

# Evaluation of the Efficacy of Alcoholic Extracts of Echinacea Purpurea and Aloe Vera Against Methicillin-Resistant Staphylococcus Aureus Isolated from Different Infections

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## Abstract

The study consisted of 100 samples (swabs of burns, wounds, skin infections, and tonsillitis) collected from Samarra Hospital and Azadi Teaching Hospital in Kirkuk from 11/5/2021 to 2/5/2022. According to the phenotypic, cultural and biochemical characteristics, 55 isolates (55%) were identified as *S.aureus*, and the methicillin bio-disk diffusion method was used to diagnose MRSA, and the results showed that 20 isolates (36%) were MRSA. The study included testing the sensitivity of 20 isolates of MRSA bacteria to ten types of antibiotics: Methicillin, Ampicillin, Erythromycin, Oxacillin, Tetracyclin, Rifampicin, Gentamycin, Clindamycin, Ciprofloxacin, Vancomycin. Methicillin (100%), Ampicillin (70%) and Erythromycin (70%), followed by the antibiotic Oxacillin, Tetracyclin, Rifampicin, Gentamycin, Clindamycin, Ciprofloxacin (65%) (65%) (50%) (45) (%) (45%) (35%), while the lowest percentage of the antibiotic Vancomycin was (10%) The inhibitory effect of the alcoholic ethanolic extract of Aloe Vera and Echinacea purpurea plants on MRSA bacteria was studied, and the results showed that Aloe vera extract gave an average inhibition diameter of 11.9 mm, while Echinacea purpurea extract gave 10.9 mm, and the treatment with a concentration of 250 mg/ml was superior and gave an average diameter of inhibition of 13.1 mm. The treatment of Aloe Vera at a concentration of 250 mg/ml showed the highest mean with a diameter of 14.8 mm. The Minimum inhibitory concentration (MIC) was determined for the extracts, and the MIC value varied according to the concentration and type of the extract and the source of infection.

**Keywords:** methicillin-resistant; alcoholic extracts; MIC

## 1. Introduction

Recent research has tended to study the effect of medicinal plant extracts on microorganisms and the possibility of using them in the treatment of many diseases resulting from microorganism infections, especially pathogens, as these plants generally produce many secondary metabolism compounds, which constitute an important source of many different pharmaceutical drugs(1). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important pathogens of modern infectious diseases and thus a challenge for nosocomial infections (2).

MRSA inhibits many antibiotics, including macrolids, aminoglycosides, and all B-lacrams, and this multi-drug resistance (MDR) makes treating MRSA difficult (3). Aloe vera contains more than 200 bioactive compounds, and the medicinal properties associated with the Aloe vera plant are due to the inner gel of the leaves (4). Studies indicated that aloe

vera gel showed its effectiveness as an antioxidant through its important role in preventing the production and scavenging of free radicals (5). *E.purpurea* is known for its medicinal properties, being one of the most widely used medicinal plants for its immune properties (6) and is considered a safe herbal medicine (7) and has been used as a treatment against a range of diseases, wound inflammation, snake bites, antioxidant, and is used in herbal supplements in the form of capsules or ointments Or tea and for the treatment of many diseases, including relieving migraine pain and anxiety, stimulating wound healing, strengthening the immune system, and treating respiratory infections (8).

## 2. Materials and methods

### 1. Sample collection

100 samples were collected using sterile cotton swabs container on a transport medium from patients arriving at Samarra General Hospital and

Azadi Teaching Hospital in Kirkuk, and those attending private clinics for the period from 5/11/2021 to 5/2/2022, from both sexes and in different age groups. The samples were planted after collecting on a medium Mannitol salt agar as a differential and selective medium to isolate *S. aureus* bacteria and blood agar medium. Then, the dishes were incubated under aerobic conditions at a temperature of 37 °C for 24 hours. After the incubation process, morphological and biochemical tests were performed.

