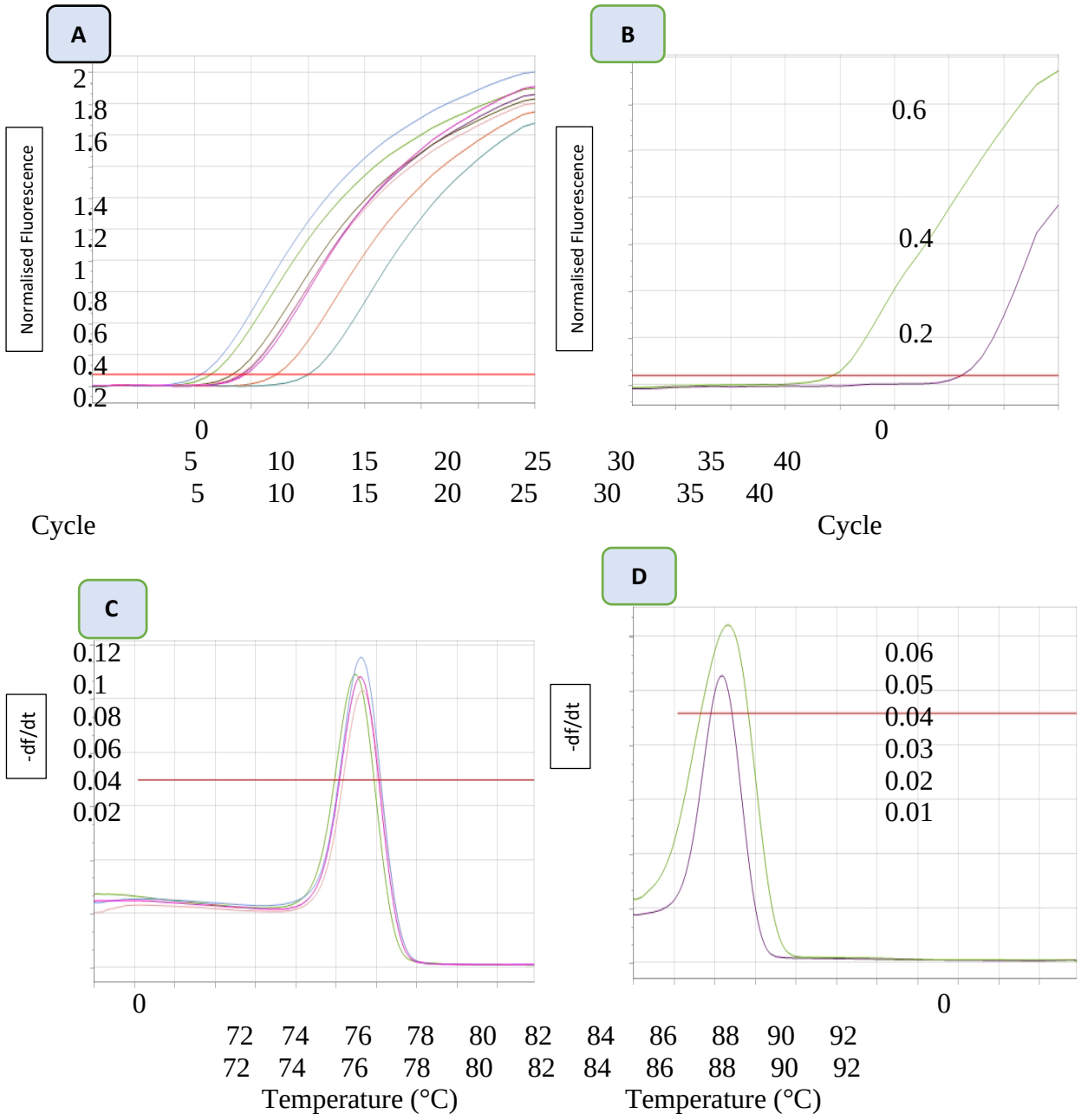

Figure 5: 16srRNA gene expression, RT-PCR amplification of the gene of interest **(A):** *hld* gene expression, RT-PCR amplification of the gene of interest **(B);** Real-time melting curves for PCR products. Single peak represents SYBER green dye's specific binding to the genes of interest 16srRNA gene in VISA treated with *R. officinalis* essential oil **(C);** Real-time melting curves for PCR products. Single peak represents SYBER green dye's specific binding to the genes of interest *hld* gene in VISA treated with *R. officinalis* essential oil **(D).**

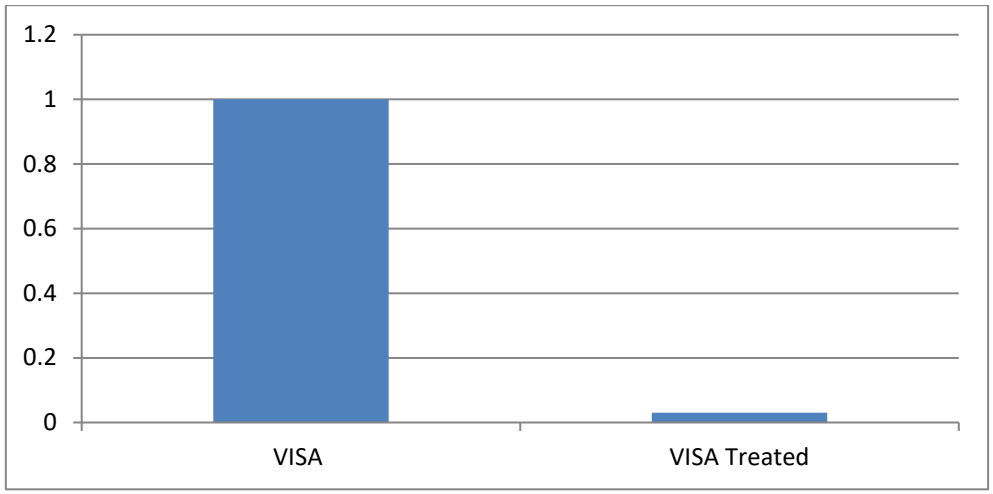

Figure 8: Inhibitory effects of the *R. officinalis* leaves extract on the VISA strain, relative expression ratios (ratio of the gene to action) of (*hld* gene) encoding delta hemolysin. And housekeeping gene 16srRNA as a normalized transcription of *hld* by the comparative CT method.

4. Conclusion

This study shows that *R. officinalis* essential oil contains active constituents that have good antimicrobial activity against the pathogen tested. So the claim of its use against infections associated with the tested pathogens is corroborated. Hence, this plant could be a reliable source for developing potential antimicrobial drugs. However, further study is warranted to screen the components of this plant that may be of value in the pharmacological industry.

5. References

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