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The Effect of Alcoholic, Aqueous and Crude Extracts of *Lemon citrus* Seeds on *Staphylococcus* and *Klebsiella* Bacteria

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Abstract

Five isolates of staphylococcus bacteria identified by phytic were obtained and activated on solid saline mannitol medium, where the colonies appeared in a golden color. Three extracts of dry lemon seeds were prepared (raw, 1% aqueous extract, 1% alcoholic extract). It was celebrated that the aqueous quotation was added operative on bacterial progress than the alcoholic and rudimentary sources. Three isolates of Klebsiella bacteria identified by VITEK were also obtained, and they were activated on solid MacConkey medium, and the colonies appeared in a pink color because they were fermented to the sugar lactose. The effect of the three extracts of dry lemon seeds (raw, 1% aqueous extract, 1% alcoholic extract) was studied. It was distinguished that the liquid isolated was extra active on bacterial evolution than the ethanolic and basic isolated extract. The derivation of the lemon is unidentified, however, lemons are supposed to have chief developed in northeast India. A genomic revision of the lemon specified that it was a mixture among bitter orange (bitter carroty) and citron. The derivation of the expression lemon could be Central Eastern. The term allurements from the Ancient French limon, then Italian limone, from the Arabic laymūn or līmūn, and from the Persian līmūn, a generic term for citrus berry, which is a similar to Sanskrit. Lemons essential a least hotness of nearby 7°C (45°F), so they are not resistant year-curved in moderate climates, but converted tougher as they matured. Citrus require minimal pruning by trimming overcrowded branches, with the tallest branch cut back to embolden bushy advance. During summer, larceny spinal tips of the greatest vigorous progression promise more profuse canopy enlargement. As mature plants may produce unwanted, fast-growing shoots.

Keywords: Mannitol, *staphylococcus*, *Klebsiella*, bacteria, MacConkey

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INTRODUCTION

Staphylococcus aureus bacteria are spherical cells that are positive for the Gram stain. They are present in the form of single globules, in pairs, or gathered in clusters. They give positive results for the detection test for the catalase enzyme, and they also can ferment glucose [1]. It grows easily on several growth media and includes a group of strains and names with varying medical importance and the ability to cause disease. In general, it lives in symbiotic balance with the body [2]. *Staphylococcus aureus* bacteria is considered the most dangerous among all the different and common types of staphylococcal bacteria.

Approximately 20% of the world's population are permanent carriers of this bacteria, which can

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be found naturally on the surface of the skin and in the nose. It also lives naturally in the lower genital tract of women. *Staphylococcus aureus* bacteria are present (usually temporarily) in the noses of 30% and on the skin of 20% of healthy people. The bacteria are more likely to be present in hospitalized patients or health care personnel.

The use of plants in medicines for a wide range of human diseases has deep roots in human history.

Lime is also used to overcome dysentery, constipation, dizziness, body odor, and increase appetite.

Increasing resistance to conventional antimicrobials has become a concern for clinical microbiologists worldwide. *C. aurantifolia* seeds can be used against various pathogenic bacteria in traditional medicine as an antimicrobial agent.

While *Klebsiella* bacteria are considered opportunistic pathogens due to their possession of an antiphagocytic capsule, they are responsible for complications resulting from acute infections, such as septicemia, pneumonia, urinary tract infection, or soft tissue infection. These bacteria constitute 6% of hospital-acquired infections and are associated with elderly patients who suffer from immunocompromised suppression, alcoholics, diabetics, and patients with chronic pulmonary insufficiency.

Klebsiella can cause damage to the respiratory system, joints, meninges, conjunctiva, genitourinary organs, as well as sepsis and purulent postoperative complications [3, 4].

Infectious diseases are responsible for a high proportion of health problems in developing countries [5]. According to the World Health Organization (WHO) report, 80% of the world population currently uses herbal medicines for some aspects of primary health care [6]. Approximately 20% of the world's plants have undergone pharmaceutical or biological testing, and many new antibiotics brought to market are obtained from natural or semi-synthetic resources [7, 8]. Many researches and practical experiments have shown that the use of medicinal plants is better than chemical and semi-synthetic drugs because of their fewer side effects, relatively low cost and effectiveness along with their synergistic effect; medicinal plants are used to treat various disorders, such as malaria, diarrhea, burns, gonorrhea, stomach disorders and other infectious diseases [9, 10]. One such plant is *Citrus aurantifolia* (lemon fruit), it is much used in herbal medicine and its bioactive activities in treating cold fever, sore throat, sinusitis and bronchitis, as well as aiding asthma and suppressing stomach pain and has been found to be an excellent cough reliever [11]. It can be beneficial for rheumatoid arthritis, obesity and cellulite, has an astringent and tightening effect to cleanse oily skin and acne, and helps in treating herpes, wounds and insect bites [12].

