

RESEARCH ARTICLE

Antimicrobial activity of aloe-emodin from Aloe-vera against *Staphylococcus aureus*

Ola Salam Znad, Zainab Razzaq Zghair

Abstract

Objective: To evaluate the antibacterial activity of aloe-emodin isolated from aloe-vera against staphylococcus aureus from human patients and cows suffering from respiratory tract infection.

Method: The experimental study was conducted at the A;-Kut Technical Institute, Iraq, from October 2021 to December 2021, and comprised human nasal swabs collected from Al-Zahraa Teaching Hospital and Al-Karama Teaching Hospital in Wassit, Iraq, in group A, cow nasal swabs collected from city centre, Al-Hay, Al-Bataar, Sheikh Saad, Al-Azizia and Al-Suwaira fields in group B. Aloe-vera was taken from the local market for which certification was obtained from the Directorate of Seed Testing and Certification, Ministry of the Agriculture, Iraq. The swab specimens were transferred to the laboratory under standard conditions. The specimens were inoculated to mannitol salt agar and blood agar media and were then incubated for 24-48hrs at 37°C under aerobic conditions. All the primary screened isolates were then subjected to various morphological and biochemical tests to ensure their identity. Data was analysed using the statistical analysis system, 2018.

Results: Of the 200 samples, 100(50%) were in group A and 100(50%) in group B. Aloe-emodin was the most effective antibiotic that inhibited human and cow pathogenic bacteria with high inhibition zone range at (1%) which increased with increasing aloe-emodin extract concentrations at (2%, 4%). Staphylococcus isolates revealed a different response to the aloe-emodin antimicrobial activity.

Conclusion: Aloe-emodin extract of alo-vera showed high antimicrobial activity against all human and cow pathogenic bacteria.

Keywords: Cattle, Aloe, Emodin, Plant, Anti-bacterial, Anti-infective, Nose, Agriculture, Ethanol, Seeds.

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Introduction

Staphylococcus (S.) aureus, a gram-positive, spherical bacteria found in the mucous membranes and on the skin, causes a wide variety of infections that require hospitalisation or spread throughout the community.^{1,2} There are various illnesses that *S. aureus* can bring on. Infections of the skin, soft tissues and respiratory system are the most common outcomes. Toxic shock syndrome (TSS), scalded skin syndrome, osteomyelitis, necrotising pneumonia, disseminated metastatic abscess formation, septic shock, and infective endocarditis are the conditions that *S. aureus* can cause.^{3,4,5} Also, *S. aureus* is often the culprit behind biofilm-associated infections, especially those that manifest on implanted medical equipment.

The aloe-vera plant can reach a height of 80-100cm and spreads via offsets and root sprouts. The lanceolate leaves are thick and fleshy and range in colour from green to greyish green; their margins are serrated. Each flower on the spike, which can reach a height of 90cm, has a yellow tubular corolla that is only 2-3mm in diameter. Aloe-vera gel, also known as aloe-gel, is extracted from the jelly-like substance found in

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the leaf's central tissue.⁶ In addition to its anti-cancer, anti-inflammatory, anti-virus, evacuating, liver-protecting, and immunity-boosting properties, aloe-vera is one of the medicinal plants that have been used as ethnic medicines in many different countries for centuries.⁷ Modern applications for aloe-vera include pharmaceuticals, nutritious foods and beauty products. Aloe's active ingredients include a wide variety of compounds.⁸ Anthraquinones and chromones, it has been claimed, are responsible for anti-cancer activity, anti-inflammatory effects and evacuation.⁹

The current study was planned to evaluate the antibacterial activity of aloe-emodin isolated from aloe-vera against *S. aureus* from human patients and cows suffering from respiratory tract infection.

