ANTIBACTERIAL ACTIVITY OF *TAMARIX APHYLLA* L. LEAVES EXTRACT ON HETEROGENEOUS VANCOMYCIN ITERMEDIATED *STAPHYLOCOCCUS AUREUS* (hVISA)

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ABSTRACT

This study was aimed to examine the antibacterial activity of the *T. aphylla* leaves ethanolic extract *in vitro* against the locally strain heterogeneous vancomycin intermediated *Staphylococcus aureus* (hVISA) at different concentrations starting from 0.03 to 200 mg/ml. *T.aphylla* leaves were extracted by ethanol 96% in the Soxhlet extraction unit. Using both gas chromatography and mass spectrometry (GC/MS), the results indicated that the composition of *T. aphylla* extract was oil and include a variety of chemicals, including 9,12-Octadecadienoic acid, methyl ester (20.06%), followed by Phytol, acetate (18.93%) and Hexadecanoic acid, methyl ester (12.14%), were in leaves. The concentration of 1.5 (mg/ml.) was determined as a minimum inhibitory concentration (MIC) for hVISA antibacterial activity. Gene expression was detected using the Quantitative Real-Time PCR technique; before and after treatment with *T. aphylla* leaves extracts; the gene expression was low after treatment with *T. aphylla* leaves extracts. In conclusion, *T. aphylla* leaves extract is a promising alternative medication that can treat the infection caused by hVISA.

Keywords: Antimicrobial activity, chemical composition, essential oil, gene expression

النشاط المضاد البكتيري لمستخلص اوراق نبات الاثل عديم الاوراق على *Staphylococcus aureus* غير المتجانسة ومتغيرة المقاومة للفانكومايسين

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المستخلص

هدفت الدراسة الحالية الى فحص النشاط المضاد للبكتريا للمستخلص الكحولي لاوراق نبات الاثل عديم الاوراق في المختبر ضد سلالة المكورات العنقودية الذهبية غير المتجانسة متغايرة المقاومة تجاه الفانكومايسين بتراكيز مختلفة تبدا من 0.03 الى 200 ملغم/مل حيث تم استخلاص اوراق الاثل بواسطة الايثانول 96% في جهاز الاستخلاص سوكسلت باستخدام الكروماتوغرافيا الغازية / المطياف الكتلي , اشارت النتائج ان مكونات المستخلصات الكحولية لاوراق نبات الاثل هي الزيوت والتي تحتوي على مجموعة متنوعة من المواد الكيمياوية بما في ذلك Octadecadienoic acid, methyl ester (20.06%)-9,12 يتبعها Phytol, acetate (18.93%) بعدها Hexadecanoic acid, methyl ester (12.14%). حدد التركيز المثبط الادنى (1.5) ملغم/مل من التركيز المثبط (MIC) للنشاط المضاد للسلالة hVISA . تم الكشف عن التعبير الجيني باستخدام تقنية تفاعل البوليميراز المتسلسل الكمي في الوقت الحقيقي قبل المعاملة بمستخلصات اوراق الاثل وبعده, كان التعبير الجيني منخفضا بعد العلاج بمستخلصات اوراق الاثل, في الختام مستخلص اوراق الاثل هو دواء بديل واعد يمكنه علاج الاصابات التي تسببها hVISA .

الكلمات المفتاحية: الفعالية المضادة للمايكروبات, المكونات الكيميائية, الزيوت الاساسية و التعبير الجيني.

