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Studying the effect of alcoholic extract of *Myrtus communis* and *Mirabilis jalapa* plants on some bacterial species of wound infections and antibiotic sensitivity of this isolates

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ABSTRACT

The study included the collection of 42 abscess samples from patients with wound infections who attended the consulting clinic in Baquba Teaching Hospital. The information about each person was recorded in a special form. The results showed that the highest infection rate was in the age group of 21–30 years, at a rate of 38.9% out of the total samples. The percentage of males infected was the highest, reaching 39.3%. The first culture results of samples were positive in 73.8% of cases, whereas 26.2% were transplant negative.

After culture was performed on differential media using a biochemical test, the results showed contamination with bacteria *E. coli*, *Pseudomonas spp.*, *Enterobacter spp.*, and *Staphylococcus epidermis*. with a percentage of 19.4%, 9.6% for *Klebsiella spp.*, and a ratio of 4% for both *Proteus spp.* and *Staph. Aureus*.

The results of the bacterial sensitivity test against antibiotics showed that the highest rate of resistance was 100% against antibiotics cefotaxime, clindamycine, and cephalaxine in bacteria *Proteus spp.* and *Klebsiella spp.* showed the same ratio towards ceftazimids and cephalaxine, while the lowest resistance rate was 33.3% to cefotaxime, ceftazimids, in *pseudomonas spp.*, and erythromycin and clindamycine in *Enterobacter spp.*

The results of testing the alcoholic extract of the *Mirabilis jalapa* showed average inhibition diameters of (3, 2, 1.5, 1.5) mm in *Proteus spp.*, *E. coli*, *E. coli*, *Enterobacter spp.*, and *Klebsiella spp.* consecutively, while it had no effect on *Pseudomonas spp.*, *Staph. aureus*, or *Staph.* As for the alcoholic extract of *Myrtus communis*, the rates of inhibition were reached (6.7, 6.6, 5.6, 4.3, 1.7) mm in *E. coli*, *Proteus spp.*, *Enterobacter spp.*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Klebsiella spp.*, and *Pseudomonas spp.* consecutively.

Key Words: *Myrtus communis* , *Mirabilis jalapa* , antibiotic resistance

Introduction

There has been a clear interest in the use of medicine and aromatic plants with the development of countries in order for these plants to enter the pharmaceutical industries because their use is safe and does not lead to the emergence of any side effects. (1). Skin diseases can be brought on by *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* (2,3,4). The skin has a complex flora, and infections can occur when the skin's integrity is compromised or when the immune system is weak. When microorganisms from a burn wound infiltrate healthy tissue, local sepsis develops; if they do so through the lymphatic and vascular systems, systemic sepsis results. (3)

The problem is made worse by the emergence of multidrug-resistant microorganisms, particularly *Staphylococcus aureus* and *Pseudomonas aeruginosa*, present in hospital and community-acquired infections. Plants are rich in alkaloids and other phytochemical content, which may be present in all

organs of the plant, including roots, stems, buds, leaves, flowers, and fruits. Infectious diseases affect millions of people worldwide and are one of the major causes of death in history. (5,6,7).

Mirabilis jalapa for medicinal and decorative uses The notion that the flower blooms in the mild afternoon or at dusk gives it one of its popular names (4 p.m. to 8 p.m.) belonging to the family Nyctaginaceae (7). From the root and aerial parts of the *Mirabilis jalapa* plant, many components have been identified, including several terpenoids, mirabilalones A to D, boeravinones C, and isoquinoline derived. (8). *Myrtus communis* is a popular spice with numerous uses in the food, cosmetics, and pharmaceutical industries. *E. coli* and other food-borne and clinical pathogens were tested for by essential oils and organic solvent extracts of leaves and stems for their antibacterial properties. *Mycobacterium tuberculosis*, *P. aeruginosa*, *P. aeruginosa* and *Proteus mirabilis* (9).

Methodology

-Collection of the plant materials

The leaves of *Mirabilis jalapa* and *Myrtus communis* were collected from one of the home gardens in the city of Baquba. The leaves were brought to the laboratory, cleaned with soft cloths to get rid of any dust or insects, dried in the shade for a week with constant turning to prevent mold, weighed, pulverized in a mortar and pestle, put in an airtight bottle, and kept in dissectors for extraction. (10).