## 2-Isolates Identification

The isolates were diagnosed based on the description of colonies growing on Mannitol salt agar medium and Blood Agar medium of shape, size, color, edge and lysis of blood. Microscopic examination also conducted a number of biochemical tests such as Catalase test, Oxidase test, Hemolysin production test, Mantol sugar fermentation test Coagulase enzyme in two ways depending on what it say(9)

## Diagnosis of methicillin-resistant staphylococci (MRSA)

It was diagnosed by a test of resistance to the antibiotic methicillin, after it was cultured on Muller-Hinton medium and incubated at 37°C for 18–24 hours(10).

## 3- Antibiotic sensitivity test

The method modified by (Kirby-Bauer) followed the Disk diffusion Method, as stated in (11). To test the sensitivity of the bacterial isolates under study to antibiotics, which included ((Methicillin, Ampicillin, Erythromycin, oxacillin, Tetracyclin, Rifampicin, Gentamycin, Clindamycin, Ciprofloxacin, Vancomycin). And as follows:

Young bacterial colonies at the age of (24) hours were taken by the carrier (Loop) and transferred in a test tube containing (2) ml of physiological saline solution, and was shaken well. Mueller-Hinton agar was inoculated with a cotton swab containing the bacterial suspension in a manner that was spread over the entire plate to ensure homogenous growth, and the plates were left to dry at room temperature. Antibiotics were placed on the medium using sterile forceps, if they were placed at equal distances by 5 antibiotics in each plate, and then the plates were placed in the incubator for 24 hours at a temperature of 37° C. The results were recorded by measuring the diameter of the inhibition zone in millimeters around each disc with a graduated ruler, then Compared with the standard inhibition diameters mentioned in (12)

## 4- Preparation of plant extracts

Aloe vera samples were collected from local markets in the city of Samarra. The leaves of the *Echinacea purpurea* were obtained from its manufacturer, Puritan's Pride (U.S.A.) The alcoholic ethanolic extract of aloe vera and *Echinacea purple* was prepared according to the method described

before(5)with some modifications and my agency: The leaves of the plant were washed Several times with distilled water to get rid of suspended dust, cut it and then dry it. Extraction was done by placing (20)gm of vegetable powder from the leaves of the two plants separately in an extraction thimble, which was placed in a continuous extraction apparatus, a Soxhlet apparatus and used (100)ml of ethyl alcohol at a concentration of (95) The extraction process lasted 7 hours, after which the solvent was evaporated using a rotary evaporator under the pressure of the pickle at a temperature of (45) C, to obtain the dry alcoholic extract, and it was placed in sterilized and opaque bottles, marked and kept in the refrigerator until use.

## 5- Studying the effect of plant extracts on bacterial growth

Diffusion method was used on Muller Hinton Agar medium and Cork borer was used to make holes with equal dimensions to avoid overlapping diameters of damping and a diameter of 6 mm to ensure that it contained the required plant extract by 0.5 ml per hole after spreading 0.1 ml of bacterial barrier on the medium to test the sensitivity of bacteria to mg/hole extracts Plants at concentrations ((250 ,125 ,62.5,31.25, 15.26 ) were left in the refrigerator for one hour to spread the plant solutions, and then the dishes were incubated at 37 °C for 24 hours and the results were read by measuring the diameter of the inhibition area with a measuring device Electronic activation (13) The minimum extract concentration in which no bacterial growth was observed is the minimum inhibitory concentration (MIC) as reported (14).

## 6- Statistical Analysis

The data were statistically analyzed using the Statistical Analysis Program (SPSS) version 23 to show the significant differences or not between the study groups and the arithmetic means and the significant variance were compared using the least significant difference test at the probability level of  $\leq 0.05$  .p

