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Plasmid Profile, Curing Analysis, and antibacterial Activity of *Alcea arebellensis* plant Against Multidrug Resistance *Staphylococcus aureus*

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Abstract
A total of 50 *Staphylococcus aureus* isolates were identified from burn , otitis media, wound and urine infection from patient admitted to the central lab and internal lab of teaching hospital and Rizgary hospital in Erbil city during the period 10thNovember 2011 to 30th March 2012. All the isolate identified according to Microscopical, cultural and biochemical tests. *S. aureus* tested for antibiotics susceptibility test using agar diffusion method .The antibiotics susceptibility testing showed that 49(98%) , 48(96%), 48(94%), 9(18%),8(10%), 7(14%), and 4(8%), were resistant to chloramphenicol , Amikacin, ampicillin, Vancomycin ,Cefotaxime, Cefazolin ,and ceftriaxone but all the isolates were sensitive to amoxicillin. Also the results showed that isolate no.38 was resistant to seven antibiotics under study which contain five plasmids with molecular weight more than 10 kbp. The results also revealed that 5(10%), 25(50%), and 20(40%) of *S.aureus* were reported to produce alpha, beta, gamma hemolysis respectively. Results of plasmid curing experiments revealed that elevated temperature at 46^{o}C were completely remove the plasmid that harbor antibiotic resistance gene for the antibiotic understudy while for bleomycin at 50µg/ml had lower effect. The antibacterial activity of aqueous and alcoholic extract of flowers and leaves of the *Alcea arebelensis* was studied against some resistant isolates of *S. aureus*, the results showed the inhibitory effect of plant extracts against selected isolates except the alcohol leaf extract which showed no inhibitory activity against all studied bacterial isolates.

Key words: *Staphylococcus aureus*, antibiotic resistance, plasmid curing, and *Alcea arebelensis*

Introduction
S. aureus one of the pathogens of major concern because of its ability to cause a diverse array of diseases ranging from minor infections to life threatening septicemia and its ability to adapt to adverse environmental conditions[1]. Microbial resistance to antibiotics, especially among staphylococcal strains, is a major threat to public health and treatment of serious *S. aureus* infections can be challenging as the widespread use of antibiotics has led some *S. aureus* becoming more resistant to antibiotics[2]. When antibiotics are used incorrectly, the target bacteria will directly adapt and develop resistance. Then, with its rapid multiplication, bacteria passes resistant genes through plasmid exchange, leading to an increasing prevalence of multi-drug resistant infections. However, not all microorganisms are equal in inducing resistance against antibiotics; this is related to many factors, such as whether the antibiotic is a concentration or time-dependent killing agent, its effects against the population of bacteria and its duration of the serum concentration in patient[3]. The levels of antibiotic resistant infections in the developing world have increased steadily in the last few decades as a result of combination of microbial characteristics and the selective pressure of antimicrobial use [4].Multidrug-resistant bacteria in both the hospital and community environment are important concern to the clinician, as it is the major cause of failure in the treatment of infectious diseases, increased morbidity, and mortality and the evolution of new pathogens[5]. Plasmids allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera[6]. Plasmid profiles determination is the earliest DNA based method used as reference patterns for detecting certain strain with possible variation in plasmid content which is very important in epidemiological studies. Now a day the most important thing which microbiologists try to control the resistant of bacteria against antibiotic and they attempt or try to know the reason behind this problem and how they controlled it, so the scientists tried to solve this problem by using medicinal plants[7,8]. *Alcea arebelensis*is a member of the Malvaceae family, popularly known as Hollyhock; it is Perennial herb, found only in a restricted range in Erbil/ North Iraq, this species like other species with simple variation [9]. *Alceaspecies* used as traditional medicine for various diseases such as antiviral [10], anti-inflammatory, astringent, demulcent, diuretic, emollient, febrifuge, blood circulation, for the treatment of constipation, dysmenorrhoea, haemorrhage [11].

This study was carried out to determine the virulence factor, plasmid profile and curing analysis of multi-drug resistant *S. aureus* with bleomycin and elevated temperature. On the other hand the inhibitory effect of aqueous and alcoholic extract of flowers and leaves of the *Alcea arebelensis* was studied against some resistant isolates of *S. aureus*.

