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Study of Dittrichia Graveolens Extracts Effect against Staphylococcus Aureus Isolated from Urinary Tract Infections and Burns

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Abstract: the current study was aimed to estimate the activity of D. graveolens extract against Staphylococcus aureus isolated from urinary tract infection and burns . In this study, 150 samples were collected from urine and burns from patients attending and lying in hospitals (Azadi General - Kirkuk General - Public Health Laboratory) after consulting the specialist doctor and referring the patient to the laboratory, taking information for each sample and recording it in the special questionnaire form for different ages and genders for the period from December 2021. Until March 2022. The effect of different concentrations of aqueous and alcoholic extracts of the D. graveolens plant on the growth of microorganisms (bacteria) was studied. The results showed The current study showed that the number of positive samples containing various bacterial growths was 102 (68%), while the number of negative samples that did not show bacterial growth was 48 (38%). the total of S. aureus isolates was 31(30.39 %) as 20(64.5%) of the isolates were isolated from urine, while 11(35.5%) were isolated from burns. The results of the study demonstrated the effect of cold water and ethanol alcohol plant extracts from the Dittrichia graveolens plant on the growth of S.aureus isolates which isolated from urinary tract and burns infections using extraction devices. These isolates are known to be pathogenic and have high resistance to antibiotics under study. The ethanol extracts showed significant results, with the highest inhibition value reaching 14.44 at a concentration of 200 mg/mL, while the cold water extracts did not show any significant results.

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Keywords: Staphylococcus Aureus, Dittrichia Graveolens, Urinary Tract Infection, Burn, Plant Extract.

1. INTRODUCTION

Dittrichia graveolens (family: Asteraceae) is one of the annual herbaceous plants, which is characterized by its conical shape and its ability to grow to a height exceeding about 91.5 cm. Dittrichia graveolens is considered a flowering plant and is one of the harmful herbaceous weeds with multiple branches, which grows upright. upward [1]. Preliminary phytochemical study showed that D. graveolens contains polyphenols, tannins, flavonoids, oil, steroidal triterpenoids, sesquiterpenes, and anthraquinones[2]. In recent years, antibiotic resistance has become an important problem worldwide, and for this reason, the search for new molecules plays a major role. Plant metabolites such as polyphenols, alkaloids, and terpenoids could represent an interesting way to do this task because they can destroy bacterial cells or inhibit their growth [3]. Many natural compounds or plant extracts, including D. graveolens, have been extensively studied to evaluate antibacterial activity against various types of fungi and bacteria. Staphylococcus aureus is part of the normal flora of the skin, nose, pharynx, and digestive tract. It is also found in the air and on surfaces. External to clothing, and since it has the opportunistic ability to cause disease, it causes infections ranging from simple skin inflammation to life-threatening systemic infections, especially when appropriate conditions are present, such as a defect in the host's immune defenses or the presence of a viral or fungal infection. & Fungal infection or the presence of chronic diseases such as psoriasis and eczema [4]. Antibiotic resistance is widespread in Staphylococcus aureus. For example, resistance to conventional beta-lactam antibiotics (penicillin and its derivatives) sensitive to beta-lactamase is almost ubiquitous in S. aureus. Furthermore, Staphylococcus aureus, often in combined form, can exhibit resistance to almost all available antibiotics. Vancomycin remains the antibiotic of last resort for MRSA infections, with highly vancomycin-resistant staphylococcus aureus VRSA strains emerging but not spreading. However, there are strains that have acquired intermediate resistance to vancomycin (VRSA) [5]. So the current study was aimed to estimate the activity of D. graveolens extract against Staphylococcus aureus isolated from urinary tract infection and burns.From this perspective, our aim in this research was to shed light on the antimicrobial bioactivity of organic and aqueous extracts of the Dittrichia graveolens plant, as well as to identify the active compounds within it and determine the effect of different concentrations of these extracts on the growth of S.aureus bacteria.