Received:11/5/2022, Accepted:7/8/2022

INTRODUCTION

Plants have a significant scientific output when used for medical research. Scopus scientific literature on medicinal plants shows rapid progress in the last 20 years, peaking in 2010. Since this year, over 5000 books have been published annually (34). With the rising occurrence of medication resistance among common diseases, as well as the accompanying risk of chemotherapeutic medicines, finding an alternative to existing drugs has become critical. In ancient times, herbs bear beenchronic within dense areas, together with nutrition, medicine, flavoring, beverages, cosmetics, etc (1, 4, 17) Researchers and scientific investigations have recently increased their interest in herbs and medicinal plants with beneficial components for medicinal and therapeutic properties. Using medicinal plants to treat gram-negative and gram-positive bacterial infections caused by *Staphylococcus* spp., *Streptococcus* spp., and *Aeromonas hydrophila* are becoming more common (14). As time passes, more aware of the nutrients found in nature beneficial to our health. Were become our desire to learn more about these elements grows due to it. *Tamarix aphylla* L**.** is a well-known species in the Tamarix genus and Tamaricaceae family (5); Among nosocomial infections, multidrug-resistant bacteria are the most concern to public health (19) *Staphylococcus aureus* is a bacterium that has the potential to cause a wide range of community and hospital-acquired illnesses (42); *Sthaphylococcus aureus* is a major human bacteria pathogen causes various clinical manifestations and fund in the environment also is normal flora on the healthiest people skin and mucous membranes (most often the nasal area (4). Bloodstream inflammation, endocarditis, Bacteremia, osteomyelitis, wound infections, and urinary tract infections are all caused by *Staphylococcus* spp. (10). Heterogeneous vancomycin-intermediate *staphylococcus aureus* (hVISA) are MRSA subpopulations with intermediate vancomycin resistance (typically 1 bacterium per CFU 10^{5}–10^{-6}); The hVISA phenotype has been discovered in clinical MRSA strain worldwide. Rates ranged from 2% to 50%, depending on the location and the method used (16). Patients with the hVISA phenotype had a higher rate of endocarditis (IE) complications such as persistent bacteremia and heart failure (16). Infection by hVISA causes great mortality and morbidity in large numbers of patients in most countries, so this study was aimed to isolated the hVISA from humans and use the ethanolic extract of the *T. aphylla* plant against hVISA, as a potential strategic option for chemical medications harmful to humans, animals, and the environment. However, more research is needed to identify the efficacy of other plants' active chemicals, which might significantly increase in value in the pharmaceutical industry and give a cost-effective treatment option with few side effects.

MATERIALS AND METHODS

***Tamarix aphylla* L. leaves extract Preparation:** The *T. aphylla* leaves Figure 1, collected from the 64th kilometer west of Baghdad (AL-Fallujah), diagnosed in the Department of Biology-College Science-Mustansiriyah University-Baghdad-Iraq. The plants were washed and dried in a 30-35°C incubator; after drying, crushed the gathered leaves in an electric mill, according to Kőszegi et al. (23), with some modification to the original technique. Using the Soxhlet apparatus at 40-60^{o}C (6 cycles per hrs.), continue for 8 h., extracted powder (25g) in ethanol (96 %) solvent (250ml) in a 1:10 ratio. The extracts were then filtered through a Whatman No. 1 filter paper, the solvent was evaporated using a rotary vacuum evaporator, and kept the extracts were at 4°C for subsequent analysis.

Fig 1. *TAMARIX APHYLLA* L. Tree

GC-MS Analysis: This study used an Agilent 7820 Gas Chromatography system (Agilent Technologies, Wokingham, United States);

coupled to an Agilent 5977 MSD with an Agilent HP-5MS Ultra Inert column (30m length x 250m diameter x 0.25m inside diameter) Gas Chromatograph-Mass Spectrometer (GC-MS) to analyze the ethanolic extract of *T. aphylla* leaves. With 70 eV ionization energy, an electron ionization device was in electron impact mode. A carrier gas of 99.999 % purity was employed as a carrier gas, with a constant flow rate of 1 ml/min and a volume injection of 1µl of extracted material (splitless). Set the temperatures of the injector and detector at 50-280°C. And arranged the column's oven temperature to be 50°C for 1 min.; 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. Determined Peaks were by comparing mass spectra to a mass spectral database.

Collection of Samples: This study collected one hundred sixty-five samples, 85 from inpatients and 80 from outpatients, including sputum, ear infections, pus, wounds, burns, blood, and foot ulcers from local hospitals in different Baghdad cities (Al-Yarmouk Teaching Hospital, Central Pediatric Teaching Hospital, Al-Karama Hospital, al Furat General Hospital, and Ibn Al-Bitar Center for Cardiac Surgery Hospital) at (February 2021 - January 2022). All research requirements were completed at the College of Science / Mustansiriyah University.

