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# The inhibitory activity of plant extracts (*Mentha Citrato L.* and *Citrus aurantium L.*) towards some bacteria strains isolated from inflamed gums

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## ABSTRACT

This study was conducted in Salahaldin for the period from 15/9/2018 to 15/3/2019, to study the inhibitory activity of aqueous and alcoholic (Eth,Meth) extract of *Mentha Citrato L.* And *Citrus aurantium L* plants by agar well diffusion method on some bacterial causing gingivitis . including *Staphylococcus aureus, Streptococcus faecalis and Klebsiella pneumonia*, were isolated. The results showed that alcohol extracts had a high inhibitory effect on the growth of the bacterial species under study compared to the aqueous extracts. These bacterial isolates were primarily sensitive to the methanolic and ethanolic extract, followed by the hot aqueous extract .

The study showed that *Mentha Citrato L* extract was more effective in inhibiting the growth of bacterial isolates compared to the extracts of the *Citrus aurantium L*. The inhibitory efficacy of these extracts increased with the concentration of the extract. The concentration was 100 (mg / ml) had a higher inhibitory value than the total concentration Study 20 and 60 (mg / ml) .

## Introduction

Allah has made the Almighty plants a food that cannot be dispensed with life, he created the Almighty from the plant disease and medicine, and herbal medicine an ancient history dating back to the early ages of history, and scientists of the Arabs and Muslims such as Razi, Ibn AL- Bitar, Ibn Al-Nafis, Ibn Sina and others a large role in this area[1]. There are many plants have an inhibitory effect towards the pathogens, plants Dossess many that have inhibitory activity of many bacteria to find resistance to them compared to antibiotics used as a treatment for these pathogenic agents[2].

Increased resistance of microorganisms (bacteria, viruses and fungi) to chemical drugs and the failure to treat infectious diseases have led to search for new sources such as natural products to counteract the effect of drugs in the killing, inhibition and spread of pathogens[3].

Gingivitis is a common oral disease that causes inflammation of the surrounding tissues. it occurs as a result of some pathogens. The continuous use of antibiotics is a major factor in the treatment of bacterial infections, but sontinuous useful long time these antibiotics results in bacterial strains resistant to antibiotics[4].

Therefore, researchers in this field had to find alternatives, such as plant extracts or medicinal herbs, as major sources of medical drugs to treat various bacterial infections, including Gingivitis[5].

The plants of *Mentha Citrato L* and *Citrus aurantium L* plants, which have many uses, especially the use of medical purposes because they contain many active compounds, so they were selected on the basis of their use in folk medicine as rich in effective substances against the growth and of bacteria and hence began the idea of this study aimed at to prepare the alcohol and hot aqueous extracts of these two plants and tested its effect on some bacterial isolates, and to be used as an alternative to antibiotics used in the treatment, which show bacterial high resistance to them.

**Materials and Methods:** Plant extracts was prepared by tacking of 50 g of dried powder from both plants; *Mentha Citrato L* leaves and *Citrus aurantium L* flowers, and soluble in 250 ml of solvents involved in the study to prepare the methanol, ethanolic and aqueous extracts[6].

Two groups of bacterial isolates were identified in the microbiology laboratory, the first positive group of Gram (G+), including *Staphylococcus aureus,*

*Streptococcus faecalis*, and(G-) group II, including *Klebsiella pneumonia*.

The microbiology suspension was obtained by taking 5 ml of the Normal salin a sterile test tube and 150 ml from bacterial cultures resting on the center of the heart and brain infusion media at an age of 18 hours for isolates under study. Thus, we aobtained the bacterial suspension of (10 Cell/ml) after comparing the turbidity of the bacterial growth formed with the turbidity of the fixed standard solution (McFarland) and the container ($1.5 \times 10^8$ cell/ml). The test of inhibitory activity of the plant extracts used in the study used to the Well diffusion method to observe the effect of the extracts on the growth of microorganisms[7].