Materials and Methods

The experimental study was conducted at the A;-Kut Technical Institute, Iraq, from October 2021 to December 2021, and comprised human nasal swabs collected from Al-Zahraa Teaching Hospital and Al-Karama Teaching Hospital in Wassit, Iraq, in group A, cow nasal swabs collected from city centre, Al-Hay, Al-Bataar, Sheikh Saad, Al-Azizia and Al-Suwaira fields in group B. Aloe-vera was taken from the local market for which certification was obtained from the Directorate of Seed Testing and

Certification, Ministry of the Agriculture, Iraq. The aloe-vera leaves were washed thoroughly with running water to remove any dirt or debris.

The beaker was weighed and then the bottoms of the leaves were chopped off, and the rest of the leaves were separated from the inner gel. The exudate that settled at the bottom of the beaker after 24hrs was dried in a 50°C oven for 1hr. With a spatula, the dried extract was scrapped off the plate, and was placed in a glass jar with a lid.¹⁰

Soxhlet extraction using 40mL of ethanol and 2.0g of sample weight were used for the separation of the active ingredients of aloe-emodin. To re-dissolve the extracts, 1mL of methanol was added after it had been evaporated to dryness. Before chromatographic analysis, the extracted solutions were filtered through a 0.45m filter. High-performance liquid chromatography (HPLC) was used to measure how much aloe-emodin was present in the final extract. HPLC testing was performed at 35°C using a stationary phase column with dimensions of 250mm by 4.6mm of pursuit xfs 3-C18. Acetonitrile was obtained for which water 50:50 was used as the mobile phase, and it was pumped at a rate of 1mL/min. With a 20L injection volume and a wavelength of 280nm, the photodiode array detector was used.¹⁰ The ethanol extract samples have been loaded on thin-layer chromatography (TLC) plates with the aloe-emodin reference standard (Sigma-Aldrich, Taufkirchen, Germany). A line with a pencil about 2cm from the base was drawn gently. The samples were recorded with at least 1.5cm between them using capillary tubes.¹¹ The chromatographic tank was filled to a depth of 1.5cm with the developing solvent, a mixture of petroleum ether and ethyl acetate 60:40v: v. After adding the sample to a silica plate and treating it with ammonia gas, the plate was gently placed in the tank and left there for about 60min to run TLC. When the solvent level reached about 2cm from the top of the plate, the plate was taken out and the solvent level was marked with a pencil. The plate was then turned upside down to dry.¹² The Aloe emodin (AE) spots, which showed up as a dark stain when examined with ultraviolet (UV) light at 245nm, were scratched out, and the silica was transferred to a polypropylene vial. The lengths of the spots and the solvent height were calculated using the standard equation from the final to the initial readings to determine the factor of delay (Rf).¹³ Preparative TLC was used for AEM purification, and the sample was spread out in a band across the layer rather than dropped in individually. From the preparative TLC plates, major bands at a desired Rf were scrapped out and extracted individually with ethanol. The purity of each of the eluates was retested on analytical TLC and the solvent was evaporated to dryness.

Before taking the human samples, consent was taken from

the patients, and the sample size was calculated after taking approval from the institutional ethics review committee by using the formula:¹⁴

$$ss = \frac{Z^2 * p * (1 - p)}{c^2}$$

The samples

included related to patients of either gender aged >5 years. The cow samples were taken regardless of gender or age from the animals that were available at the time of sample collection. The swab specimens were transferred to the laboratory under appropriate conditions, and were inoculated to mannitol salt agar (MSA) and then incubated in the blood agar media at 37°C under aerobic conditions for another 24-48hrs. All the primary screened isolates were then subjected to various morphological and biochemical tests to ensure their identity.¹⁵

Kirby/Bauer technique was used¹⁶ to carry the anti-microbial susceptibility test for 6 different antibiotics.

Different concentrations of aloe-emodin, along with Dimethyl sulfoxide DMSO as a control, were poured into agar wells and the resulting microbial communities were examined using the in vitro agar well diffusion screening method.

Data was analysed using statistical analysis system, 2018. The least significant difference (LSD) test and analysis of Variance (ANOVA) were used to compare the mean values.