2. MATERIALS AND METHODS

Specimen Collection

A total of 150 clinical samples were collected from both male and female patients of all ages who visited or were admitted to Azadi General Hospital, Kirkuk General Hospital, and the Public Health Laboratory. The collection period was from December 2021 to March 2022. Samples were obtained from burn patients using sterile cotton swabs, and urine samples were collected in sterile containers. The samples were then cultured on mannitol salt agar to

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differentiate between Staphylococcus bacteria species. Afterward, the samples were streaked, and the plates were inverted and incubated at a temperature of 37°C for 24 hours.

Isolation and Diagnosis

Morphological and Cultural Examinations

Colonies were initially identified through the phenotypic characteristics of isolated bacterial colonies grown on mannitol salt agar medium and blood agar media, which included the shape, color, texture, odor, and size of the colonies. Staphylococcus aureus bacteria were grown on Mannitol agar.

Microscopic Diagnosis

The isolates were identified under the microscope by removing a single pure colony with a sterile lube from mannitol salt agar medium, then placing it on the glass slide, mixing it well with water and passing it three times quickly over the flame in order to fix the colony cells on the slide. Then they were stained with Gram stain according to the steps. The sequence of the staining process was examined microscopically using an optical microscope and using an oil lens to see the shape, colors, and arrangement of the cells, according to their interaction with the Gram stain[6].

Biochemical Tests

The isolates were initially diagnosed based on biochemical tests, which included Indole test, Methyl-red, Voges-proskauer test ,Citrate utilization test, Catalase Test ,Oxidase Test ,Coagulase ,Urease test, Motility Test, Then the diagnosis was confirmed by using the API Staph system.

Collecting and Preparing Plant Samples

Samples of the D. graveolens plant were collected and prepared for this study. The plants were collected from their locations in Northern Iraq and southern Turkey. Then, the plants were washed and cleaned from soil and other impurities attached to them. They were placed in a dry and dark place, away from sunlight, at an appropriate temperature for the purpose of drying. After that, they were finely ground to obtain a ready-to-extract plant powder, which was then placed in sterilized plastic containers until used.

Preparation of D. Graveolens Extracts

Preparing the Crude Alcohol Extract of the D. Graveolens

Ethanol was chosen as a suitable solvent for all plant parts. The alcohol extracts of Dittrichia graveolens plant were prepared based on the method described by[7]. A certain amount of plant powder (200g) was taken and placed in an extraction vessel with 200ml of ethanol. The extraction was carried out using a Soxhlet apparatus, which is an effective tool for extracting all active compounds using dry heat. The apparatus was operated for 4-6 hours. After the extraction, the crude liquid extract was transferred to a rotary evaporator to remove the alcohol and retain the concentrated, dried crude plant extract without any alcohol. The samples were then stored under refrigeration at 4°C in sterilized glass bottles until used.

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Preparing the Aqueous Extract of the D. Graveolens

In this study, the aqueous extract of Dittrichia graveolens plant was prepared based on the method described by[8]. A mixture of 20g of plant powder and 400ml of cold, sterilized distilled water was prepared with continuous stirring using a magnetic stirrer for 24 hours at room temperature. The extracts were then filtered first using a medical sieve and then using Whatman no.1 filter paper. The precipitate or liquid extract was then placed in a sterilized Petri dish and left to dry at room temperature. This process was repeated several times to obtain a sufficient quantity of the plant extract, which was then collected and stored in a sterilized glass container at 4°C until used.

Preparation and Sterilization of the D. Graveolens Extracts Dilutions

The dilutions for the plant extracts were prepared by dissolving 1g of each raw extract separately (ethanol extract, petroleum ether extract, and cold aqueous extract) in 5ml of Dimethyl Sulphoxide (DMSO) to obtain a standardized concentration of 200mg/ml. This concentration was then used in the preparation of subsequent dilutions, which were 100mg/ml, 50mg/ml, and 25mg/ml. The dilutions were then sterilized by passing them through Millipore filter membranes with a diameter of 0.045µm. The samples were stored in tightly sealed sterilized bottles at 4°C until used (Srinivasa et al., 2001).