The dishes containing the Muller-Hinton Agar medium were sterilized using the Swab needle, which was immersed in the bacterial lobe, and was performed on the middle surface using the lumbar end and all directions. Excavation was performed in the irrigated plant medium with a diameter of 5 mm using a core borer, and was added at the three wells in each dish. After that, 0.1 mL of each concentration of prepared vegetable concentrates was distributed on the three pits in a sequence (20, 60 and 100 mg / ml -1). The dishes were incubated for half an hour to dry after incubating the dishes at 37 °C for 24 hours at a rate of three replicates for each bacterial isolations. The effect of the extract was determined by measuring the diameter of the inhibition area around each hole measured in millimeters.

## Results and discussion

**Effect of ethanolic, methanolic, and hot aqueous extract of *Mentha Citrato L*, and *Citrus aurantium L* to Staphylococcus. aureus bacteria:**

It is clear from Table (1) that the effect of plant extracts on *Staphylococcus aureus*. isolates from inflamed gums varies according to the concentrations used and the type of extract. The most inhibitory is the concentration (100 mg/ml) of the two plants compared with the other concentrations (20, 60 mg / ml). The different plant extracts showed a different effect on the growth of bacteria. The alcoholic (Eth, Meth) extracts were more effective than the hot aqueous extracts, which also showed a difference in their effect on the growth of the bacteria. The effect of these extracts may be proportional to the concentration, and there were significant differences (0.05>p) between the rates of the inhibition diameters and the effect of each concentration in the isolated germ. The same in Table (1) showed that the most effective plant extracts in isolated bacteria and at concentration (100mg/ml) is the methanolic extract of *Mentha Citrato L* plant, which showed inhibitory effects against bacteria in all three repeater, the highest inhibitory diameter is (24.00mm) in isolated bacteria and then the Ethanol extract of the *Mentha Citrato L* plant, which had the highest inhibitory effect and with an inhibition diameter of 21.00 mm. The results showed that alcoholic extracts of *Citrus aurantium L* was less effective than the alcoholic extracts of *Mentha Citrato L***.** The highest inhibitory value of the extract of the *Citrus aurantium L* was the value were methanol extract at 100 mg/ml and reached 8.00 mm.

These extracts were more effective in comparison with hot aquatic plant extracts, which also showed a different inhibitory effect, the most effective extract was the hot aqueous extract of the *Mentha Citrato L* plant, which showed the highest inhibitory effect in the bacteria in diameter (11.00 mm). The hot aqueous extract was effective at concentrations (20, 60, 100 mg / ml) with the exception of the hot aqueous extract of the *Citrus aurantium L*, which showed no inhibitory effect on the bacteria and at the concentrations used. We conclude that the *Mentha Citrato L* extract is more effective on the isolated bacteria compared to the *Citrus aurantium L* extract . The reason is that the *Mentha Citrato L* contains more active ingredients than the *Citrus aurantium L* plants.

The results also showed that the methanol extract was highest in the concentration of 100 mg / ml. It was noted that both alcohol extracts (methanolic and ethanolic) in all concentrations gave higher values of inhibition comparative to hot aqueous extracts and all sources of extracts. The active substances are well soluble in organic solvents[8].

In addition, the efficacy of the alcohol extract was due to its containment of higher content of the active compounds. The study agreed with the findings of Al-Zawahiri (2007)[9] which found that the alcohol extract was more effective, followed by aqueous extracts due to the containment of the alcohol extract high content of active soluble substances that have the potential to inhibit microbial growth through the ability of these substances to penetrate the cellular wall, or through its effect on vital parts of the cell such as cytoplasm, ribosomes, DNA or other parts[10]. Flavonoids Inhibition of microorganisms through their ability to form a complex with the bacterial cell wall and to tear the membrane[11]. The reason for the lack of effectiveness of water extracts is due to the inhibition of bacteria because they do not contain flavonoids because they cannot melt well in water, Its inhibitory efficacy compared to alcoholic extract[12] .