-Preparation of the alcoholic extract of the plant *Mirabilis jalapa* & *Myrtus communis*

25 grams of dry leaf powder were weighed for *Mirabilis jalapa* and *Myrtus communis*, then put in one liter of ethanol alcohol, and left for 24 hours at 35 degrees with constant stirring in the shaking incubator. After that, the mixture was filtered through filter paper, the filtrate was centrifuged for 10 minutes at 2500 rpm, and the extract was dried out for 24 hours at 40 °C. (11,12).

-Patient and specimens collection

obtained 42 diagnoses isolated from patients suffering from infection wounds from hospitals and laboratories in Baquba during October-December 2017. All bacteria isolates were re-diagnosed and identified in the microbiology laboratories of the Biology department, the College of Basic Education, and Diyala University according to their colony morphology, microscopic gram stain, investigation, and biochemical test.

-Antibacterial activity assays

The Kirby Bauer method was employed to evaluate the extracts' antibacterial activity (13). In all, 14 different bacterial strains were tested on Muller-Hinton agar. To ensure an even distribution of inoculums, plates were streaked with the test organism (prepared in a sterile saline tube). Each experiment was repeated three times. A circular well with a 6 mm diameter was aseptically punched, and 100 liters of crude metabolic extracts from the leaves of *Mirabilis jalapa* and *Myrtus communis* were poured into the control well.

Results & Discussion

-Distribution of wound infection by age group and sex.

Forty-two patients (28 males and 14 females) with various wound infections were in the consulting clinic at the Baquba teaching hospital. It is clear from the table that in the age group 21–30, which is most susceptible to wound infections, the percentage of males is highest, reaching 39.3%, compared to the female percentage of 35.7 in the same age group. This is due to the fact that this category is the most engaged in business, especially men.

Table 1: Distribution of wound infection and sex by age group

Category/sex	Males		Females		Total	
	N	%	N	%	N	%
Less than 10 year	1	36	3	21.4	4	9.5
11-20	3	10.7	3	21.4	6	14.3
21-30	11	39.3	5	35.7	16	38.9
31-40	6	21.4	2	14.3	8	19.4
41-50	6	21.4	0	0	6	14.3
1-60	7	3.6	1	7.1	8	19.4
Total	28	100	14	100	42	100

-Bacterial species isolated from wound infections.

42 samples were collected from patients with wound infections according to the microscopic character, shape of the bacterial cell, staining with gram stain, biochemistry test, and agricultural traits of colonies on growth media. The proportion of positive samples was 73.8%, while the remaining samples did not show any bacterial growth, as shown in table 2.

Table 2: percentage of contamination of samples collected from wound infection

Type of samples	N	%
Culture positive samples	31	73.8
Culture negative samples	11	26.2
Total	42	100

As for negative isolates, the researcher (14) attributed the reason for the absence of bacterial isolates to the possibility of treatment with antibiotics that the patient receives, which may be by using broad-spectrum antibiotics as well as the use of external disinfectants that may have an effect in reducing the proportion of isolated bacteria.

The results of the study are shown in table (3), which shows that the highest percentage of contaminants was with the bacteria *Pseudomonas spp.*, *Enterobacter spp.*, *Escherichia coli*, and *Staph epidermis* with a ratio of 19.4%; the other types were *Klebsiella spp.*, *Proteus spp.*, and *Staph. aureus* with a ratio of (9.6, 6.4, and 6.4)% consecutively, also (15). mentioned that bacterial isolation from wound infection was consistent with other earlier research, which also noted that the nosocomial transmission of microorganisms to the burn wound happened via transfer from the hands of health care personnel.

Table 3: bacterial species isolated from 42 patients with wound infections

bacterial isolates	N	%
<i>E.coli</i>	6	19.4
<i>Proteus spp.</i>	2	6.4
<i>Pseudomonas spp.</i>	6	19.4
<i>Enterobacter spp.</i>	6	19.4
<i>Klebsiella spp.</i>	3	9.6
<i>Staph.aureus</i>	2	6.4
<i>Staph.epidermis</i>	6	19.4
Total of positive culture isolates	31	100

- Bacterial sensitivity to the antibiotics used (16) reported that *Pseudomonas* spp. was the most common pathogen isolated from wound infections; (17) reported that the rate of gram-negative bacteria isolation from burn wounds was more than twice that of gram-positive bacteria; and they observed that *Klebsiella* spp. was the pathogen most frequently isolated, constituting 24.4%, followed by *P. aeruginosa* (29%) *Staph. aureus* (26.8%).