Results

Of the 200 samples, 100(50%) were in group A and 100(50%) in group B. Isolates of *S. aureus* grew as round, smooth colonies on MSA that fermented mannitol and turned the medium golden yellow colour, while other staphylococcus isolates failed to do so (Figure 1). Large, round, smooth, white to yellow, glistening, opaque *S. aureus* isolates were seen on blood agar (Figure 2). After incubation for 18-24 hours at 37°C, under the microscope, the bacteria appeared as clusters of grapes and were identified as gram-positive, cocci, single-cell pairs (Figure 3). The *S. aureus* isolates examined were positive in catalase, gelatinase, deoxyribonuclease (DNase), coagulase and haemolysis assays. (Figure 4A-E).

The total *S. aureus* isolates were 48(24%); 22(22%) from among the human samples, and 26(26%) from among the cow samples. Most human isolates showed resistance to tetracycline, while all of them (100%) cv were sensitive to penicillin (Table 1, Figure 5). A similar pattern was noted in the cow isolates (Table 2, Figure 6).

Aloe-emodin was the antibiotic that inhibited human and cow pathogenic bacteria, with high inhibition zone range increasing with increased concentrations (Tables3-4).

Table-1: Antibiotic susceptibility testing of human *staphylococcus (S.) aureus* isolates.

Antibiotic	Concentration (µcg/ disc)	Number of isolations	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Penicillin G 10U	P	22	22 (100)	0 (0)	0 (0)
Vancomycin 30 µcg	VA	2226	15 (68.2)	0 (0)	7 (31.8)
Tetracycline 10 µcg	TE	22	1 (4.5)	1 (4.5)	20 (90.9)
Ciprofloxacin 10 µcg	CIP	22	1 (4.5)	1 (4.5)	20 (90.9)
Chloramphenicol 30 µcg	CP	22	20 (90.9)	1 (4.5)	1 (0)
Clindamycin 10 µcg	DA	22	19 (86.4)	2 (9.09)	1 (4.5)

Table-2: Antibiotic susceptibility testing of *staphylococcus (S.) aureus* isolated from cows.

Antibiotic	Concentration (µcg/ disc)	Number of isolations	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Penicillin G 10U	P	26	23 (88.4)	2 (7.6)	1 (3.8)
Vancomycin 30 µcg	VA	26	5 (19.23)	2 (7.6)	19 (73.07)
Tetracycline 10 µcg	TE	26	2 (7.6)	3 (11.5)	21 (80.7)
Ciprofloxacin 10 µcg	CIP	26	1 (3.8)	1 (3.8)	24 (92.3)
Chloramphenicol 30 µcg	CP	26	22 (84.6)	2 (7.6)	1 (3.8)
Clindamycin 10 µcg	DA	26	23 (88.4)	1 (3.8)	2 (7.6)

Table-3: Antibacterial activity of aloe-emodin concentrations 0.5, 1, 2 and 4µg/ml against human *staphylococcus (S.) aureus* isolates.