**Table (1) the inhibitory activity of plant extracts against *Staphylococcus. aureus* bacteria**.

| Concentration and type of extract. | Diameter of inhibition zone in (mm) | |
|---|---|---|
| | Source of extract | |
| | *Mentha Citrato L* | *Citrus aurantium L* |
| 20 mlg.ml$^{-1}$ hot aqueous | 8.00± 2.64 fg | 0.00± 0.00 h |
| 20 mlg.ml$^{-1}$ ethanolic | 10.00± 1.00 deg | 2.00± 1.00 h |
| 20 mlg.ml$^{-1}$ methanolic | 12.00± 1.00 de | 0.00± 0.00 h |
| 60 mlg.ml$^{-1}$ hot aqueous | 9.00± 1.00 ef | 0.00± 0.00 h |
| 60 mlg.ml$^{-1}$ ethanolic | 14.00± 1.00 cd | 4.00± 1.00 gh |
| 60 mlg.ml$^{-1}$ methanolic | 18.00± 3.60 bc | 2.00± 1.00 h |
| 100mlg.ml$^{-1}$hot aqueous | 11.00± 2.00 def | 0.00±0.00 h |
| 100 mlg.ml$^{-1}$ ethanolic | 21.00± 3.60 ab | 6.00± 1.00 g |
| 100mlg.ml$^{-1}$ methanolic | 24.00± 4.00 a | 8.00± 1.00 fg |

- Similar letters mean that there are no significant differences at the probability p≤ 0.05
- Different letters mean significant differences at the probability p≤ 0.05.

**Effect of ethanolic, methanolic, and hot aqueous extract of *Mentha Citrato L, and Citrus aurantium L,* to *Streptococcus. faecalis* bacteria:**

Table (2) showed the effect of plant extracts in this study against *S. faecalis* bacteria isolated from inflamed gums. The results showed that the most effective concentrations were (100mg/ml) compared to other concentrations used in the experiment for all types of extracts. The extracts showed a different effect on the isolated bacteria, as alcoholic extracts were better than the water extracts, which were also different in terms of their inhibitory effect. The inhibitory effect of the extracts could be directly proportional to the increase of concentrations with significant differences at (p≤0.05) in diameters of inhibitions rates, and uses concentrations.

The most important plant extracts affecting the bacteria at the concentration (100 mg/ml) is the methanolic extract of the *Mentha Citrato L* plant with an inhibition diameter (25.00 mm), followed by the ethanolic extract of the *Mentha Citrato L* plant and the diameter of the inhibition (23.00 mm). The results of the present study showed that the methanolic extracts (60 mg/ml) had the highest inhibitory value of (8.00 mm) inhibition diameter towards the bacteria, so that the *Citrus aurantium L* was less effective than the *Mentha Citrato L.*

For the hot aquaeous extracts, the extracts of the *Mentha Citrato L* plant at (100 mg/ ml) concentration were more inhibitor than the hot aqueous extract of the *Citrus aurantium L* plant, which was actually inhibited in the isolated bacteria and with an inhibition diameter of 11.00 mm, followed by the hot aqueous extract of the *Citrus aurantium L* plant which showed inhibitor effect in the bacteria with a diameter of 2.00 mm.

As a result, the most effective extracts of isolated bacteria are the alcoholic extracts of *Mentha Citrato L* and *Citrus aurantium L*. The most vulnerable types of plant extracts are the hot aqueous extracts for all the concentrations used. This is due to the ability of the active substances to dissolve well in organic solvents, The effectiveness of the extract is due to the content of the active substances, which makes it more effective in inhibiting the growth of microorganisms by blocking the work of the cell wall and its impact in biological processes such as its ability to generate hydrogen bonds with the Proteins that lead to the destruction of protein building in bacterial cells, which in turn inhibits the growth of bacteria, as well as their effect on the genetic material of these bacterial growt[12]. .