Bacterial isolation: Mannitol salt agar and Brain heart infusion agar were used to isolate *Staph. aureus*, based on morphological, biochemical, physiological were carried out according to Bergey's Manual of Systematic Bacteriology (11).

Detection of Local Methicillin-resistant *Staphylococcus aureus* (MRSA) strain: MRSA strains were detected with CHROMagar^{TM} MRSA medium as a selective MRSA medium. The color changed from pink to mauve, indicating positive colonies. The *mec* gene was discovered via PCR, a gene Methicillin resistance gene (10).

Standard E-test for Screen heterogeneous vancomycin intermediated *Staphylococcus aureus* (hVISA): The Etest is a screening method for hVISA that determines Vancomycin MICs. This way, we inoculated 10 µl of 0.5 McFarland of all MRSA on plates of Mueller-Hinton agar (MHA) (Oxoid, UK) with 5% blood, using vancomycin Etest strips (BioMerieux, Marcy l'Etoile, France) with incubation 48 h. We Evaluated MIC values at the actual endpoint, which the manufacturer recommends for results reporting. The test is positive when the MIC of the vancomycin is ≥8 µg/ml. After complete inhibition, we read the zones to visualize hazy development or microcolonies with care (9,16,18).

population analysis profile and area under the curve (PAP-AUC) technique: A small number of colonies were cultured into BHI broth and incubated at 37°C overnight; then, this study prepared logarithmic serial dilution (10^{-3}, 10^{-6}, and 10^{-8}) in 0.9% sterile saline there after inoculated 100 µl of each dilution on the surface of BHI agar plates containing vancomycin of concentration 0-8 µg/ml. GraphPad Prism 9.3.0 software (GraphPad Software Inc., San Diego, CA, USA) used CFU/ml numbers to calculate AUC. In the PAP technique, hVISA (ATCC 700698, Mu3), VISA (ATCC 700699), and *Staph. aureus* (ATCC-29213) was used as standard strains; if the AUC ratio of test to control was between ≥0.9 and ≤1.3, the strain was classed as hVISA, and if it was ≥1.3, the strain was classified as VISA (16).

Determination plant extracts inhibitory concentration: A 96-well microtiter plate and the microdilution technique determined the minimum inhibitory concentration (MIC); according to Jouda *et al*. (21), plant extract stock solutions were two-fold serial dilutions ranging 0.39-200 mg/ml prepared in 50 µl of Mueller Hinton broth (Oxoid, UK). Then, adjusted the inoculam to 0.5 McFarland; the inocula added 10 µl of to each well except for the positive control and incubated plates at 37°C for 18–20 hours. Positive control was plant extract with media, and negative control was inoculum with media. After incubation, add 50 µl of 0.1%. 2, 3, 5-triphenyl tetrazolium chloride (TTC) solution for each well as an indicator solution, then incubate the plates for 45-60 minutes at room temperature to determine bacterial growth. The MIC of plant extract concentration was the lowest

cons., after incubation, the growth reduction of tetrazolium dye to red/pink. Growth inhibition occurs if the well solution stays clear following TTC incubation.

RT-Qpcr: The experiment was run befor and after treating hVISA with *T.aphylla* leaves extract. The TRIzol Reagent extraction method was used to extract the total RNA (6). The *hld* gene expression was evaluated using 100 ng/µl of RNA. Following the master amplification process with the One-Step RT-PCR software, used GoTaq®1-Step RT-qPCR System (Promega, USA) to assess the expression of (*hld*) genes. It's a reagent system that uses a one-step RT-qPCR protocol to perform quantitative RNA analysis. Perform the procedure with a 10 µl reaction volume, as directed by the manufacturer. A critical component of the bacteria regulatory system is the delta-lysin gene (*hld*), which is positively controlled by the gene *agr* and is transcribed from an upstream promoter. Using the primers for the *hld* gene were forward primer (FP) 5`-ATTTGTTCACTGTGTCGATAATCC-3` and reverse primer (RP) 5`-GGAGTGATTTCAATGGCACAAG-3` (38) 16srRNA: FP 5`-CTGCTGCCTCCCGTAG-3` and RP 5`-CCGACCTGAGAGGGTGA-3` (20). It was created by Macrogen (Korea). The ΔΔCt method was used to analyze the expression of selected genes that normalized against housekeeping (6).