Material and methods
Specimen collection
Fifty isolates of *S. aureus* were identified among 175 sample were collected from clinical specimens including burn ,otitis media , wound ,and urine from different hospitals in Erbil cityas clarified in Table (1). All isolates were identified depending on morphological, cultural and some biochemical tests.

Table (1) Distribution of *S. aureus* according to the source of isolation

Source of isolate	Number of sample	Number of *S. aureus*
Burn	37	10
Otitis media	46	12
Wound	43	8
Urine	49	20
Total	175	50

Antibiotic Resistance test

To study the effect of different antimicrobial on *S.aureus* ,isolates, Muller Hinton agar was used as growth media, after sterilization and cooling at 45°C, final concentration of antibiotics as cleared in Table (2) were added to media and poured into sterile plates. After solidification, the plates were inoculated by streaking method with *S. aureus* isolates then incubated at 37°C for 24 hours. The results were recorded next day[13,14].

Table (2) Represent Antibiotics that used in this study

Antibiotics Names	Symbol	Stock Solution mg/ml	Final Concentration µg/ml
Chloramphenicol	Chl	10	10
Ceftriaxone	Cefn	10	30
Cefotaxime	Cefom	10	30
Amoxicillin	Amk	10	25
Ampicillin	Amp	10	50
Cefazolin	Cefz	10	30
Amikacin	Amk	10	25
Vancomycin	Van	10	30

Plasmid profiling and agarose Gel electrophoresis

The plasmid of S. *aureus* was extracted using GF-1 kit manufactured by Vivantis Company and including following steps:

Preparation of stock culture growing 5-10ml plasmid containing bacteria cells in LB medium with appropriate antibiotic overnight (12-16 hours)at37°C with agitation. Centrifugation: Pellet 1.5-5mlof bacterial culture containing the plasmid by centrifugation at 6,000 × g for 2Min .If 15ml or 50ml centrifuge tube is used to harvest the cells, centrifuge at 6.000 × g for 5Min. The supernatant Decanted completely. Resuspension of pellet: Two hundred fifty µl S1 will be added to the pellet and resuspend the cells completely by vortexing or pipetting, then the suspension transferred to a clean.1.5ml microcentrifuge tube. Alkaline lysis: Two hundred fifty µl of S2 was added and gently mixed by inverting tube several times (4-6 times) to obtain a clear lysate. Incubated on ice or at room temperature for not longer than 5 min. Neutralization: To neutralize the lysate, 400 µl of Buffer NB was added and gently mixed by inverting the tube several times (6-10 times) until a white precipitate forms. Centrifugation at 14.000-16.000× g for 10 min. loading to column: the supernatant transferred into a column assembled in a clean collection tube (provided). Centrifuge at10, 000 × g for 1min. Flow through discarded.

Column washing: the column will be washed with 700µl Wash Buffer and centrifugated at 10,000× g for 1 min. flow through discarded. Column drying: the column Centrifugated at 10,000× g for 1min to remove residual ethanol. DNA elution: The column Placed into a clean microcentrifuge tube. 50 - 100µl of Elution Buffer added TE buffer or sterile water directly onto column membrane and stands for 1 min. Centrifuge at 10,000×gfor 1 min to elute DNA and stored at 4°C or -20°C.

Agarose gel electrophoresis techniques

Plasmid samples were visualized under UV light after electrophoresis in a 1% agarosegel[15].One g of powder added into 100 ml 1X TBE buffer (Tris /borate EDTA buffer). The mixture heated to boiling and cooled at 55 °C on water bath. Five µ1 of ethidium bromide (10mg/ml) added to the agarose gel solution to a final concentration of 0.5 µg/ml. The comb inserted into the electrophoresis gel chamber (10×12) cm and the agarose gel solution poured. After the gel solidification, the comb was removed gently and the gel was soaked in a gel tank contain 1XTBE buffer. Five µl of each sample were mixed with 1.5 µ1 of loading buffer dye, and the mixture was loaded into the agarose wells. DNA separations were performed using ~80V for 75-120 minutes. The gel was exposed to UV light and then photographed by digital camera.

Test for hemolytic activity

The hemolytic activities of the *S .aureus* were identified by the presence of diffuse (α-hemolysis) or clear (β-hemolysis) halos around the colonies and gamma (absence of hemolysis). A colony of each of the bacterial isolates was subcultured onto freshly prepared blood agar (nutrient agar containing human blood) plates incubated at 37°C for 24 hours, after which the colonies were examined for hemolytic activity[16].