## 3. Result and discussion

### Isolation

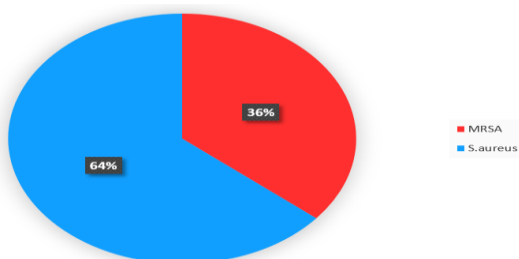
Table (1) shows the areas of collection of pathological samples from people with different disease areas, the number of isolates from each area, the number and percentage of isolates of *S. aureus*, as well as the number of MRSA-resistant *Staphylococcus aureus* isolates. ) A bacterial isolate was related to *S. aureus* bacteria, and this result is consistent with (15), as the number of isolates reached 68 total isolates, percentage of (58%) of the total samples, It agreed with a similar study (16), which obtained (50%) of the total samples, and the fact that the bacteria is one of the main causes of nosocomial infections, this is due to the reason for its increase in the samples taken (17), and gave 20 (36%) isolates of *S. aureus* with the characteristic Methicillin

resistance after growing it on Muler Hinton agar medium. Figure (1) shows the incidence of S.aureus and MRSA. in the initial isolation. The obtained results were similar to what was reached by (18),

where the percentage of MRSA isolates from S. aureus bacteria was (47.19%) and less than what was reached by (19), where the percentage was (66%), while (10) the percentage of MRSA was (84%).

**Table (1): Type of infection, number of samples, numbers and percentages of S.aureus and MRSA**

percentage	The number of MRSA isolates	percentage	Number of S.aureus isolates	number of samples	The type of disease
45.00%	9	25.45%	14	21	burns
25.00%	5	18.18%	10	19	wounds
10.00%	2	20.00%	11	20	skin infections
20.00%	4	36.36%	20	40	tonsillitis
100.00%	20	100.00%	55	100	Total



**Figure (1) Percentage of appearance of S.aureus and MRSA in primary isolate.**

Table (2) Distribution of S.aureus and MRSA bacteria by sex \shows the numbers and percentages of S.

aureus and MRSA bacteria by sex, as the results showed that the prevalence of MRSA is that the percentage of MRSA isolates isolated from males carrying S. aureus was (40%), while its percentage was from females (%) 60) The results obtained were similar to those reached by (15) with a rate of (55.11%) isolated from females, and 21 isolates with a rate of (44.89%) belonging to males,. The difference in incidence between the two sexes may be due to the quantity and quality of the natural flora in the bodies of the different sexes, as well as the difference in the method of collecting samples for both sexes during the study (20).

**Table (2) Distribution of S.aureus and MRSA bacteria by sex**

Sex	Isolated S.aureus number	percentage	MRSA number	percentage
Male	30	54.5	8	%40
Female	25	45.5	12	%60
Total	55	100	20	%100

Table (3) shows the numbers and percentages of the distribution and prevalence of S.aureus and MRSA by age groups. The patients were divided into three age groups (10-29) (30-49) ( $\leq 50$ ) and the results showed that the highest rate of MRSA infection is in the first age group (45%), and this percentage decreases as we progress in age groups and (%) 25) (30%) for the

second and third age groups, respectively. These obtained results were similar to what was reached (19), where the percentages were (50%) (41%) (33.3%) for the first, second and third age groups, respectively. Like wise, it is similar to what was reached(21) all of the MRSA isolates that were isolated were in the first age group.

**Table (3) Numbers and percentages of the distribution and prevalence of S.aureus and MRSA by age groups.**

	Age group	Number of S.aureus	percentage	Number of MRSA	percentage
1	29-10	30	%54.5	9	%45
2	49-30	15	%27.3	6	%30
3	$\leq 50$	10	%18.2	5	%25
Total	-	55	%100	20	%100

**S.aureus Laboratory diagnosis**

S.aureus were identified morphologically,

microscopically and biologically to confirm the isolated species, as shown in Table (4)

**Table (4) Results of culture, microscopic and biochemical tests to diagnose S.aureus**

Test type	The result
Agricultural properties on mannitol medium, blood agar medium	golden yellow colonies Complete hemolytic cream
microscopic properties	Spherical in shape, in clusters or pairs
Coagulase	+
Oxidase	-
Catalase	+
(+) the result is positive for the test, (-) the result is negative for the test	

Colonies appeared on solid local manitol salt agar in a circular shape, smooth edges, slightly raised on the

surface, golden in colour. As this medium is considered one of the selective and differential media for isolating the bacteria S. aureus, where Staphylococcus aureus, S.

aureus, is fermented for mannitol sugar and produces acid, and then leads to an increase in the acidity index, leading to the conversion of the color of the medium from red to yellow, and this is consistent with (22) As for the colonies isolated and grown on blood agar medium, they are large, with a creamy color close to golden, surrounded by a clear transparent halo, indicating hemolysis. This result is consistent with (23) that it was found that *S. aureus* isolates produce B. haemolysin enzyme.