RESULTS AND DISSCUSION

Chemical component of *Tamarix aphylla* L. leaves oils extract: After analyzing the chemical compositions of *T. aphylla* leaves extract by GC-MS comparing the constituents with the NIST11 library, the main compounds were observed 99.98% of the essential oil content The quantitative values of essential oil compounds to identified compounds with retention time indices are in Table 1. The highest was 9,12-Octadecadienoic acid, methyl ester (20.06%), followed by Phytol, acetate (18.93%), and Hexadecanoic acid, methyl ester (12.14%), follwed 6-Octen-1-ol, 3,7-dimethyl-, acetate (9.34%), then Hexadecanoic acid, ethyl ester (7.48%), were in leaves. 2-Methoxy-4-vinylphenol was the first component detected, with a retention time of 13.785 minutes, and 2-Dodecen-1-yl(-) succinic anhydrid was the final element noticed, with a retention time of 29.592 minutes, as indicated in (Table 1). According to peak areas, the most abundant chemical in the *T. aphylla* leaves extract was 9,12-Octadecadienoic acid, methyl ester, with a peak area of 20.06. While 2-Methoxy-4-vinylphenol, Oleic Acid, and cis-9-Hexadecenoic acid were the lowest chemicals, having a peak area of 0.3.

Table 1. Chemical composition of *Tamarix aphylla* L. leaves extract

Reaction Time	Constituent	Area %
13.785	**2-Methoxy-4-vinylphenol**	**0.32**
14.392	**2,2,6-Trimethyl-1-(3-oxo-but-1-enyl)-7-oxa-bicyclo[4.1.0]hept-4-en-3-one**	**0.34**
14.444	**Ethanone,1-[5-(1-hydroxyethylidene)-1,3-cyclopentadien-1 yl]-**	**0.48**
17.094	**1-Cyclohexene-1-acetaldehyde, 2,6, 6-trimethyl-**	**0.38**
18.564	**Acetic acid, 1-methyl-3-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-propenyl ester**	**0.73**
18.709	**Homovanillic acid**	**0.33**
19.017	**Phytol, acetate**	**18.93**
19.342	**6-Octen-1-ol, 3,7-dimethyl-, formate**	**5.66**
19.590	**6-Octen-1-ol, 3,7-dimethyl-, acetate**	**9.34**
20.240	**Hexadecanoic acid, methyl ester**	**12.14**
21.035	**Hexadecanoic acid, ethyl ester**	**7.48**
21.872	**Geranyl isovalerate**	**0.61**
22.394	**9,12-Octadecadienoic acid, methyl ester**	**20.06**
22.625	**Heptadecanoic acid, 10-methyl-, methyl ester**	**6.97**
23.086	**1,2-15,16-Diepoxyhexadecane**	**2.74**
23.120	**11,13-Dimethyl-12-tetradecen-1-ol acetate**	**2.63**
23.351	**Octadecanoic acid, ethyl ester**	**3.22**
24.642	**cis-Vaccenic acid**	**0.39**
24.839	**Oleic Acid**	**0.32**
25.471	**cis-9-Hexadecenoic acid**	**0.32**
26.078	**5H-3,5a-Epoxynaphth[2,1-c]oxepin, dodecahydro-3,8,8,11a-tetramethyl-, [3S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.)]-**	**0.80**
26.232	**Ethyl iso-allocholate**	**1.17**
28.121	**Vitamin E**	**3.46**
28.164	**dl-.alpha.-Tocopherol**	**0.66**
29.592	**2-Dodecen-1-yl(-)succinic anhydride**	**0.50**

Bold values represent the majority compounds of the essential oil

***Staphylococcus aureus* identification**

Staphylococcus aureus was detected in 89 (53.9%) samples from 165 inpatients and outpatients using the usual culture method and microscopic features. Eighty-nine strains developed smooth, creamy, transparent, yellow-pigmented colonies on mannitol salt agar. When examined microscopically, the bacterial cells reacted positively to the gram stain and appeared as clusters of grapes. When tested for catalase and coagulase, 89 strains were found to be positive, but none were positive for oxidase in biochemical assays.