Curing with Bleomycein

Elimination of antibiotic resistance plasmid DNA from *S. aureus* isolate was done by Bleomycein using the method described by[17]. Ten ml of nutrient broth containing 50 µg/ml Bleomycein was inoculated with 0.3 ml of overnight culture of *S. aureus* isolate, incubated at 37°C for 24hours, and then0.1 ml samples were diluted up to 10^{-7} and 0.1 ml of last three dilutions were plated on nutrient agar plates. Incubated for 24 hours, then 100 colonies replica plated on nutrient agar plated containing final concentration of appropriate antibiotics separately and the percentage of curing colonies were calculated.

Curing of plasmid DNA by elevated temperature

Ten ml of nutrient broth inoculated with a single colony of *S .aureus*isolate , after inoculation for 24 hours at 37 °C,5 ml nutrient broth culture inoculated with 0.2 ml of bacterial culture, incubated with shaking 100rpm at 46°C for 24 hours, serial dilutions were prepared up to 10^{-7}, then 0.1 ml of last three dilutions spread on plates of nutrient agar, then

incubated at 37°C till the colonies appeared, then 100 colonies replica plated on nutrient agar plated containing final concentration of appropriate antibiotics separately and the percentage of curing colonies were calculated [17].

Medicinal plants

Flowers and leaves of *A. arebelensis* were collected from gardens and parks in different places in Erbil city, Iraq and the plant was classified in the Education Salahaddin University Herbarium (ESUH).

Preparation of plant extracts

The fresh flowers and leaves were harvested, washed properly with tap water and rinsed with sterile distilled water and dried at room temperature to constant weight separately. The water and ethanol extracts of *A. arebelensis* (85 g) were obtained by solvent-distillation method using a soxhelt apparatus for 3 h. The solvent was evaporated at reduced pressure to constant and preserved until it used.

Preparation of the Test organisms

Colonies of fresh cultures of the more resistance isolates number 1, 10, 21 and38 of *S. aureus* were selected and suspended in 10ml of nutrient broth. Using tenfold serial dilution, in sterile normal saline the population density for the entire test isolates was determined. All cultures were incubated for 24hrs at 37 °C[18].

Determination of antimicrobial activity

Muller Hinton Agar was poured to sterilized plates. The plates were allowed to dry in the incubator for 30minutes at 37 °C and with the aid of a sterile standard cork borer, 6 wells were bored at equidistant, then 100 µl afresh cultures of a standardized inoculum (1–2 X 10^7 CFU/ml)of the selected isolates were inoculated. Different concentrations (1,5, 25, 75, 100, 125, 250, 375, 500, and 750) µg/ml of the aqueous and alcohol extracts of flowers and leaves of *A. arebelensis* was aseptically introduced into wells in addition to control. The plates were then incubated at 37 °C for 24 h and the diameters of inhibition zones were measured and evaluated. The average of the three readings was taken to be zone of inhibition of the bacterial isolates in question at that particular concentration [19,20].

Results and discussion

Bacterial isolation and identification

Fifty isolates of *S. aureus* were identified among175 isolates collected from different clinical specimen using Microscopical, cultural, and biochemical tests. From smear preparation are spherical cocci arranged in irregular cluster. Non spore forming and non-motile. It was characterized by their ability to ferment mannitol and turn the mannitol saltagar to yellow color; the colonies produce hemolysis blood agar. On nutrient agar the colonies were smooth raised,circular glistening, entire, translucent and the pigment range from deep golden to cream or white. The biochemical tests showed ability of *S. aureus*to ferment mannitol, sucrose, maltose, lactose, galactose, glucose also its positive for catalase, coagulase, citrate, but it was negative for indole, methylred ,and gelatin hydrolysis[21].