The results of the microscopic examination of all the glass slides prepared for 55 isolates after staining them using Gram stain showed that the bacteria cells are single or arranged in pairs or in the form of tetramers or are arranged in the form of clusters resembling grapes, which are positive for the Gram stain as they appear in violet color and this corresponds to (10). All isolates of *S. aureus* bacteria were positive for the catalase test, as this test is important in distinguishing Streptococci (negative for the catalase test) and Staphylococcus aureus (positive for the catalase test), as all the isolates produced the catalase enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen (24). These results are consistent with (18), where all isolates were positive for the catalase test. All isolates of *S. aureus* bacteria were negative for the oxidase test, and the negative result in this test is caused by the inability of *S. aureus* to change the blue or violet color of the oxidase detector, and this means the inability to have the cytochrome oxidase that stimulates the transfer of electrons from these donor compounds to the electron acceptors (25). All *S. aureus* isolates were positive for this test. A Coagulase blood clotting test was conducted to distinguish between *S. aureus* that is positive for this test, that is, it is able to produce the Coagulase enzyme and convert plasma into a thrombus, and Staphylococcus aureus that is negative for the Coagulase test (26). And that the production of this enzyme is a criterion for the diagnosis *S. aureus* (27)

### Identification of Methicillin Resistant *S. aureus*

#### Method of spreading the antibiotic Methicillin

The results of Figure (2) show that all 55 isolates of *S. aureus* were tested for resistance to the antibiotic thistle and it was found that out of 55 isolates of *S. aureus*, 20 isolates (36%) were MRSA, and this result does not agree with what was found by (10). It was found that out of 46 *S. aureus* isolates, 39 (84.8%) isolates were MRSA.



Figure (2): Shows the mode of spread of the antibiotic Methicillin

### Sensitivity test for Antibiotic

Figure (3) shows the percentages of sensitivity testing of MRSA isolates to (10) antibiotics, which are (Oxacillin, Ampicillin, Meticillin, Vancomycin, Erythremycin, Ciproflaxian, Centamicin, Clindamycin, Rifampicin, Tetracyclin) using the disc diffusion method as stated in the The Laboratory Standards Institute(12), and as shown in the table, the percentages of susceptibility testing for MRSA isolates to the above-mentioned antibiotics. (100%) and this result is identical to what was stated (19), while the resistance to Ampicillin was (70%) and Oxacillin by (65%), and this result is less than the results of (18), where The ratio was for both antibiotics (100%) and methicillin is a narrow-spectrum  $\beta$ .Lactams antibiotic of the class of penicillins. Methicillin works by inhibiting the gliding of bacterial cell walls as it works by blocking the binding of bacterial cells (28). Alternating between chains Peptidoglycan polymer is a major component of the Gram-positive bacterial cell wall. Bacterial resistance to methicillin is caused by various mechanisms, one of which is the production of an unusual penicillin-binding protein (PBP) that forms PBP2, thus weakening the affinity of the antibiotic  $\beta$ . The mec A gene (29). The study also showed that the isolates under study showed resistance to the antibiotic at a rate of (70%), while the sensitive isolates (25%) and the medium (5%). 28.3%) that the antibiotic resistance of macrolids is mainly a plasmid mediated that shares the adenine homologousness in the RNA of the riboflavin subunit S 50, and this leads to a decrease in the binding of anti-macrolids to their usual targets on the opossum (30). For the antibiotic Clindamycin, the percentage of resistant isolates to it was (45%) while the sensitive to it was 55%. Clindamycin is one of the preferred antibiotics for the treatment of MRSA because of its excellent pharmacological properties such as optimal penetration into tissues and accumulation in abscesses, however, the random use of antibiotics led to an increase Staphylococcus number (31).As for the antibiotic Gentamycin, the percentage of resistance was (45%), while the percentage of sensitivity to it was (55%). While the results were close to what was stated (32), where the resistance was (32%)(10), where the resistance was (34.8) and sensitive (58.7),