Methicillin resistant *Staphylococcus aureus* (MRSA) identification: Isolated MRSA by cultivating on CHROMagar™ MRSA, which inhibited all MSSA strains while allowing MRSA strains to grow (Figure 2). 30 *Staph. aureus* isolates developed rose to mauve color and were recognized as MRSA. Detection of the *mecA* gene discovered that 30 (33.7%) of the 89 *Staph. aureus* isolates were MRSA, 19 (63.3%) and 11 (36.7%) coming from inpatients and outpatients, respectively.

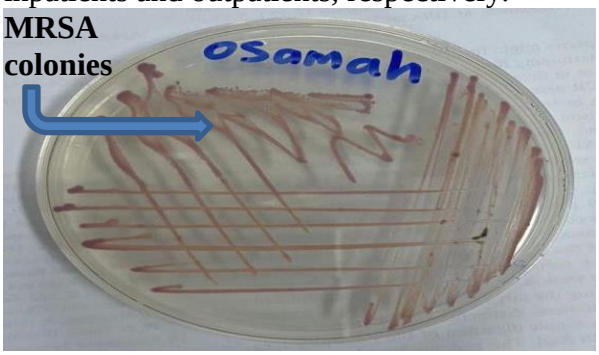
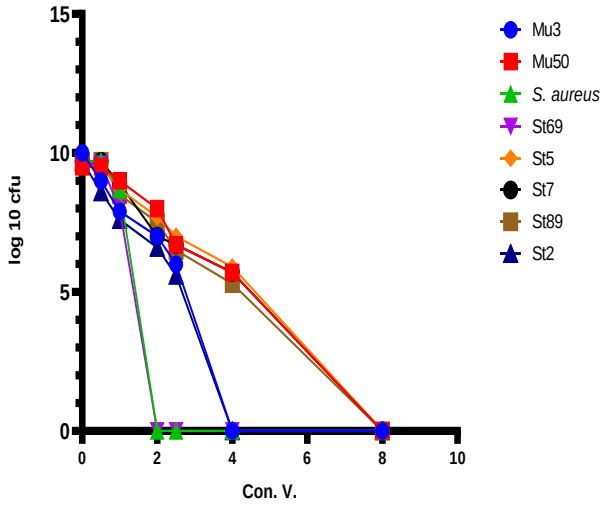


Fig 2 . MRSA isolate on CHROMagar™ MRSA media

Heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) identification: With the (PAP-AUC) technique, one MRSA strain (3.3%) displayed the hVISA phenotype; this was isolated from an older man (73 years patient with bacteremia Two MRSA strains (6.6 %) from this study were positive for hVISA according to the Etest, with ≥8 µg/ml MIC values. Most of the studies initially used a variety of screening tests before confirming positive screening tests by the PAP method, the isolation screen by the Agar screen method, and the E-test, most of which were initially positive. In a study by **Sancak *et al*. (35),** they tested 256 clinical MRSA isolates from 256 individuals; they confirmed positive results using the PAP-AUC technique after screening with BHI agar containing vancomycin; their research discovered 46 (17.97%) hetero VISA isolates.In this study used both methods to test all 30 strains. This study confirmed 1 (3.3%) as hVISA, with 3 strains (10%) as VISA and one strain as VSSA by the PAP-AUC method, which remains one of the gold standard techniques for confirming VISA and hVISA from specimens. Figure 3. The Strain St69 was non-viable at 2 µg/mL in this study (Figure 3) Mu3 showed hetero-resistance, with another population surviving at 2.5 µg/mL, and St2 had no surviving colonies at 4 µg/mL. Mu50 had consistently high counts until the vancomycin concentration reached 8 g/mL; the population profiles for St5, St7, and St89 remained constant until the vancomycin concentration reached 8µg/mL, at which point the counts for these strains dropped to 0 CFU/mL.