Plant extracts have similar behavior with all bacterial strains in terms of effect of the extract. The plant extracts gave higher effect values in terms of inhibiting the growth of *S.faecalis* bacteria compared to the effect of hot aqueous extracts, and high concentrations gave higher inhibitory effect than low concentrations. The effect was more severe at the concentration of 100 mg/ml and gradually decreased to the lowest values at 20 mg /mL concentration for all types of extracts, whether alcoholic or aquatic, which was consistent with what was found (Tortora *et al.*, [13] . The inhibitory effect increases with the increase of concentration and inhibitory efficacy of plants have many effective compounds such as tannins and volatile oils with inhibitory activity of microorganisms[14] , and are consistent with the study of (Thawini and others)[14].

**Table (2) the inhibitory activity of plant extracts against *Streptococcus.faecalis* bacteria.**

| Concentration and type of extract. | Diameter of inhibition zone in (mm) | |
|---|---|---|
| | Source of extract | |
| | *Mentha Citrato L* | *Citrus aurantium L* |
| 20 mlg.ml$^{-1}$ hot aqueous | 7.00 ±2.00 ef | 0.00±0.00 g |
| 20 mlg.ml$^{-1}$ ethanolic | 11.00 ±1.73 cd | 3.00 ±1.00g |
| 20 mlg.ml$^{-1}$ methanolic | 13.00± 1.73 bc | 5.00± 1.00 fg |
| 60 mlg.ml$^{-1}$ hot aqueous | 10.30 ±1.52 cd | 2.00± 1.00 g |
| 60 mlg.ml$^{-1}$ ethanolic | 15.00 ±1.00 b | 3.00± 0.00g |
| 60 mlg.ml$^{-1}$ methanolic | 17.00± 1.00 b | 8.00± 1.73 ef |
| 100mlg.ml$^{-1}$hot aqueous | 11.00± 1.73 cd | 2.00±0.00g |
| 100 mlg.ml$^{-1}$ ethanolic | 23.00± 2.00 a | 5.00 ± 1.73 fg |
| 100mlg.ml$^{-1}$ methanolic | 25.00± 1.00 a | 7.00± 2.00ef |

- Similar letters mean that there are no significant differences at the probability p≤ 0.05
- Different letters mean significant differences at the probability p≤ 0.05.

**Effect of ethanolic, methanolic, and hot aqueous extract of *Mentha Citrato L, and Citrus aurantium L,* to *Klebsiella Pneumonia* bacteria:**

Table (3) showed the inhibitory effect of the plant extracts used in the study toward *P. pneumonia* isolated from inflamed gums. The extracts showed a mixed inhibitory effect against the bacteria according to the type of extract and the concentration used. The effect of these extracts may be directly proportional to the concentration of each type of extracts in the effect on the bacteria with significant differences between the rates of diameters, concentrations used and the type of extract at a significant level (p ≤ 0.05).

Alcohol extracts showed a higher inhibitory effect than the inhibitory effect shown by hot aqueous extracts. The results showed that the extracts of the *Citrus aurantium L* plant had a low effect of all kinds and all its concentrations compared to the extracts of *Mentha Citrato L* plant. The highest value was the extract of the *Citrus aurantium L* extract (13.00 mm) and the methanolic extract at concentration (100 mg / ml). The most effective extracts in the bacteria at the concentration (100 mg/ml) were the alcoholic extracts of *Mentha Citrato L* plants, which were affected by their three concentrations at different levels. The highest inhibitory activity was shown by methanolic extract at 27.00 mm inhibition dimeter, Followed by Ethanolic extract of *Mentha Citrato L* plant, and

inhibition diameter (24.0 mm). The aqueous extracts were different in terms of the effect of the bacteria. The hot aqueous extract of the *Mentha Citrato L* plant showed an inhibitory effect on the bacteria at concentrations of 100 mg/ml and 15.00 mm, the highest inhibitory value given by hot aqueous extracts of all kinds and different concentrations. We conclude from this that the alcoholic extracts exceeded the aqueous extracts in terms of their inhibitory capacity towards the bacteria, noting the high diameters of the alcoholic extracts of the two types (ethanolic, methanolic and aqueous) gradually and significantly until reaching the concentration (100 mg / ml). The reason for the superiority of alcohol extracts to contain the higher content of active, solubility in alcoholic extract compared to aqueous extract in inhibiting the growth of microorganisms through its impact on the cell wall and thus its impact on the biological processes of bacteria such as their ability to generate hydrogen bonds with proteins, which leads to the destruction of protein in the cells Which in turn inhibits the growth of bacteria as well as their effect on the DNA of bacteria[12].