Antibiotic susceptibility testing

The antibiotic susceptibility of *S. aureus* isolates were described in Table (3). Of the fifty *S. aureus* screened for susceptibility to the 8 antibiotics the results showed that 49(98%) of the isolates were resistant to chloramphenicol, 48(96%) were resistant to Amikacin but for others were 47(94%), 9(18%), 8(16%), 7(14%), and 4(8%) for ampicillin, Vancomycin, Cefotaxime, Cefazolin and ceftriaxone respectively. On the other hand the results revealed that all isolates were sensitive to amoxicillin which is considered the most effective antibiotic against *S.aureus*. Data from the table also showed that most of the isolates showed multiple drug resistance (MDR) toward the antibiotics used and the percentage ranged between (87.5-12.5%). Furthermore S38 was resistant to seven antibiotics, and this may due to that this isolate produce enzyme that modify the antibiotics or have active efflux pump that pump the antibiotic out of the cell or carry five plasmids with molecular weight more than 10kbp that harbor resistance genes against large number of antibiotics while isolate S46 that sensitive to seven antibiotics carry only one plasmid with molecular weight more than 10kbp as shown in Figure (1).

Table (3) Results of antibiotic resistnat test for *S. aureus*

Isolate No.	Antibiotics								Percentage
	Chl.	Cefn.	Cefom.	Van.	Amp.	Amk.	Cefz.	Amx.	
1.	R	S	R	S	R	R	S	S	50%
2.	R	S	R	S	R	R	S	S	50%
3.	R	S	R	S	R	R	S	S	50%
4.	R	S	S	R	R	R	S	S	50%
5.	R	S	S	R	R	R	S	S	50%
6.	R	S	S	R	R	R	S	S	50%
7.	R	S	S	S	R	R	S	S	37.5%
8.	R	S	S	R	R	R	S	S	50%
9.	R	S	S	S	R	R	R	S	50%
10.	R	S	S	S	R	R	R	S	50%
11.	R	S	R	S	R	R	S	S	50%
12.	R	S	R	S	R	R	R	S	62.5%
13.	R	S	S	S	R	R	S	S	37.5%
14.	R	S	S	S	R	R	R	S	50%
15.	R	R	R	S	R	R	S	S	62.5%
16.	R	S	S	S	R	R	S	S	37.5%
17.	R	R	S	S	R	R	S	S	37.5%
18.	R	S	S	S	S	R	S	S	25%
19.	R	S	S	S	R	R	S	S	37.5%
20.	R	S	S	S	R	R	S	S	37.5%
21.	R	R	R	S	R	R	S	S	62.5%
22.	R	S	S	S	R	R	S	S	37.5%
23.	R	S	S	S	R	R	S	S	37.5%
24.	R	S	S	S	R	R	S	S	37.5%
25.	R	S	S	S	R	R	S	S	37.5%
26.	R	S	S	S	R	R	S	S	37.5%
27.	R	S	S	S	R	R	S	S	37.5%
28.	R	S	S	S	R	R	S	S	37.5%
29.	R	S	S	S	R	R	S	S	37.5%
30.	R	S	S	S	R	R	R	S	50%
31.	R	S	S	S	R	R	S	S	37.5%
32.	R	S	S	S	R	R	S	S	37.5%
33.	R	S	S	R	R	R	S	S	50%
34.	R	S	S	S	R	R	S	S	37.5%
35.	R	S	S	S	R	R	S	S	37.5%
36.	R	S	S	R	R	R	S	S	50%
37.	R	S	S	S	R	R	S	S	37.5%
38.	R	R	R	R	R	R	R	S	87.5%
39.	S	S	S	S	R	R	S	S	25%
40.	R	S	S	S	R	R	S	S	37.5%
41.	R	S	S	R	R	R	S	S	50%
42.	R	S	S	R	R	R	S	S	50%
43.	R	S	S	S	R	R	S	S	37.5%
44.	R	S	S	S	S	R	S	S	25%
45.	R	S	S	S	R	R	S	S	37.5%
46.	R	S	S	S	S	S	S	S	12.5%
47.	R	S	S	S	R	R	S	S	37.5%
48.	R	S	S	S	R	S	S	S	25%
49.	R	S	S	S	R	R	R	S	50%
50.	R	S	S	S	R	R	S	S	37.5%
Total	**98%**	**8%**	**16%**	**18%**	**94%**	**96%**	**14%**	**0%**	

These results were in agreement with [22] who found that *S.aureus* isolates from different clinical specimens were resistance 100% to methicillin, 86%, to penicillin, 76% to ampicillin, 56% to cephalosporin, 54% to chloramphenicol, 34% to Amikacin, and 28 % to Vancomycin. Furthermore [23] described among116 *S.aureus* isolates 75% were resistant to ampicillin and chloramphenicol, 100% to penicillin but all of them were sensitive to Amikacin. [23] showed that the resistance in bacterial population can be spread either by transfer of bacteria between people or transfer of resistance genes between bacteria usually on plasmid and by transfer of resistance genes between genetic element within bacteria on transposons or may chromosomally located. Susceptibly to antibiotics is changed in general and increase in antibiotic resistance has been shown worldwide. The main reason for this trend is

the increase in antibiotic consumption and the abused of broad spectrums antibiotics [24].