The antibiotic resistance pattern of Gram –positive isolates is shown in(figure 1,2) the isolates of *Staph.aureus* , *Staph.epidermis* showed 100% of resistance to Cloxaciline, Ampiciline, Vancomycine , while the resistance ratio was (50,100) % to the antibiotic polymixine B IN *Staph .epidermis* & *Staph.aureus* consecutively.

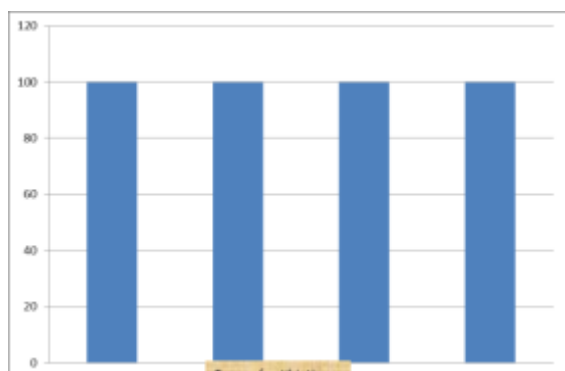


Fig. 1: Percentage of *Staph.aureus* resistant to antibiotics

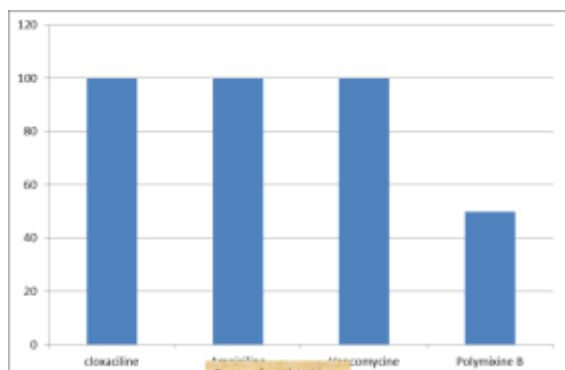


Fig. 2: Percentage of *Staph.epidermis* resistant to antibiotics

While the Gram-negative bacteria isolated from wounds exhibit a pattern of drug resistance in figures (3,4,5,6,7), *Proteus* spp. showed 100% resistance to cefotaxime, ceftazidime, Erythromycine, Clondamycine, Clindamycine, cephalixine while the lowest resistance rate was 33.3% to ceftazidime in *Pseudomonas* spp., *E.coli*, Erythromycine in *Enterobacter* spp., and *Klebsiella* spp.

Our study a relevant percentage 100% of *Staph aureus* , *Staph epidermis* resistance to cloxaciline , Ampiciline , Vancomycine MRSA poses two issues, the first of which is related to the chronic wound and the second of which is related to the influence of MRSA on the chronic wound life. MRSA has always

been a significant source of infection in acute-soft-tissue wounds. (18).

Also study showed the high resistant of *proteus* spp. it is due to all the antibiotics used a significant decrease in susceptibility to 3rd- generation cephalosporine & ciprofloxacin the prevalence of ESBL remained stable but AmpC β – lactamase – producing *P. mirabilis* in creased significantly (19).