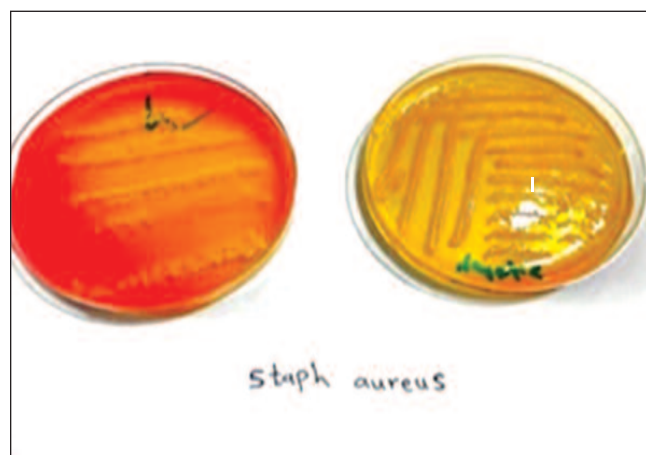
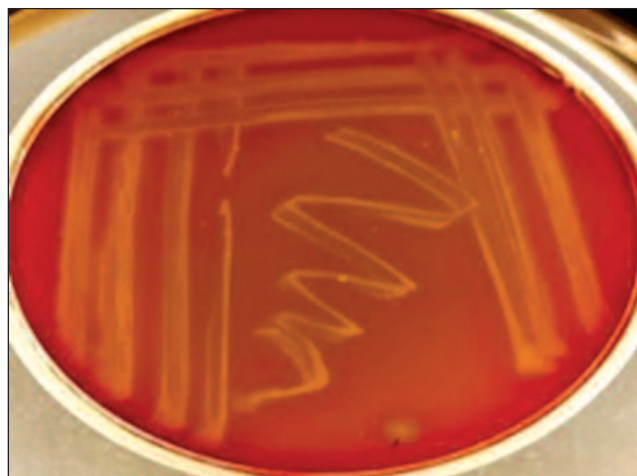
		Mean	SD	t-test	Df	Sig. (2-tailed)
Pair 1	0.5% and 1%	-0.58974	2.01838	-1.490	25	0.149
Pair 2	0.5% and 2%	-2.51282	2.55122	-5.022	25	0.000
Pair 3	0.5% and 4%	-5.01282	2.35981	-10.832	25	0.000
Pair 4	0.1% and 2%	-1.92308	2.28679	-4.288	25	0.000
Pair 5	0.1% and 4%	-4.42308	2.66092	-8.476	25	0.000
Pair 6	0.2% - and 4%	-2.50000	1.79691	-7.094	25	0.000

SD: Standard Deviation

Table-4: Antibacterial activity of aloe-emodin concentrations 0.5, 1, 2 and 4µg/ml against *staphylococcus (S.) aureus* isolated from cows.

Paired Samples Test		Paired Differences				
		Mean	SD	t-test	df	Sig. (2-tailed)
Pair 1	0.5% and 1%	-0.58974	2.01838	-1.490	25	0.149
Pair 2	0.5% and 2%	-2.51282	2.55122	-5.022	25	0.000
Pair 3	0.5% and 4%	-5.01282	2.35981	-10.832	25	0.000
Pair 4	0.1% and 2%	-1.92308	2.28679	-4.288	25	0.000
Pair 5	0.1% and 4%	-4.42308	2.66092	-8.476	25	0.000
Pair 6	0.2% - and 4%	-2.50000	1.79691	-7.094	25	0.000

SD: Standard Deviation.

**Figure 1:** *Staphylococcus (S.) aureus* on mannitol salt agar (MSA) shows golden-yellow pigment colonies of mannitol fermentation (yellowish colour).**Figure 2:** *Staphylococcus (S.) aureus* on blood agar (β-haemolysis).

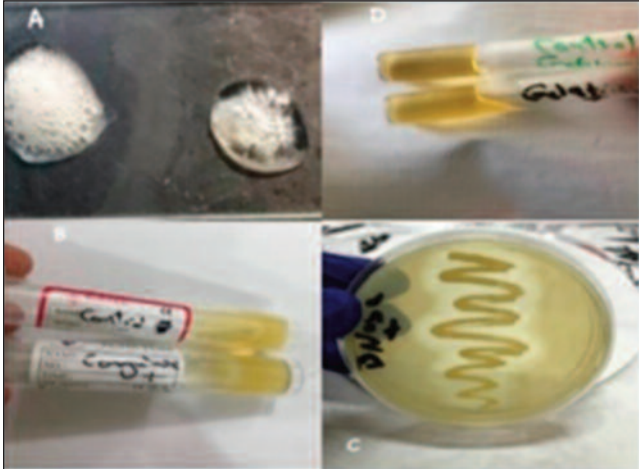


Figure 3: Biochemical tests of *staphylococcus (S.) aureus*. A: catalase-positive, B: coagulase-positive, C: deoxyribonuclease-positive, and D: gelatinase-positive.