MATERIALS AND METHODS

Obtaining Bacterial Isolates

The pathogenic bacteria were obtained from the postgraduate laboratory of the College of Education for Girls, where the bacteria were identified using phytic and the number of isolates were 5. They were activated using manitol salt ager medium to form golden colonies.

The pathogenic *Klebsiella* bacteria were obtained from the graduate laboratory of the College of Education for Girls, where the bacteria were diagnosed using phytic acid. The number of isolates were 3, and they were activated by culturing them in Macconkya agar.

Preparation of Extracts

a. *Preparation of the crude extract:* An extract was made from the lemon seeds, after grinding them well.
b. *Preparation of aqueous extract of Lemon citrus seeds:* According to the source [13], it was prepared by dissolving 1 gram of well-ground seeds and mixing them with 100 ml of heated

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distilled water. It was left for a period, then it was filtered using filter paper and then left to dry. In the incubator, the dry extract is then scraped, weighed, and its amount was 0.1 g. It was dissolved in 10 ml of water to obtain a concentration of 1% and placed in the designated hole in the medium of Mueller-Hinton to determine the effect of the extract on bacterial growth [14].

c. *Preparation of alcoholic extract of Lemon citrus seeds:* According to the source [13], it was prepared by dissolving 1 gram of well-ground seeds and mixing them with 100 ml of absolute alcohol. Leave it for a period, then filter it using filter paper, then leave it to dry in the incubator, then rub the dry extract and take a specific weight of 0 g. 1. It is dissolved in 10 ml of water to obtain a concentration of 1% water, and the extract is placed in the designated hole on Mueller-Hinton medium to test its effectiveness on coliform bacteria.

Study the Effect of the Extract on Bacterial Growth

Hinton-Muller agar medium was prepared, and a dilution of the pathogenic bacteria was prepared and compared with a 0.5 Macfet Land tube to obtain 1.5 Drill the aqueous, alcoholic, crude, and control extracts. Then, the aforementioned aqueous, alcoholic, and crude extracts and control (distilled water) were added, and the dishes were incubated in the incubator at a temperature of 37° for 24 hours, after which the results were read by calculating the area of bacterial growth activation using a ruler.

Study of the Effect of the Extract on the Growth of *Staphylococcus aureus* Bacteria

Muller-Hinton agar medium was prepared, and a dilution of the pathogenic bacteria was prepared and compared with a Macfer Land tube (0.5) to obtain 15 x 10^8 bacteria per ml. The bacteria were then spread with a cotton swab on the medium and left for a period, after which the the aqueous, alcoholic, crude, and control extracts were etched, then the aforementioned aqueous, alcoholic, crude, and control extracts were added (distilled water), and the dishes were incubated in the incubator at a temperature of 37° for 24 hours, after which the results were read by calculating the bacterial growth activation zone using ruler.

Study of the Effect of the Extract on the Growth of *Klebsiella* bacteria

Muller-Hinton agar medium was prepared, and a dilution of the pathogenic bacteria was prepared and compared with a Macferland tube (0.5) to obtain 1.5 x 10^8 bacteria per ml. The bacteria were then spread with a cotton swab on the medium and left for a period, after which holes were made. The aqueous, alcoholic, raw extract, and control were then added. The aqueous, alcoholic, and control extracts (distilled water) were added, and the plates were incubated in the incubator at a temperature of 37° for 24 hours. Then the results were read by calculating the bacterial growth activation area using a ruler.

