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Comparison of the effect of *Zingiber officinale* extract and antibiotics on *Staphylococcus aureus* isolated from door handles in the

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ABSTRACT

This study was conducted at the College of Science - Department of Life Sciences where it examined the effect of *Zingiber officinale* alcoholic extract and some antibiotics (Levofloxacin, Vancomycin, Nitrofurantion, Oxacillin) on isolates of *Staphylococcus aureus*, which were isolated from door handles in the College of Sciences, University of Tikrit, Department of Biology, and through the obtained results the *S. aureus* was sensitive to all three concentrations (25 – 50 – 75)% of the alcoholic extract, but it was differentially sensitive to the antibiotics as the strongest effect and the largest area of inhibition was for Levofloxacin 5 µg (LEV-5), the diameter of the inhibition zone was (22 mm) compared to the other antibiotics that showed clear and convergent inhibition zones, but less in diameter than LEV-5.

Introduction

S. aureus is Gram-positive, belonging to the family Micrococaceae, positive for coagulation enzyme test, not capsule forming, facultative aerobic or aerobic fermentation for mannitol, glucose and maltose, not spores forming and arranged in bunches of grapes, grown in many agricultural media [1]. It has the ability to survive heat, salinity and can survive for 14 weeks in dry pus, immobile, its colonies are medium in size, with a diameter of up to (1.3 mm), yellow or golden in color, smooth, circular and regular in edge, It grows on solid media such as the nutrient agar, but on the blood agar its colonies are larger with a transparent edge because it causes complete hemolysis from the type hemolysis-β. *S. aureus* are normal flora present in the human body, as the upper respiratory tract is their normal flora. They often grow in respiratory membranes and on the surface of the skin, especially in wet areas. They enter tissues through wounds and burns, as it was found that they are the main responsible for their contamination. *S. aureus* is one of the most isolated pathogens from hospitals, due to its resistance to antibiotics, as it is characterized by its resistance to penicillin and methicillin, so it is called Methicilli Resistant *Staphylococcus aureus* or (MRSA) [2].

In addition for that *Staphylococcus aureus* having virulence factors and its great ability to settle and reproduce in many conditions, so it causes a large

number of bacterial infections and also causes abscess, which is a purulent gathering that causes acute inflammation in tissues with its spoilage and fermentation[3].

Natural plants are an important source of many pharmaceutical materials since ancient times to this day, used by people in the treatment of many diseases, because they contain a large number of biologically active compounds [4]

The wrong using of these compounds resulting development of bacterial resistance towards many antibiotics, which increased the interest of specialists in the natural substances that were previously used as treatment, Also the increase in the number of researches proving the effectiveness of these substances and their sufficient ability to find a solution to the problem of resistance, and among these natural substances are the roots of *Z. officinale*. The development of bacterial resistance towards many antibiotics resulted from the wrong use of these compounds, which increased the interest of specialists in natural materials that were previously used as treatment, in addition to the increase in the number of researches that prove the effectiveness of these materials and their sufficient ability to find a solution to the problem of resistance, and among these natural materials are the roots of *Z. officinale*. Alcoholic *Z. officinale* extract contains

carbohydrates, flavonoids, alkaloids, phenols, and terpenes, and this agrees with most studies that dealt with the chemical composition of *Z. officinale*[5] that these compounds are effective against many germs and bacteria.

The aim of study

Knowing the effect of *Z. officinale* extract on *S. aureus* bacteria and comparing the results with the results of the effect of some antibiotics on the same bacteria.

Materials and Methods

1- Preparation of Culture Media

The culture media was prepared according to the manufacturer's instructions installed on the packages, then it was sterilized by Autoclave at a temperature of 121°C and at a pressure of 15 pounds/inch² for a period of 15 minutes. After pouring the media, it was incubated at 37°C for 24 hours to ensure that it was not contaminated. Then it was kept in the refrigerator at 4°C until use.

1-1 Methyl red Reagent

The reagent was prepared by dissolving (0.1) g of methyl red powder in (300) ml of (95%) ethanol and then filling the volume to (500) ml by adding (200) ml of distilled water.

2- Biochemical tests:

2-1 Indol Test

The tubes containing peptone water medium were inoculated with bacteria and then incubated for 24 hours at a temperature of 35°C. After incubation and observation of bacterial growth, 1-5 drops of Kovacs reagent were added to the tube walls and the appearance of a red ring at the top of the medium indicated the positivity of this test[6]

2-2 Methyl Red Test

Test tubes containing MR_VP medium were inoculated with bacteria, then the tubes were incubated for 24 hours at 35°C. After incubation and observation of bacterial growth, 6 drops of the reagent (VP1) were added and the tube was agitated, then two drops of potassium hydroxide (VP2) reagent were added to it, and after 30 minutes The appearance of a dark pink or red color above the middle indicates a positive test[6].