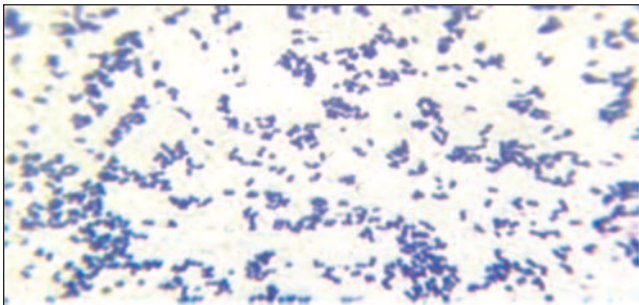


Figure 4: *Staphylococcus (S.) aureus* gram stain showing gram-positive cocci as either single cells, pairs of cells, or clusters of grape-like cells.

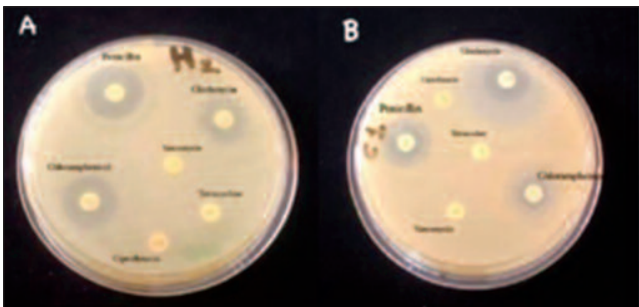


Figure 5: Antimicrobial disc diffusion test of *staphylococcus (S.) aureus* isolates (A): from a human, and (B): from a cow.

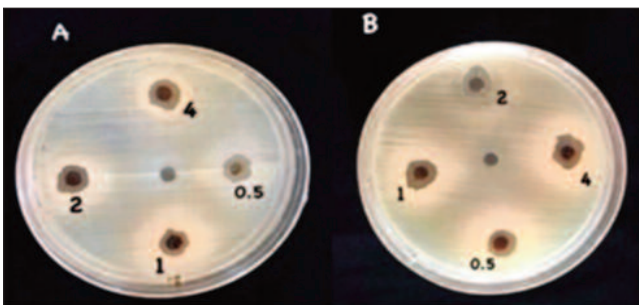


Figure 6: Effects of aloe-emodin extract from aloe-vera on *staphylococcus (S.) aureus* isolates from humans (A) and from the cows (B).

Discussion

Staphylococcus species (spp.) were found in different shapes and colours in the bacterial culture. These results agreed with previous reports.¹⁶ Among the most useful phenotypic identifiers of *S. aureus* are colony shape, gram staining, and tests for coagulase, catalase, gelatinase, haemolysis and DNase.¹⁷ The current results in this regard were similar to those reported elsewhere regarding *S. aureus* isolates.¹⁶ The isolation rate for *S. aureus* in the present study was 22(22%) in human samples, which was in line with literature.^{18,19} One study involving dogs²⁰ reported a sharp rise compared to humans.²¹ Bacterial pathogens around the world are becoming increasingly resistant to antibiotics, rendering these drugs useless for treating infections in humans and animals.²² The emergence of multidrug-resistant bacteria is being closely monitored.²³ The current study revealed that aloe-emodin had high antibacterial activity against human and cow pathogenic bacteria with high inhibition zone range increasing with increased aloe-emodin extract concentrations. *Staphylococcus* isolates revealed a different response to the aloe-emodin antimicrobial activity. The minimum inhibitory concentration (MIC) for aloe-emodin ranged from 62.5g/ml in *bacillus (B.) subtilis* and *escherichia (E.) coli* to 250g/ml in *S. epidermidis* and *shigella (S.) sonnei*, demonstrating a broad spectrum of antimicrobial activity.²⁴ Some biological extracts, such as those derived from plants or animals, have been used for centuries, while others have only been discovered recently.^{25,26}

Conclusion

Aloe-vera aloe-emodin extract was highly antimicrobial against all human and cow pathogenic bacteria. The zone of inhibition increased with increasing aloe-emodin concentration

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Disclaimer: The text based on an academic thesis.

Conflict of Interest: None.

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