vancomycin concentration

Fig 3. Population analysis profile curves, one hVISA positive isolate. Three isolates were VISA, and one was vancomycin-susceptible *S. aureus* (VSSA) compared to the Mu3 reference strain

Determination of MICs

The results revealed that the MIC value was 1.5 mg/ml as a sufficient to stop the growth of heterogeneous vancomycin intermediated *Staphylococcus aureus* (hVISA) strain, which was vulnerable to this Essential oil. The MIC values of serial dilutions of *T. aphylla* leaves extracts on microbial growth with (hVISA) are shown in Table 2.

Table 2. Minimum inhibitory concentration of *Tamarix aphylla* L. essential oil (MIC).

MIC (mg/ml) / Bacteria	200	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39
hVISA ST1	-	-	-	-	-	-	-	-	+	+

: Inhibition; + : Growth

Determination of gene expression

The Quantitative RT-PCR technique evaluates the *hld* gene expression, which is responsive to the generation of delta-hemolysin, by comparing hVISA growth before and after treatment with *T. aphylla* leaves extract. Housekeeping gene (H.K) was used as a calibration; the results showed a decrease in gene expression. The amplification was recorded using quantitative RT PCR software as a Ct value (cycle threshold). Low gene expression is indicated by high Ct values, whereas low Ct values indicate high gene expression. Total RNA was successfully extracted from the isolated after confirmation by the PAP-AUC in this study. The control group's RNA concentrations ranged from 70 to 160 ng/µl. At the same time, it varies from 80 to 160 ng/µl in the treated group. Purity levels in the control group ranged from 1.7 to 1.9, and the purity of RNA-treated samples ranged from 1.8 to 1.9. The gene expression level was quantified using the ΔCt value and folding ($2^{-\Delta\Delta Ct}$) method, which normalized to the level of a housekeeping gene. The expression of the *hld* gene after treatment with *T. aphylla* leaves extract is shown in Figures 4. Low gene expression is indicated by a significant difference in the mean increased Ct values. After treatment, the expression of the *hld* gene was not equal. Table 3 with Figure 4 shows the expression *hld* gene according to treatment with *T. aphylla* leaves extract. The mean Ct value of *hld* gene amplification was [22.74] in control. The Ct values treatment was a mean [29.39]. A significant difference in the mean increased Ct values indicates low gene expression. Expression of the *hld* gene was low after treatment.

Table 3. Genetic expression values for *hld* gene after and before treatment

Sample	16srRNA	*hld*	DCT	DDCT	Folding
hVISA	8.75	19.74	11.00	0.00	1.0
hVISA Treated	9.76	26.36	16.60	5.61	0.02

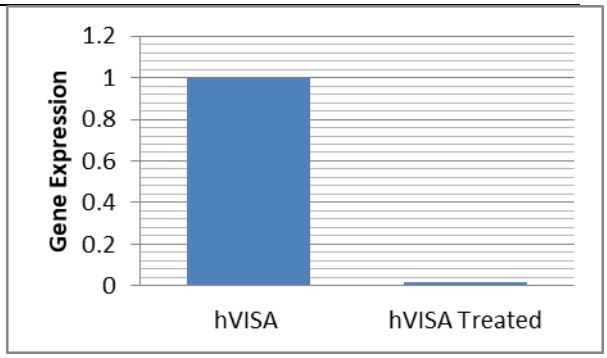


Figure 4. Inhibitory effect of the *T. Aphylla* leaves extract on the hVISA strain, relative expression ratios (ratio of the gene to action) of (*hld* gene) encoding delta hemolysin. And housekeeping gene 16srRNA as a normalized transcription of *hld* by the comparative CT method.