2-3 Voges Proskauer Test

Test tubes containing MR-VP medium were inoculated with bacteria and then incubated for 24 hours at 35°C. After incubation and observation of bacterial growth, 6 drops of alpha-naphthol reagent (VP1) were added and the tube was agitated, then two drops of potassium hydroxide (VP2) reagent were added to it, after 30 minutes The appearance of a dark pink or red color above the middle indicates a positive test,[6].

2-4 Citrate Utilization Test

Test tubes were inoculated amid citrate agar consumption by planting bacteria on the surface of the medium and then incubated for 24 hours at a temperature of 35° C. When the color of the medium changed from green to blue, this test was positive,[6].

3- Preparation of the alcoholic extract:

Mix (20 g) of *Zingiber officinale* powder with (200 ml) of ethyl alcohol 70% and leave the solution with continuous stirring by a shaker device for 24 hours at a temperature of (25) °C, then filter the solution through layers of gauze and then put the extract in a Petri dish and left uncovered to dry at room temperature (25 °C) and then stored in sterile glass bottles to test its effectiveness against isolates used later,[7].

4- Bacterial isolates:

- Samples and swabs taken from door handles were transported directly to the laboratory.
- Mannitol agar medium was prepared according to the manufacturer's instructions.
- 10 samples were cultured on mannitol media and the cultures were incubated in aerobic conditions at a temperature of 37°C for a period of 24 hours.
- *S. aureus* was diagnosed according on the agronomic, microscopic and biochemical characteristics based on [8]for colonies growing on mannitol agar in terms of golden colony color and the color of the media changed from pink to yellow.

As for the biochemical tests, a smear of the developing colonies was placed on a slide on which a drop of hydrogen peroxide was previously placed, where we noticed the release of a bubble, evidence of the release of oxygen, which is resisted by bacteria and appears as an accidental product.

5- Preparation of plant alcoholic extract:

Used (2 g) of alcoholic *Z. officinale* extract was weighed and dissolved with (10 ml) of dimethyl sulfoxide (DMSO) organic solution by stirring it and then placing it in a water bath at a temperature of 50 ° C for 10 minutes, and then the solution was purified by using a micro filter for the purpose of purification and filtering to obtain a pure solution As much as possible, then different dilutions were made of the extract as the following concentrations were prepared: (25 - 50 - 75) % of the original solution [9]

6- Antimicrobial sensitivity test:

• Preparing the vaccines:

According to the manufacturer's instructions, an appropriate amount of Mueller_Hinton Agar was prepared and poured into several sterile Petri dishes. Then, the diagnosed isolates of *Staphylococcus aureus* were transferred to these dishes by a sterile swab, then the sensitivity test was applied by antibiotics and plant extract

• Application of antibiotic tablets:

After the vaccine had dried on the medial surfaces, (4) tablets of antibiotics were placed using sterile forceps, (4) tablets in one dish, with firm pressure to fix the tablets of the following antibiotics:

- Nitro furantion 100 mcg (F-100)
- Vancomycin 30 mcg (VA)
- Levofloxacin 5 mcg (LEV-5)
- Oxacillin 5 mcg (OX)

The dishes are left for 15 minutes, then turned and incubated at a temperature of 37°C for 18-24 hours.

After that, the result is read by measuring the diameter of the inhibition zone, which represents the area of no bacterial growth surrounding the hole using a ruler.

• **Bacterial sensitivity test against pre-prepared alcoholic *Zingiber officinale* extract:**

In the bacterial sensitivity test for alcoholic *Z. officinale* extract, wells were made in the culture media inoculated with bacteria using a sterile cork piercing with a diameter of 5 mm and by means of a Micropipette 10 microliters of each concentration of the prepared alcoholic *Z. officinale* extract were transferred and placed inside the hole and at the same time control dishes were made by placing 10 microliters of sterile distilled water in the holes instead of *Z. officinale* extract, then incubating the dishes at a temperature of 37°C for 18-24 hours. After that, the result is read by measuring the diameter of the inhibition area, which represents the area of no bacterial growth surrounding the hole using a ruler

Results and discussion

Table 1: shows the diameters of the inhibition zones (mm) for the pathogenic bacteria *S. aureus* by *Z. officinale* alcohol extract.