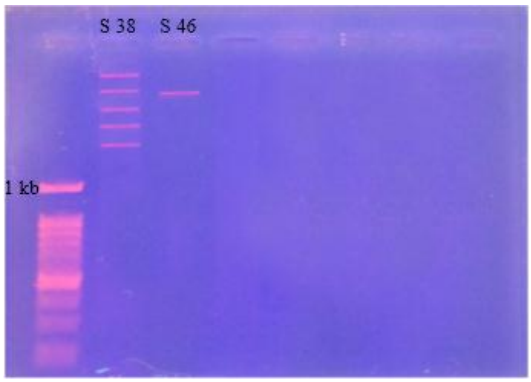

Figure (1) Plasmid profile of *S. aureus* isolates
Lane1: 1 kb DNA ladder , Lane2: Plasmid profile of isolate no.38 , Lane3: Plasmid profile of isolate no.46

Hemolysis production in *S.aureus*

Virulence factor (haemolysin production) of *S.aureus* isolates are shown in Table (4) with 5(10%), 20(40%), and 25 (50%) of *S.aureus* production alpha (diffuse hemolysis), beta (clear) hemolysis) and gamma (absence of hemolysis) respectively. Similar results were obtained by[25] who isolate the *S.aureus* from otitis media with 10(38.1%), 22(52.4%) and 4(9.5%) producing alpha, beta and gamma hemolysis. The hemolytic properties of this bacteria lead to the hemolysis of the erythrocyte, necrotizing the skin and cause unnecessary release of cytokines causing toxic shock syndrome [26].

Table (4) Number of occurrence and type of hemolysis produced by *S. aureus*

Bacterial sp	No. of isolates	α %	β%	δ%
S. aureus	50	5(10%)	25(50%)	20(40%)

Plasmid curing experiment

By elevated temperature

Elevated temperature at 46°C was used to cure plasmid DNA that confer resistance to antibiotics, from *S.aureus* isolate S38. Table(5) show that treated isolate appeared sensitive with percentage ranged between 89- 100% to all antibiotics under study. From the Figure (2) of plasmid DNA on agarose gel of cured cells for *S.aureus* isolates appear that the plasmid was lost (cured) by elevated temperature completely. From the above results, conclusion can be made that curing by elevated temperature is the efficient method for plasmid curing. This is may be due to that the enzyme of DNA replication are affected by these temperature. Our interpretation involve changing the shape (folding of the polypeptide) of the enzyme responsible for DNA replication of plasmid DNA including the plasmid that confer resistance to the antibiotics. This change in folding of DNA polymerase may convert it in to inactive enzyme at this temperature, i.e. the enzyme is sensitive to the elevated temperature .On the other hand, enzymatic activity decline above the specific temperature that is the characteristic of heat stability of the particular enzyme [27].[28] obtained more antibiotic sensitive cells after shifting the temperature from 30 to 40°C.

Also [17] found that 43°C effect on genes responsible for β-lactam antibiotics in *S.aureus* isolate and some isolates remain resistance to elevated temperature on curing of antibiotic resistance plasmid.

Table (5) Plasmid curing percent analysis of *S. aureus* with elevated temperature at 46 °C

Isolate No.	Antibiotics					
	Chl.	Amp.	Amik.	Ceft.	Cefom.	Van.
S 38	95	100	93	95	89	100

By Bleomycin

Bleomycin was used for curing of plasmid DNA that confers antibiotic resistance in *S.aureus* isolates. The concentration of bleomycin used was 50µg/ ml with the culture of isolateS38. Table(6) shows the effect of blyomycein on curing the plasmid DNA from isolates ofS38 .From the table it is clear the effect of bleomycin on antibiotic resistance gene harbored on plasmid DNA and all the antibiotic resistance genes were affected between 80-100% and the plasmid DNA was cured as shown in Figure (2).