As for the antibiotic Ciprofloracin, the percentage of MRSA resistant to it was (35%), while it was sensitive to it (55%). It is a broad-spectrum antibiotic against Gram-positive and Gram-negative bacteria.

As for the antibody Rifampicin, the percentage of resistant isolates to it was (50%), and this result is close to what was reported (10), where the percentage of resistance was (37%) and sensitive (52.23%) and far from what it came (19), where The percentage of resistance was (26%) As for the antibiotic Vancomycin, the percentage of resistance to it was (10%) and sensitivity to it (90%).

The reason for the different rates of resistance and

sensitivity to the antibiotics used in this test is that these antibiotics, such as penicillins, are popular, cheap and more available compared to the more modern and more expensive ones such as Vancomycin. As they are frequently used by the general public even without consulting a doctor, and resistance to these antibiotics leads to a high case of treatment and a longer stay in the hospital, and treatment failure thus leads to the limits of life-threatening diseases (33).

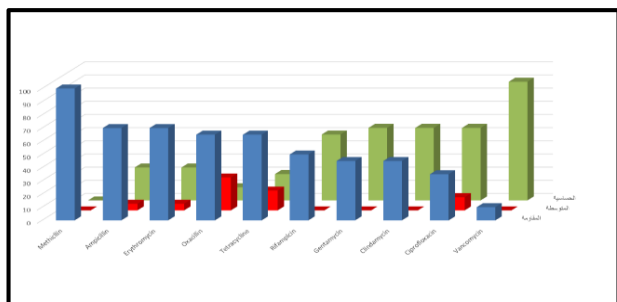


Figure (3) Percentages of MRSA isolates susceptibility testing to the above-mentioned antibiotics

Effect of plant extracts on the growth of methicillin-resistant Staphylococcus aureus

Table (5) shows the effect of *Aloe vera* and *Echinacea purpurea* plant extracts, the concentrations used, and the interaction between them on the sensitivity of methicillin-resistant staphylococcus bacteria. With regard to the concentration, the results showed that there were significant differences between the means, as the treatment was superior to the concentration 250 mg/cm<sup>3</sup> and gave the average diameter of inhibition 13.1 mm, followed by the concentration 125 and gave the diameter of inhibition 12.8. As for the bilateral interaction between the two types of extract and concentration, it was noted that there are differences Significant in the effect on bacterial sensitivity, if *Aloe vera* treatment at a concentration of 250 mg/ml showed the highest mean with a diameter of inhibition of 14.8 mm, followed by treatment with *Echinacea*

*purpurea* a concentration of 125 mg/ml with a diameter of inhibition of 13.8, while the treatment gave a concentration of 12-15 mg/ml and a diameter of inhibition of 8.3 mm, followed by *aloe vera* with a diameter of 7.4 mm inhibition, and the picture (4) shows the effect of *Aloe vera* extract the highest value of MRSA bacteria and the concentrations used, while picture (5) shows the effect of *echinacea* extract and the affected concentrations serve.

The results of *aloe vera* extract agreed with (34), as they found that the alcoholic extract of *aloe vera* had an inhibitory effect on *S.aureus* bacteria and gave a diameter of 23 mm at the highest concentration of 100%. The results also agreed with the results of (35). Those who demonstrated the effectiveness of the ethanolic extract of *Aloe vera* plant in inhibiting the growth of *S.aureus* bacteria, and the results are also consistent with (36) who noticed an increase in the diameter of inhibition when the concentration of the extracts increased, and the ethanolic extract gave the highest inhibitory activity against *S.aureus* and the results were in agreement *Echinacea* with (37), as they found that the ethanolic extract of *E. purpurea* leaves has an inhibitory effect on the growth of MARS, as well as the results agree with the value (38), as they also found that the ethanolic extract of *E. purpurea* has an inhibitory effect on the growth of MARS.