The Effectiveness of D. Graveolens Extracts was Tested Using the Agar well Diffusion Method

To perform the test, 0.1ml of the pre-prepared bacterial suspension was spread evenly on the surface of a Muller-Hinton agar medium using a sterilized cotton swab. The medium was then punctured using a sterilized 6mm diameter cork borer at regular intervals. Next, 0.1ml of each plant extract dilution was added to its respective well. The plates were left to incubate at 37°C for 24 hours to allow the extracts to diffuse into the medium. After incubation, the inhibition zones were measured using a millimeter ruler.

Statistical Analysis

All the results of the current study underwent statistical analysis. For this purpose, the SPSS software version (17) was used to estimate the means and standard deviations.

3. RESULTS AND DISCUSSIONS

Samples Isolation

In this study, 150 samples were collected from urine and burns from visitors and patients hospitalized in (Azadi Educational Hospital, Kirkuk General) and the Public Health Laboratory (Bacteriology Unit) for both gender and for different age groups for the period from 20/12/2021 to 20/3/2022. The results showed The current study showed that the number of positive samples containing various bacterial growths was 102 (68%), while the number of negative samples that did not show bacterial growth was 48 (38%), as in Table [1].

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Table [1] Distribution of study samples according to bacterial growth

	Positive bacterial growth +ve	**Negative bacterial growth –ve**	**Total**
Isolates	**102(68.0%)**	**48(32.0%)**	**150(100%)**

Regarding sample sources, the number of urine samples was 84 (56.0%) out of a total of 150 samples, while the number of burn samples in the current study was 66 (44.0%) out of a total of 150 samples. While the number of positive samples for bacterial growth taken from urine was 57 (55.9%) out of a total of 102 positive samples. On the other hand, the number of samples positive for bacterial growth taken from burns was 45 (44.1%) out of a total of 102 positive samples, as in Table [2].

Table [2] Distribution of study samples according to bacterial growth and sample source

Type of specimens	Positive bacterial growth +ve	Negative bacterial growth –ve	Total
Urine	57(55.9%)	27(56.3%)	84(56.0%)
Burns	45(44.1%)	21(43.7%)	66(44.0%)
Total	102(68.0%)	48(32.0%)	150(100.0%)

Finally, the current study showed that 71(69.6%) of the isolates were from other bacterial species, as 37(52.1%) of the isolates were isolated from urine, while 34(47.9%) were isolated from burns. While the total of S. aureus isolates was 31(30.4%) as 20(64.5%) of the isolates were isolated from urine, while 11(35.5%) were isolated from burns shown in Table [3].

Table [3] Distribution of study samples according to bacterial growth and type of bacteria

Type of specimens	S. aureus	Other types	Total
Urine	20(64.5%)	37(52.1%)	57(55.9%)
Burns	11(35.5%)	34(47.9%)	45(44.1%)
Total	31(30.4%)	71(69.6%)	102(68.0%)

Morphological Identification

The bacteria under study were diagnosed based on microscopic examination using Gram staining, where their colonies appeared under the microscope in the form of clusters of purple grape-like structures. They were also diagnosed visually by growing the bacteria on Mannitol Salt Agar medium, and their colonies appeared as golden yellow. They were able to change the color of the medium from pink to yellow due to their fermentation of mannitol sugar. Additionally, biochemical tests and the API Staph test were performed, which confirmed the definitive diagnosis of this type of bacteria, as these tests confirmed that the non-growth isolates belonged to S.aureus bacteria. As shown in Figure 1.