Table (6) Plasmid curing percent analysis of *S.aureus* with Blyomycein

Isolate No.	Antibiotics					
	Chl.	Amp.	Amik.	Ceft.	Cefom.	Van.
S 38	100	88	88	90	86	80

Bleomycin act by induction of DNA strand breaks. Some studies suggest bleomycin also inhibit incorporation of thymidine into DNA strand. DNA cleavages by bleomycin depend on oxygen and metal ions, at least in vitro. It is believed bleomycin chelate metal ions (primarily iron) producing a pseudo enzyme that reacts with oxygen to produce superoxide and hydroxide free radicles that cleave DNA [29]. Our results coincide with [30] who used bleomycin to eliminate multi copy plasmid R6K from growing cells of *E. coli* AB1157. Measurement of R6K- encoded β–lactamase and covalently closed plasmid DNA indicated that the drug causes a progressive reduction in plasmid copy number.

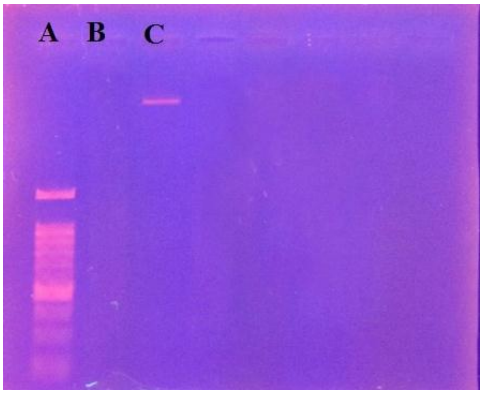

Figure (2) The curing analysis of plasmid in S 38 isolate
A: Ladder., B: Plasmids of S38 after curing with elevated temperature., C: Plasmids of S38 after curing with bleomycin.

Antibacterial activity of tested plant against some local isolates of *S. aureus*

The results of the present studies showed that the antibacterial activities of aqueous and alcohol extract of *A. arebelensis* flowers gave different mean zone diameter of inhibition on the most tested isolates (Table 7). The aqueous flower extract gave the mean zone diameter of inhibitions ranging from 12–14mm at concentration 500-750µg/ml for *S.aureus* isolate no. 1, 12–15mm for isolate no.10 at concentration 125-750µg/ml and 5–16mm at concentration 250-750µg/ml for isolate no.38, while isolate no. 21 was not inhibited. Whereas the alcohol flower extracts inhibited the growth of all the isolates giving a mean zone diameter of inhibition ranging from 5–25mm at concentration 250-750µg/ml for *S. aureus* isolate no. 1, while isolate no. 21 gave the mean zone diameter of inhibition 18 mm only in concentration 750µg/ml, 10–25mm for isolate no.10 at concentration 250-750µg/ml and 10–26mm at same concentration 250-750µg/ml for isolate no.38 respectively. The obtained results were similar with that reported by [31] when they found the inhibitory effect of *Alcea rosea* flowers extracts on different species of bacteria. The flowers of *A. rosea* are reported to contain mucilaginous polysaccharides, antocyanins and flavonoids [32,33].

Table (7) Antibacterial activity of *A. arebelensis* flowers extract against some local isolates of *S. aureus* understudy

No. of Isolate	Concentrations µg/ml										Extract
	1.5	25	50	75	100	125	250	375	500	750	
	Mean zone Diameter of Inhibition (mm)										
1	0	0	0	0	0	0	0	0	12	14	Aqueous extract
10	0	0	0	0	0	12	14	15	15	15	Aqueous extract
21	0	0	0	0	0	0	0	0	0	0	Aqueous extract
38	0	0	0	0	0	0	5	14	14	16	Aqueous extract
1	0	0	0	0	0	0	5	25	25	25	Alcohol extract
10	0	0	0	0	0	0	10	16	25	25	Alcohol extract
21	0	0	0	0	0	0	0	0	0	18	Alcohol extract
38	0	0	0	0	0	0	0	0	15	15	Alcohol extract