The indiscriminate use of antibiotics has led to an increase in multidrug-resistant bacterial pathogens, and this increase has led to the inability of existing antibiotics to combat pathogens, so it has led to the need for new antibiotics (39). Microbes have alternatives in the development of new antibiotics (40). *Aloe vera* acts as an antibacterial agent as it contains anthraquinones that inhibit bacterial protein synthesis by blocking the translation process (38). *Echinacea purpurea* also acts as an antibacterial agent. The inhibitory activity of *Echinacea purpurea* is due to the presence of the phenolic acids in its leaves (38) and also contains flavonoids that have an inhibitory activity against bacteria (41).

Table (5): Effect of Aloe vera and Echinacea purple plant extracts and the concentrations used and the interaction between them on the susceptibility of methicillin-resistant Staphylococcus aureus						
Average effect of the extract	15.12	31,25	62.5	125	250	Concentrations / plant type
11.9 a	7.4 e	11.7 c	12 c	13.8 b	14.8 a	<i>Aloe vera</i>
10.9 b	8.3 d	11.2 c	11.8 c	11.9 c	11.4 c	<i>Echinacea purpurea</i>
	7.8 c	11.4 b	11.9b	12.8 a	13.1 a	Average focus effect

Similar letters mean that there are no significant differences at the level of significance  $p \leq 0.05$ , different letters mean that there are significant differences at the level of significance  $p \leq 0.05$

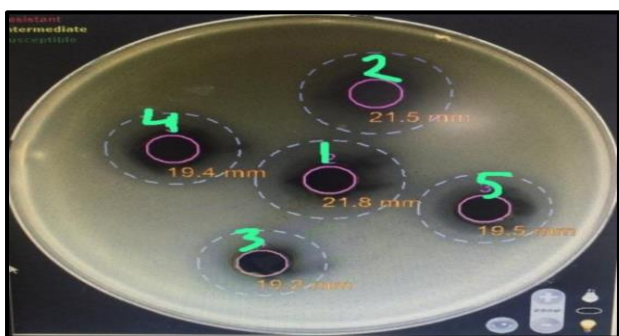


Figure (4): Effect of Aloe vera extract on MRSA bacteria.

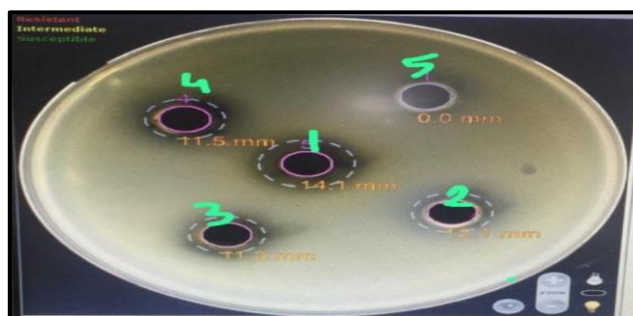


Figure (5) Effect of Echinacea purpurea extract on MRSA.

## Minimum Inhibitory Concentration (MIC)

Table (6) shows the minimum inhibitory concentration (MIC) of aloe vera and echinacea extracts against MRSA according to the source of the answer. Cutaneous, tonsillitis and burns were equal to 31.25 mg/ml. While the (MIC) of aloe vera extract against the bacterium isolated from the wounds was equal to 62.5 mg/ml. As for the extract of echinacea, the MIC was equal to 62.25 mg/ml against bacteria isolated from wounds, burns and tonsillitis, while the MIC was equal to 31.25 mg/ml.

**Table (6): Minimum inhibitory concentration (MIC) of plant extracts on MRSA isolates (mg/ml)**

Extract type	source of injury			
	skin infections	wounds	tonsillitis	burns
	MIC	MIC	MIC	MIC
Aloe vera extract	31.25	62.5	31.25	31.25
Echinacea extract	31.25	62.5	62.25	62.25

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