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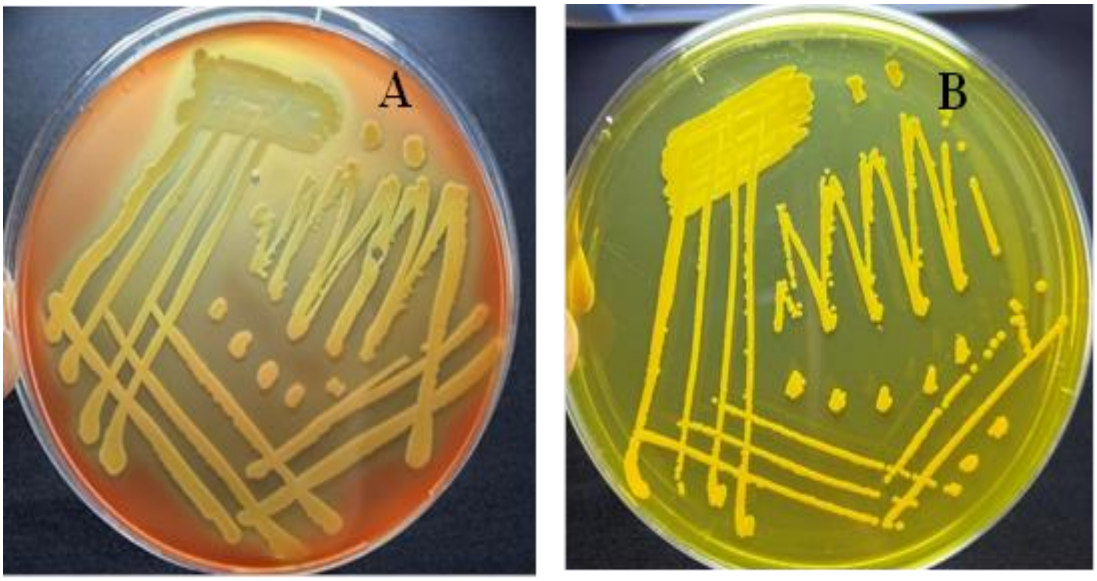

Fig.1 showing the phenotypic appearance of S.aureus bacteria colonies on blood medium (A) and mannitol saline medium (B).

Through the diagnostic results, 31 isolates of S. aureus bacteria were obtained from the total number of samples. The isolates obtained were distributed into 11 isolates, representing 35% of burns, and 20 isolates of this bacteria were obtained, representing 65% of urine, and this result contradicted the researcher [9], who obtained a rate of 83% of the isolates for S. aureus taken from various sources from patients suffering from various degrees of burns. Regarding urine samples, the results of our study showed that the number of isolates of S. aureus bacteria reached 20 isolates, a rate of 65%. This result is contrary to the result of the researcher[10], who obtained a rate of 17% of S. aureus isolates from urine samples, and our results are consistent with The findings of the researcher[9], who obtained a high percentage of 86% of S. aureus bacteria isolates from urine samples. The reason for the difference in these percentages in each study mentioned above may be due to bias in collecting samples or to the nature of the size of the research sample included in the study, in addition to the health and immune status of the patients included in each study and the type of colonizing bacteria for each individual. It may be attributed to the endemicity of this bacteria, especially in hospital lobbies and burn units, which causes infections in patients hospitalized who suffer from burns or major wounds. In addition to what was mentioned above, the difference in methods and means used in isolating samples and increasing health awareness, as well as the difference in the locations and number of swabs taken and the treatment taken by patients, or because of the level of cleanliness of the environment and the tools used in hospitals, may play an important role in whether or not infection with this bacteria occurs[11]. The percentage difference obtained agrees or disagrees with other previous studies. The agreement and difference are due to a number of reasons, the first of which is the difference in the methods and means by which the samples were taken. It is also due to the locations and number of swabs taken, in addition to how the samples were handled, such as storage conditions, growth, and destruction of samples, all of which led to a noticeable variation in the percentage [12].

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The Antimicrobial Activity Test of the Plant Dittrichia Graveolens

In recent years, antibiotic resistance has become a significant problem worldwide. For this reason, many studies have attempted to uncover the role of some active compounds in plants as effective antibacterial agents. Plant metabolites such as polyphenols, alkaloids, and terpenoids can be an interesting approach to exert an effective antibacterial role as they can destroy bacterial cells or inhibit their growth[3] . Therefore, this study aimed to extract some active compounds from D. graveolens plant extracts and investigate their effective role against S. aureus bacteria by measuring their inhibitory diameter.