**Table (3) the inhibitory activity of plant extracts against *Klebsilla. Pneumonia* bacteria.**

| Concentration and type of extract. | Diameter of inhibition zone in (mm) | |
|---|---|---|
| | Source of extract | |
| | *Mentha Citrato L* | *Citrus aurantium L* |
| 20 mlg.ml$^{-1}$ hot aqueous | 9.00±2.00 de | 3.00± 0.00fg |
| 20 mlg.ml$^{-1}$ ethanolic | 13.00±2.00 bc | 5.00±1.00 efg |
| 20 mlg.ml$^{-1}$ methanolic | 14.00± 2.00 bc | 8.00±2.00 ef |
| 60 mlg.ml$^{-1}$ hot aqueous | 11.00± 2.00 cd | 5.00±2.00 efg |
| 60 mlg.ml$^{-1}$ ethanolic | 15.00 ± 1.00 bc | 6.00±1.00 ef |
| 60 mlg.ml$^{-1}$ methanolic | 19.00± 1.00 b | 9.00± 1.73 de |
| 100mlg.ml$^{-1}$hot aqueous | 15.00± 1.73 bc | 5.00 ±0.00 efg |
| 100 mlg.ml$^{-1}$ ethanolic | 24.00±1.00 a | 11.00±3.00 cd |
| 100mlg.ml$^{-1}$ methanolic | 27.00± 3.00 a | 13.00±1.00 bc |

• Similar letters mean that there are no significant differences at the probability p≤ 0.05

• Different letters mean significant differences at the probability p≤ 0.05

## Resources

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# الفعالية التثبيطية لمستخلصات نباتي النعناع Mentha Citraro L وقداح شجرة النارنج Citrus aurantium L تجاه بعض أنواع البكتريا المعزولة من اللثة الملتهبة

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**الملخص**

اجريت هذه الدراسة في محافظه صلاح الدين لفترة من 15/9/2018 ولغايةً15/3/2019، حيث درست الفعالية التثبيطية للمستخلصات الكحولية (الايثانول ,الميثانول), والمائية الحارة لنباتات النعناع وقدح شجرة النارنج بطريقة الانتشار بالحفر على عدد من الانواع البكتيرية الممرضة المسببة لإلتهاب اللثة اذ عزلت العديد من الاجناس البكتيرية من اللثة الملتهبة ومن بينها الاجناس *Streptococcus* ,*Staphylococcus aureus* , *klebsiella pneumonia* ,*faecalis* وقد بينت نتائج الدراسة ان المستخلصات الكحولية اعطت تاثيراً تثبيطياً عالياً على نمو الاجناس البكتيرية قيد الدراسة مقارنة بالمستخلصات المائية الحارة اذ ان هذه العزلات البكتيرية ابدت حساسية بالدرجة الاولى للمستخلص الكحولي الميثانول ثم الايثانول يليهما المستخلص المائي الحار , وقد بينت الدراسة ايضاً ان مستخلصات نبات النعناع كانت اكثر فعالية في تثبيط نمو العزلات البكتيرية مقارنة بمستخلصات نبات قداح شجرة النارنج، وان الفاعلية التثبيطية لهذه المستخلصات ازدادت بزيادة تركيز المستخلص اذ كان التركيز 100(ملغم/ مل) ذي قيمة تثبيطية اعلى من بقية التراكيز المستخدمة قيد الدراسة 20,60 (ملغم/مل).