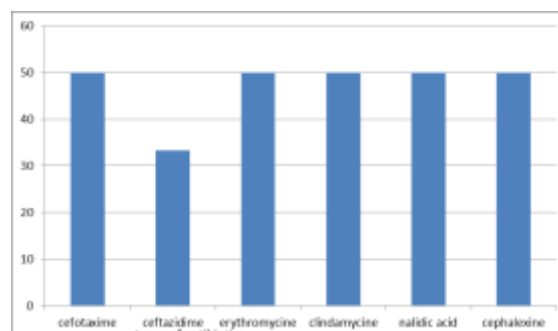


Fig. 3: Percentage of *Pseudomonas* spp.resistant to antibiotics

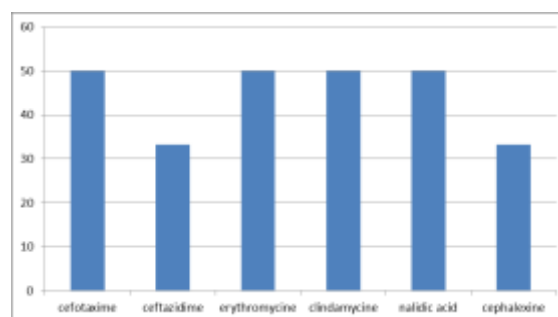


Fig. 4: Percentage of *E.coli* .resistant to antibiotics

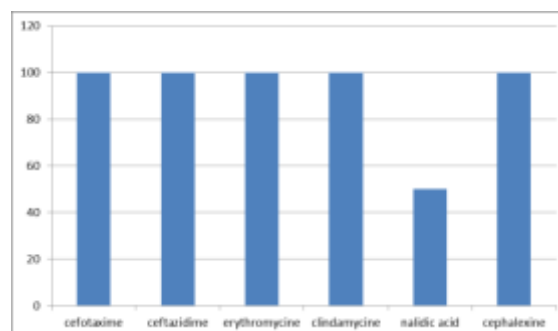


Fig. 5: Percentage of *Proteus* spp. resistant to antibiotics

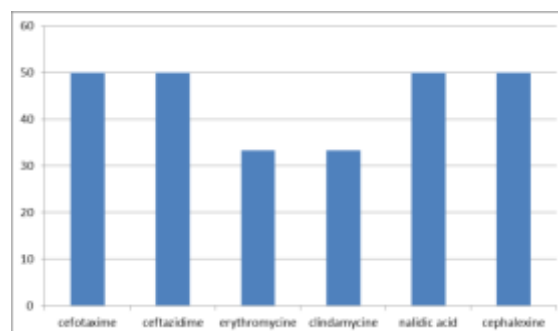


Fig. 6: Percentage of *Enterobacter* spp. resistant to antibiotics

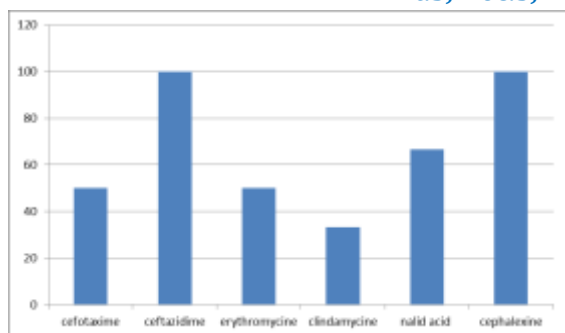


Fig. 7: Percentage of *Klebsiella* spp. resistant to antibiotics

-Effect of the alcoholic extract of *Mirabilis jalapa* & *Mirtus communis* plants on the growth of bacteria isolated from wound infection.

The results of the alcoholic extract of *Mirabilis jalapa* and *Mirtus communis* plants on bacteria contaminating wounds showed that the rates of *Mirabilis jalapa* inhibition diameters reached 3, 2, 1.5, 1.5 mm in bacteria including *Proteus* spp., *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. consecutively. whereas the crude alcoholic extract did not show any effect on the bacteria *Pseudomonas* spp., *Staph. aureus*, and *Staph. epidermis* This is evident in figures 8 and 9. The leaf extracts of *Mirabilis jalapa* contained glycosides, anthraquinone, tannins, carbohydrate, and protein (20); according to a preliminary phytochemical screening of the extract for the bioactive compounds, the presence of tannins, cardiac glycosides, and saponins was detected, while phlobatanins and antraquinones were absent. The presence of these phyto constituents may be responsible for the antimicrobial, antioxidant, free radical scavenger, and hemolytic properties of the plant extract (21);

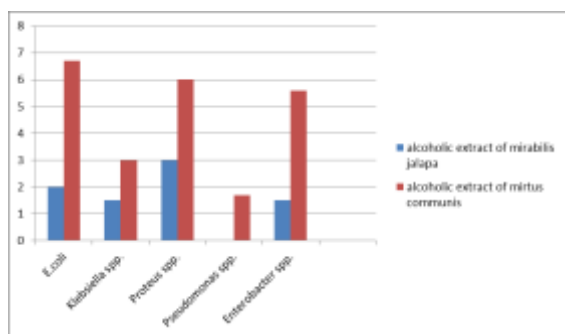


Fig. 8: Inhibition rates of the crude alcoholic extract of *Mirabilis jalapa* & *Mirtus communis* plants against Gram-negative bacteria

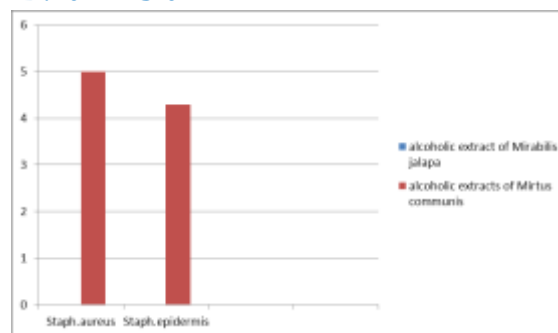


Fig. 9: Inhibition rates of the crude alcoholic extract of *Mirabilis jalapa* & *Mirtus communis* plants against Gram-positive bacteria