Inhibitory Effectiveness of D. Graveolens Extracts on S. Aureus

The results of the study demonstrated the effect of cold aqueous and ethanolic plant extracts of Dittrichia graveolens on the growth of S. aureus isolates obtained from urinary tract and burn infections, as these isolates are known to be pathogenic and highly resistant to antibiotics under investigation. The study examined the effect of different concentrations of ethanolic extracts on S. aureus isolates obtained from burn wounds. The concentrations of 25, 50, 100, and 200 mg/mL showed inhibitory effects, with inhibitory diameters of 14.44, 10.22, 7.33, and 4.89 mm, respectively. Similarly, the ethanolic extract exhibited inhibitory effects on S. aureus isolates obtained from urinary tract infections, with inhibitory diameters of 12.13, 10.19, 6.81, and 3.75 mm for the mentioned concentrations. On the other hand, no inhibitory effect was observed for the cold aqueous extract of Dittrichia graveolens on the isolates of S.aureus. There is an increasing interest in natural compounds derived from plants that possess antibacterial activity[13]. These compounds are being explored to combat the rapid spread of antibiotic-resistant bacteria, including S. aureus, due to the vast diversity of plants. In recent years, medicinal plants have gained significant attention in research. Plants have been used to treat various microbial infections, and Dittrichia graveolens has been used as an antiseptic for wounds and as an antibacterial and antifungal agent. Additionally, leaf and branch infusions have shown potential for treating diabetes and high blood pressure[14][15] . Dittrichia graveolens possesses inhibitory activity due to its content of glycosides, saponins, tannins, flavonoids, and alkaloids [16]. These findings align with a study conducted by [17], which concluded that Dittrichia graveolens extracts exhibit high efficacy against certain microorganisms, including S. aureus. This is consistent with the findings of[18], who reported the effectiveness of Dittrichia graveolens extracts against burn and urinary tract pathogens and the prevention of biofilm formation. Our results also agree with the findings of [19], who demonstrated that the ethanolic extract of coriander had a larger inhibitory diameter on S. aureus compared to the cold aqueous extract, attributed to the presence of active compounds with inhibitory effects on pathogenic bacteria. The ineffectiveness of the cold aqueous extract may be due to the low efficacy of its compounds against bacterial isolates. [20] reported the antimicrobial effects of 13 plant species used in wound and bacterial infection treatment in South Texas against S. aureus. They observed that the aqueous extracts of the plants showed no effect against the isolated bacteria, while the ethanolic extracts exhibited inhibitory effects. This supports the potential use of plants as antibacterial agents against S. aureus. In a study by[21], the inhibitory activity of 15 plant extracts against S. aureus was tested, and 8 plants demonstrated effective effects. Dittrichia graveolens is characterized by its ability to form complexes with extracellular proteins and

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cell walls, leading to the disruption of the bacterial outer membrane, resulting in ion leakage and cell content release [22]. Gram-positive bacteria, which retain the crystal violet dye, are more sensitive to plant extracts than gram-negative bacteria[23][24] . This sensitivity is attributed to the absence of a protective outer membrane in gram-positive bacteria. The antibacterial substance can easily penetrate the bacterial cell wall and cytoplasmic membrane, leading to cytoplasmic leakage and clotting [25][26].

Table [4] Inhibitory effectiveness of D. graveolens extracts on S. aureus

Statistics								
Type of bacteria	Extracts			Control	25mm	50mm	100mm	200mm
S.aureus	Ethanol	Burn	N Valid	9	9	9	9	9
			Mean	0	4.89	7.33	10.22	14.44
			SD	0	5.862	7.176	7.965	6.126
		Urine	N Valid	16	16	16	16	16
			Mean	0	3.75	6.81	10.19	12.13
			SD	0	5.791	7.241	7.626	8.846
	Aqueous	Burn	N Valid	9	9	9	9	9
			Mean	0	0	0	0	0
			SD	0	0	0	0	0
		Urine	N Valid	16	16	16	16	16
			Mean	0	0	0	0	0
			SD	0	0	0	0	0