According to our knowledge, this study is unique in Iraq because obtain the it isolates of Heterogeneous Vancomycin Intermediated *Staphylococcus aureus* (hVISA) locally from patients with (bacteremia) with vancomycin treatment failure. And studies the effect of *Tamarix aphylla* L. extract those isolated. With estimated the gene expression after being treated with *Tamarix aphylla* L extract; many pathogenic like hVISA have developed antibiotic resistance due to increased use. As a result, scientists began to find a replacement for chemotherapeutic medications, particularly those derived from plants that are easily obtainable, inexpensive, and have less documented side effects (14). This study investigated antibacterial activity of Iraqi *T. aphylla* leaves against hVISA. The findings revealed that an ethanolic extract of *T. aphylla* leaves has antibacterial activity against the human pathogen hVISA. This result are comparable with Adnan et al. (2), who discovered that *T. aphylla* leaf extract has antibacterial activity against *Staphylococcus aureus*. As shows in Tables 2, revealed that *T. aphylla* leaves extract had activity against hVISA. This activity may be due to different chemical compounds which play the primary role in inhibiting pathogenic growth. The GC–MS analysis results demonstrate the various chemical compounds that showed many biological activities, such as 9,12-

Octadecadienoic acid and methyl ester, found to have an antioxidant and antimicrobial activity effect (30). Moreover, the phytol acetate showed antioxidant and antimicrobial inhibitory action against *B. Subtilis* and *Staph. aureus* (15). Addition, Hexadecanoic acid methyl ester showed antibacterial potency against *Pseudomonas aeruginosa* D31, *Staphylococcus aureus* W35 and *Klebsiella pneumoniae* DF30 (36). Alrumman (7) indicated that the main component of the *T. aphylla* leaves extracts grown in Saudi Arabian were propenoic acid (28.99%), beta-d-mannofuranose, (23.04%) and Hexadecanoic acid (13.29%). Alhourani et al. (4) pointed out that the components of the essential oil of *T. aphylla*, which is grown in Jordan, contained: 6,10,14-trimethyl-2-pentadecanone, (32.39%), β-ionone, (13.74%) dodecanoic acid (6.00%), and tetradecanoic acid (3.35%), were present in its aerial parts. The difference in the significant elements of the extract is due to variation in the cultured area for the plant; The change in the region's geographical nature and environmental conditions significantly impacts the quality and quantity of extracted essential oil from the same plant species and cultured in different areas (8); the procedures employed to extract the oil impact the quality and amount of the essential oil produced (13). In this study, detected MRSA with Chromogenic agar makes it easier to isolate from primary isolation plates for up to 24 hours after enrichment, without additional biochemical tests (25). On the other hand, PCR-based methods are the most reliable for detecting MRSA (33). This study Identified 30 MRSA, of which 19(63.3%) and11 (36.7%) were from the inpatients and outpatients, respectively. The results showed that (3.3%) and (6.6%) displayed hVISA phenotype by using (PAP-AUC) and Etest methods, respectively from the total MRSA isolated. Chaudhari et al. (9) observed that the prevalence of hVISA was 6.9% with PAP-AUC and macro method Etest. This study prevalence rate of hVISA is consistent with a study by Iyer and Hittinahalli from India that shows an hVISA prevalence rate of 2% (18). In Italy, France, and The Netherlands, the majority of hVISA in MRSA was 1.1% (27), 0.6% (31), and 6% (39), respectively. Thus, hVISA prevalence appears to occur with slight differences among countries, although this is partially due to detection methods. Nevertheless, some actual differences among countries do occur. According to this study, the hVISA strain is more prevalent in blood-borne MRSA, similar to Zhang *et al.* **(43),** who discovered hVISA frequencies in blood culture samples, Linked vancomycin heterogeneous resistance to many clinical outcomes. Moore et al. (28) confirmed the failure of vancomycin therapy in MRSA endocarditis caused by an hVISA strain. Maor *et al.* (26) defined 27 (hVISA bacteremia) cases to 223 control individuals (MRSA bacteraemias). Compared to controls, the hVISA bacteremia was linked substantially with extended endocarditis and osteomyelitis infection rates. In addition, Rose *et al.* (32) found that vancomycin exhibited inadequate efficacy against clinical strains of hVISA at suitable dosages (AUC/MIC, 105–317) in an *in vitro* pharmacokinetic/pharmacodynamic model. The result also estimated the antibacterial activity of *T. aphylla* leaves ethanolic extract using MIC assays. Present study showed that the essential oil of *T. aphylla* leaves extracts possesses an antibacterial activity in the tested strain; with the MIC value was 1.5 mg/ml, sufficient to stop the growth of hVISA. This value was consistent and lower than the value reported by Al Sobeai (3), who revealed that the minimum inhibitory concentration that inhibits the growth by ethanol extract of *T. aphylla* leaves were 1.5, 22, 20, 15, 15, 15, 10, 10, 5, and 5 (mg/ml) for *Listeria monocytogenes, Klebsiella pneumoniae, Streptococcus pyogenes, Shigella sonnei, Enterococcus faecalis, Salmonella enterica subsp. enterica Typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis,* and *Proteus mirabilis,* respectively. Also, the work carried out by Shabani and Sayadi **(37)** on the antibacterial activity of *T. aphylla* smoke showed a significant efficiency on all the following strains (*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus faecalis, and Streptococcus pyogenes*). Differences in the antimicrobial effects of different *T. aphylla* extracts could be due to strains with varying resistance profiles. It could be due to the