RESULTS AND DISCUSSION

The inhibition zones for the growth of *Staphyllococcus* bacteria were measured due to the use of the extracts, and the inhibition zones were calculated using a ruler as mentioned in Table 1 and according to Figure 1. It was noted that the aqueous extract had a greater effect on bacterial growth than the alcoholic and raw extract. The inhibition is mainly due to the seeds containing vitamin C, which has a major role. The reason for this is that citrus fruits in general, due to their properties, containing vitamin C, and containing many effective compounds that have an important role in preventing the occurrence of chronic infections, especially flaronoids and lemons, which give an acidic taste and which can remain in their natural state for a long time [14].

Then, the inhibition zones for the growth of *Klebsiella* bacteria were measured due to the use of the extracts, and the inhibition zones were calculated using a ruler as mentioned in Table 2 and according to Figure 2.

It was noted that the aqueous extract had a greater effect on bacterial growth than the alcoholic and raw extract. The inhibition is mainly due to seeds containing vitamin C, which has a major role [15].

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The reason for this is that citrus fruits in general, due to their properties, contain vitamin C, and also contain many effective compounds that have an important role in preventing the occurrence of chronic infections, especially flaronoids and lemons, which give an acidic taste and which can remain in their natural state for a long time [16, 17].

Table 1. Measurement of the diameters of inhibition of *Staphylococci* due to *Lemon citrus* seed extract.

Inhibition Zone in mm			Bacterial Isolation Number
Crude	***Alcoholic Extract***	***Aqueous Extract***	
5	8	15	1
3	6	14	2
2	8	10	3
2	7	12	4
4	5	15	5

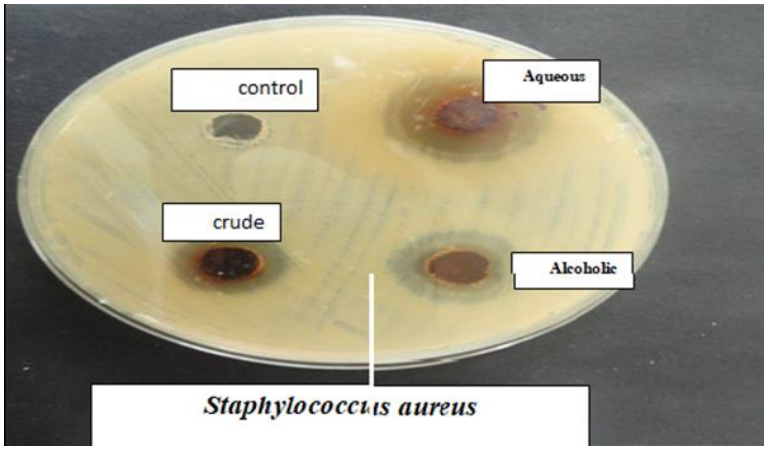

Figure 1. Shows the diameters of inhibition for the three types of extract.

Table 2. Shows the areas of inhibition of *Klebsiella* bacteria by extracts (crude, aqueous, and alcoholic).

Inhibition Zone in mm			Bacterial Isolation Number
Crude	***Alcoholic Extract***	***Aqueous Extract***	
10	16	22	1
9	14	25	2
5	13	20	3

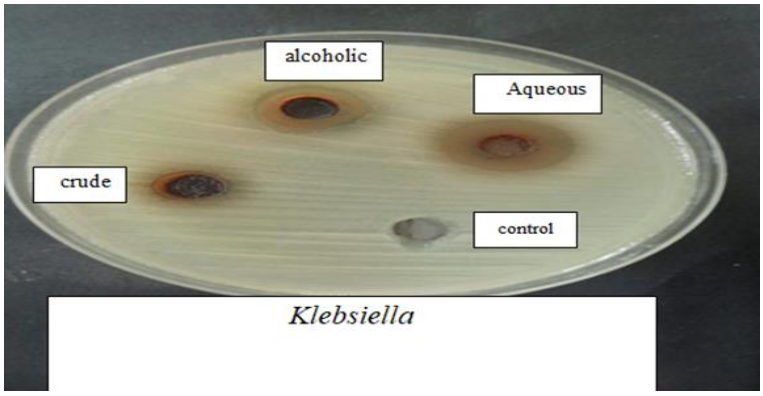

Figure 2. Shows the effect of (crude, aqueous and alcoholic) extracts on the growth of *Klebsiella* bacteria.

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CONCLUSIONS

It was noted that the aqueous extract had a greater effect on bacterial growth than the alcoholic and raw extract. The inhibition is mainly due to seeds containing vitamin C, which has a major role.

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