As mentioned by the researcher (22): "metal nanoparticles' ability to interact closely with microbial membranes due to their small size and high surface-to-volume ratio has been credited as the cause of their bactericidal impact rather than only the release of metal ions in solution or on culture plates." The results of the crude alcoholic extract of *Mirtus communis* also showed the rates of inhibition diameters reached (6.7, 6.6, 5.6, 4.3, 3.17 mm) in bacteria *E. coli*, *Proteus* spp., *Enterobacter* spp., *Staph. aureus*, *Staph. epidermis*, *Klebsiella* spp., and *Pseudomonas* spp. Consequentially, the oxygenated terpenes such as 1,8-cineole, linalool, and -terpineol exhibit potent antimicrobial activity. Several studies reported that the antimicrobial activity of herbs is due to a phenolic compound (23), and the diameter of the inhibition zone revealed that the ethanol extract of myrtle leaves tested had significant antimicrobial activity against all the strains. (24) Multiple mechanisms of action are possible for polyphenolic substances. They can break down cell walls and cytoplasm membranes, causing the leakage of cellular components, altering fatty acid and phospholipid composition, influencing DNA and RNA production, and destroying protein interactions. (25). Myrtus communis is a suitable option for a herbal mouthwash, as studies have shown that it has an antibacterial impact on oral Gram positive (26) and Gram negative pathogens (27). Iranian natural herb Myrtus communis is found there. It grows in the provinces of Khorasan, Manjil, Kazeran, and Sarab (28). In order to cure periodontal disease or to enhance periodontal regeneration, the use of Myrtus communis oil or extract in local delivery methods such strips, chips, and fibers should be investigated. By doing this, you might be able to avoid the negative effects of antibiotics on periodontitis. Antibiotics and Myrtus communis used together may be effective in combating the problem of growing drug resistance. due to the fact that the bacteria were sensitive to all concentrations.

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دراسة تأثير المستخلص الكحولي لنباتي الأس و لآلة عباس على بعض الأنواع البكتيرية الملوثة للجروح وحساسيتها للمضادات الحيوية

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الملخص

اشتملت الدراسة على جمع 42 عينة خراج من المرضى الذين يعانون من خمج الجروح والمراجعين للعيادة الاستشارية في مستشفى عام بعقوبة وقد دونت المعلومات الخاصة لكل شخص. وقد أظهرت النتائج أن أعلى نسبة خمج كانت الفئة العمرية (21-30) سنة ونسبة 38.9% من مجموع العينات وكانت نسبة الذكور المصابين بالخمج هي النسبة الأعلى وبلغت 39.3%، وقد كانت نتائج الزرع الأولي للعينات ايجابية الزرع في 73.8% في حين كانت 26.2% سالبة الزرع، وبعد إجراء الزرع على الأوساط التفريقية والانتخابية والتشخيص باستخدام *Enterobacter*, *Pseudomonas spp.*, *E.coli* الاختبارات الكيموحياتية التلوث بنسبة 19.4% لكلا من *Staph.aureus*, *proteus spp* ونسبة 6.4% لكلا من *Klebsiella spp*, ونسبة 9.6%، *proteus spp.* , *Staph.epidermis* وقد أظهرت نتائج اختبار حساسية البكتريا تجاه المضادات الحيوية أن أعلى نسبة مقاومة بلغت 100% تجاه مضادات *ceftazidime* , *cephalexine* ادات في حين كانت أقل نسبة مقاومة 33.3% *cefotaxime*, *ceftazidime*, *cephalexine* بكتريا وقد أظهرت نتائج اختبار المستخلص الكحولي لنبات لآلة عباس معدلات أقطار تثبيط بلغت (1.5,1.5,2,3) ملم على التوالي. أما المستخلص الكحولي لنبات الأس فقد بلغت *Pseudomonas spp.*, *Enterobacter spp.*, *Klebsiella spp.*, *E.coli*, *Proteus* *Staph.epidermis*, *Staph.aureus*, *Klebsiella spp.*, *Proteus Enterobacterspp.*, *E.coli* 1.7,3,4.3,5,5.6,6,6.7spp., ملم في بكتريا