different solubilities of various compounds found in *T. aphylla* and the various methodologies used, such as other solvents for extract preparation or different MIC determination techniques. The accuracy, sensitivity, and speed of reverse transcription-quantitative PCR (RT-qPCR) distinguish it from other gene expression methods. For gene expression analysis, this technology has become the gold standard. Focused the experiments on comparing the expression levels of the *hld* gene across different samples. In this investigation, the SYBR green fluorescent dye was utilized, which identifies any double-stranded DNA, including cDNA The optimal approach for measuring the *hld* gene is real-time RT-PCR. It quickly performs absolute number and repeatable multi-analysis on many samples (24). By encoding the *hld* gene and regulated by *agr*, delta-hemolysin is unique from the other secreted virulence factors encoded by the *hld* gene and regulated by *agr*. Formed from the translation of RNAIII, the *agr* effector molecule (41). utilized Delta-hemolysin expression can be as a marker of *agr* function in an hVISA strain *Tamarix a phylla* L. leaves extract were tested on the hVISA strain. The PCR (RT-qPCR) was used to evaluate the expression of the *hld* gene after 18 to 24 hours of exposure to *T. aphylla* leaves extracts, with the 16srRNA gene serving as an internal control (12). According to the findings, when *T. aphylla* leaf extracts were given at a concentration of 1.5 mg/mL, the expression of the hVISA *hld* gene was decreased compared to the control sample before exposure. Prior research of three medicinal herbs (*Ballota nigra*, *Castanea sativa*, and *Sambucus ebulus*) found a dose-dependent response in the production of δ-hemolysin in a pathogenic MRSA strain suggesting considerable anti-quorum sensing action (29). Understanding the quorum sensing system could benefit disease prevention management and antimicrobial therapy, as it modulates the expression of δ-hemolysin. Can control Bacterial infections with quorum sensing inhibitors (QSIs), which effectively prevent multidrug-resistant organisms from developing (22). In response, researchers are looking for novel compounds with QSI potential. Plant cells may be able to create QSI chemicals to combat QS infections since they lack a robust immune system to fight bacterial infections (22). QSI capabilities have been demonstrated in prior investigations on plant species like garlic, ginger, and turmeric (22,40). Following treatment with 1.5 mg/ml of *T. aphylla* leaves essential oil, the *hld* gene was significantly down regulated. This study revealed that *T. aphylla* essential oil acts as a QSI agent against hVISA cells; this research establishes *T. aphylla* essential oils as a viable agent for preventing and removing the hVISA effect at 1.5 mg/ml sub-MIC concentrations. Nonetheless, will be required more effort in the future to conduct clinical trials of these essential oils.

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