On the other hand the alcohol leaves extracts showed no inhibitory activity against some studied bacterial isolates and these results were in agreement with that reported by (34) when they found no inhibitory effect of *Alcea apterocarpa*on growth of different species of bacteria. Moreover, the Table (7) indicated that the aqueous leaves extracts of tested plant inhibited the growth of the tested isolates giving a range of 15–25mm at concentration 250-750µg/ml for *S. aureus* isolate no. 1, while isolate no. 21 gave the mean zone diameter of inhibition 19 mm only in concentration 750µg/ml, 10–25mm for isolate no. 10 at concentration 125-750µg/ml and 15–16mm at concentration 375-750µg/ml for isolate no.38 respectively. These results indicate that different parts of same plant species have different antimicrobial effects. Even if extracts are prepared using the same parts (flowers, leaves or fruits or seeds) of the same plant species, and physiological growth cycle of the plant may cause variations in the chemical compounds of the plant. In addition to these factors, the parts of plants, extraction process, solvent, and the species of bacteria that are used are also variables [35,36]. *Alcea* species has attracted the attention of researchers because of their antimicrobial potential besides antioxidant, anti-inflammatory and cytotoxic activities, particularly due to flavonoids and other phenolic constituents [37].

Table (8) Antibacterial activity of *A. arebelensis* leaves extract against some local isolates of *S. aureus* understudy

No. of Isolate	Concentrations µg/ml										Extract
	1.5	25	50	75	100	125	250	375	500	750	
	Mean zone Diameter of Inhibition (mm)										
1	0	0	0	0	0	0	15	25	25	25	Aqueous extract
10	0	0	0	0	0	10	24	25	25	25	Aqueous extract
21	0	0	0	0	0	0	0	0	0	19	Aqueous extract
38	0	0	0	0	0	0	0	15	16	16	Aqueous extract
1	0	0	0	0	0	0	0	0	0	0	Alcohol extract
10	0	0	0	0	0	0	0	0	0	0	Alcohol extract
21	0	0	0	0	0	0	0	0	0	0	Alcohol extract
38	0	0	0	0	0	0	0	0	0	0	Alcohol extract

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المحتوى البلازميدي, تحليل التحييد والفعالية ضد البكتيرية لنبات *Alcea arebelensis* ضد العنقوديات الذهبية متعدد المقاومة للمضادات الحيوية

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الملخص

تم تشخيص خمسون عزلة من بكتريا *Staphylococcus aureus* من الحروق ,االتهاب اذن الوسطى, الجروح والادرار من المرضى الوافدين الى المختبر المركزى والداخلى لمستشفى اربيل التعليمى ومستشفى الرزكارى في مدينة اربيل من فترة ما بين الاول من شهر ايلول الى شهر الاذار لعام 2013. تم تشخيص العزلات استنادا على الصفات المزرعية ,المظهرية, الاختبارات الكيمياوية. تم اختبار الحساسية لبكتريا *Staphylococcus aureus*باستعمال طريقة الانتشار بالحفر واظهرت النتائج بان (14%)7, (94%)47, (10%)8, (18%)9, (96%)48,(98%)49 و (8%)4 من عزلات كانت مقاومة لـ كلورامفينيكول ,اميكاسين, امبيسيلين, فانكومايسين, سيفوتاكسيم, سيفازولين و سيفترياكسون على التوالى. جميع العزلات كانت حساسة اموكسيسيلين. ايضا اظهرت النتائج بان العزلة رقم 38 كانت مقاومة لسبعة مضادات حيوية وكانت حاوية على خمسة بلازميدات ذو وزن جزيئى اكثر من kbp10. ايضا اوضحت النتائج بان (10%) 5, (50%)25 و(40%) 20 كانت منتجة لالفا ,بيتا وكاما تحلل الدم على التوالي.اوضحت نتائج التحييد بان الحرارة المرتفعة عند $46^{o}C$ ازال البلازميدات بصورة كاملة في حين ان البليومايسين عند /50µg كانت ذو تأثير اقل. تم دراسة الفعالية ضد البكتيرية للمستخلص المائى والكحولي لزهرة واوراق نبات الالسيا ضد بعض العزلات المقاومة *S.aureus* واظهرت النتائج التاثير المثبط لهذه المستخلصات ما عدا المستخلص الكحولي لاوراق النبات حيث لم يظهر اي تاثير تثبيطي ضد العزلات المقاومة قيد الدراسة.

الكلمات المفتاحية: العنقوديات الذهبية ، مقاومت المضادات الحيوية ، تحييد البلازميدات و نبات الالسيا