Concentration of <i>Z. officinale</i> alcohol extract	Diameter of inhibition Zone in mm.
75%	10
50%	9
25%	8

Table 3: shows the results of the biochemical tests for *S. aureus*.

Number of samples	Indol test	Methyl Red test	Voges Proskauer test	Citrate Utilization test
10	-ve	-ve	-ve	-ve

To convert glucose to acetylmethyl carbinol (Acetoin), the first reagent (VP1) alphanaphthol and the second reagent (VP2) Potassium hydroxide did not react with the resulting acetoin, and it was also negative for the citrate test, which led to no center color change from green to blue.

Table (1) indicates the results of the effectiveness of the alcoholic extract using the three concentrations (75-50-25%), where there was inhibition of *S. aureus* bacteria in the alcoholic extract, Where the results of inhibition for the three concentrations were as follows, 75% >10 mm, 50% >9 mm, and 25% >8 mm.

Z. officinale extracts showed high efficacy against the tested bacteria and this is consistent with what was mentioned[11] in terms of *Z. officinale* containing about 400 compounds of different compounds, which are a mixture of volatile oils and non-volatile components such as Gingerols, Sesquiterpenoids, Zingerone, Shogaols.

The alcoholic extract shows its effectiveness against *S. aureus* bacteria in all concentrations (75-50-25%), respectively.

Table 2: shows the results of antibiotic susceptibility to *S. aureus* bacteria.

Standard antibiotic	Diameter of inhibition zone in mm.
Nitrofurantion (F-100)	14
Vancomycin (VA)	15
Levofloxacin (LEV-5)	22
Oxacillin (OX)	16

As for the results of the biochemical tests as shown in table (3), *S. aureus* was negative for each of the Indol test, as a red ring did not appear on the surface of the media as a result of the failure of the tryptophase enzyme to analyze the amino acid tryptophan using the Kovacs reagent. This test is one of the important tests in differentiating between *S. aureus* and the rest other types of intestinal family, and negative for the methyl red test as a result of the lack of acid from consuming and fermenting glucose and peptose, which leads to the lack of acid production and the low pH of the media, and then the color of the media does not turn red, and these two characteristics are important for its diagnosis [10]and it was negative for the voges-proskauer test as a result of the appearance of a yellow-brown color in the liquid medium and this is due to the inability of bacteria.

Compared with what is shown in Table No. (2), where they showed the effectiveness of the mentioned antibiotics against *S. aureus* bacteria, where the results showed that the mentioned bacteria showed a very clear sensitivity to the antibiotics used and with slightly different inhibition diameters, where it was found that the inhibition results are close to the antibiotics (OX-VA-F). -100) compared to the effect of anti-LEV, which showed a relatively high sensitivity of *S. aureus* bacteria towards it.

Where the results of inhibition for the three antibiotics were as follows, OX < 16 mm, VA > 15 mm, and F-100 > 14 mm. As for the anti-LEV, its inhibition diameter was about 22 mm, and this shows the stronger effect of this antibacterial compared to the rest of the mentioned antibiotics against *S. aureus* bacteria.

The results of this study agreed with the results of the previous studies of [12]and[13], which recorded the antagonistic activity of some plant extracts, including ginger, against bacteria.

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مقارنة تأثير مستخلص الزنجبيل والمضادات الحيوية على بكتريا *Staphylococcus aureus*

المعزولة من مقابض الأبواب في قسم علوم الحياة-جامعة تكريت

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الملخص

أجريت هذه الدراسة في كلية العلوم - قسم علوم الحياة حيث درست تأثير مستخلص الزنجبيل (*Zingiber officinale*) الكحولي وبعض المضادات الحيوية (Levofloxacin, Vancomycin, Nitrofurantion, Oxacillin) على عزلات من بكتريا (*Staphylococcus aureus*) والتي تم الحصول عليها من مقابض الأبواب من كلية العلوم جامعة تكريت قسم علوم الحياة، ومن خلال النتائج التي تم الحصول عليها كانت بكتريا (*S. aureus*) حساسة لجميع التراكيز الثلاثة (25 ، 50 ، 75 %) للمستخلص الكحولي للزنجبيل، غير انها كانت حساسة بشكل متباين للمضادات الحيوية المذكورة حيث أن أقوى تأثير وأكبر منطقة تثبيط كانت لمضاد (Levofloxacin 5 µg) (LEV-5) حيث كان قطر منطقة التثبيط له ٢٢ ملليمتر مقارنة مع بقية المضادات التي اظهرت مناطق تثبيط واضحة ومقاربة مع بعضها لكنها اقل قطرا من المضاد LEV-5.