On the other hand, [17] reported that D. graveolens essential oil (10 mg) was effective against two Gram-positive bacteria, Staphylococcus aureus and Bacillus subtilis, showing an inhibition zone diameter of 33.0 and 22.0 mm, respectively. The antibacterial activity of D. graveolens on S. aureus was also studied by [18], who indicated that the minimum inhibitory concentration value is 5 mg/ml and the minimum antibacterial concentration value is 10 mg/ml, which are very close to each other, showing antibacterial activity. In addition, pretreatment with essential oil at a concentration of (10 mg) showed that after two hours of exposure, an antibacterial endpoint of (99%) was obtained. They noted that the mechanism responsible for this strong antibacterial role is the direct effect on the cell wall and changes in cytoplasm density and distribution, which represent potential sites for the antibacterial action of the active substances. This explains the results of the current study on the role of raw extracts of the D. graveolens plant against bacteria in the study current[27] reported the

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antibacterial activity of acetone/water crude extract (4:1) and hexane fraction against Staphylococcus aureus. In contrast, a recent study reported no activity against Staphylococcus aureus, while D. graveolens extract (1.25 mg) was able to inhibit the Gram-positive bacteria B. subtilis with an inhibition zone of 10 mm[28] . Extraction processes affect the chemical profile, which may explain the difference in antibacterial activity results obtained between different studies and the present study. The presence of some active compounds of D. graveolens such as aldehydes and phenols is associated with antimicrobial activity[29][30] studied the activity of some flavonoids extracted from the aerial parts of D. graveolens. They reported weak antibacterial activity against S. epidermidis with MIC values of 40, 100 and 80 µg/ml, respectively.

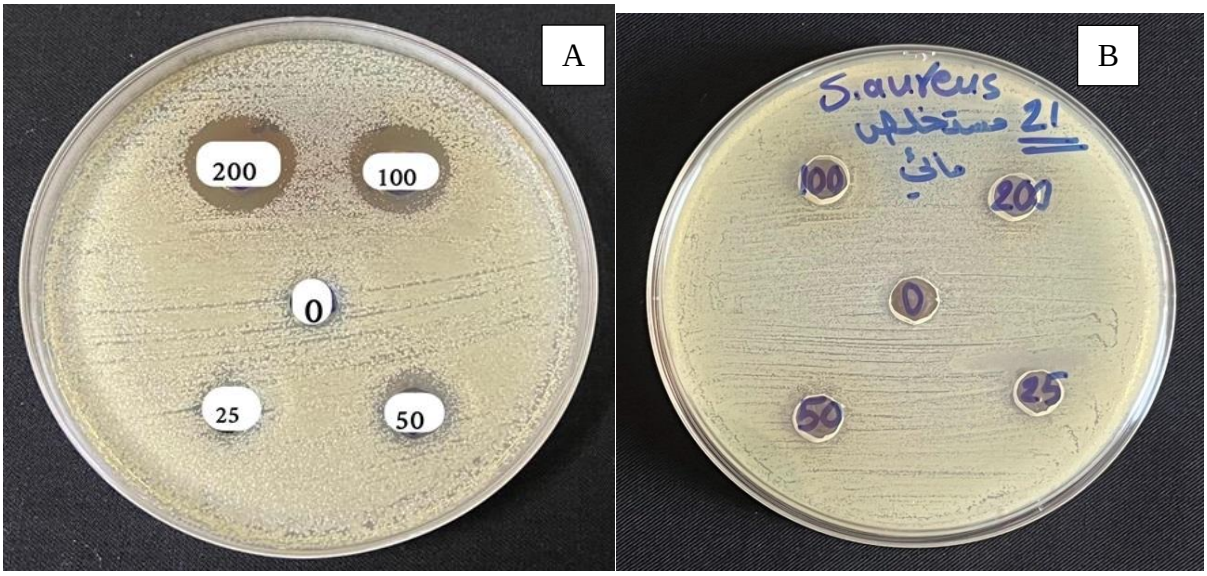

Fig.2 The effect of ethanol(A) and aqueous extracts(2) of the blow plant on S.aureus

4. CONCLUSIONS

Based on the results of the current study, it was found that alcoholic extracts of the D. graveolens were effective against bacteria, especially Staphylococcus aureus, which was isolated from urinary tract infections and burns.

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