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INVESTIGANDO A CAPACIDADE DE MATERIAIS NATURAIS PARA A INIBIÇÃO DE ALGUMAS BACTÉRIAS PATOGÊNICAS

INVESTIGATING THE ABILITY OF NATURAL MATERIALS TO INHIBITION OF SOME PATHOGENIC BACTERIA

دراسة قدرة المواد الطبيعية على تثبيط بعض أنواع البكتريا الممرضة

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RESUMO

Introdução: Bactérias patogênicas representam uma grande preocupação, pois podem afetar a saúde pública. Assim, a corrida entre os fabricantes de medicamentos e o aumento da resistência bacteriana aos antibióticos está em seu auge. Para superar esse obstáculo, estudos recentes estão direcionados para o uso de alternativas naturais que podem substituir os antibióticos e ter um impacto significativo sobre essas bactérias patogênicas. **Objetivos**: os principais objetivos deste estudo foram (1) encontrar uma alternativa natural e ecologicamente amigável aos antibióticos químicos tradicionais, capaz de inibir o crescimento de algumas bactérias patogênicas conhecidas; (2) testar a capacidade de cinco suspensões vegetais — cravo, hibisco, mostarda, canela e suco de limão —, além de ácido acético (4%), de inibir o crescimento de quatro espécies de bactérias patogênicas: Klebsiella pneumoniae, Enterobacter cloacae, Escherichia coli e Staphylococcus aureus; (3) comparar os efeitos das suspensões vegetais com o efeito de vários antibióticos conhecidos e explicar seus modos de ação. **Métodos**: o extrato vegetal aquoso foi preparado a partir de partes secas das plantas, e as colônias de bactérias purificadas foram obtidas de amostras hospitalares. Em seguida, a capacidade dos extratos vegetais contra essas bactérias foi testada usando o método de difusão em poços de ágar, e a zona de inibição para cada bactéria foi determinada. **Resultados**: Os resultados mostraram que Klebsiella pneumoniae foi sensível aos antibióticos usados, exceto cloranfenicol, nitrofurantoína e ampicilina, e foi sensível a todos os extratos, exceto o de mostarda. Já Enterobacter cloacae foi resistente à tetraciclina, co-trimoxazol, ampicilina e nitrofurantoína, apresentando sensibilidade intermediária ao cloranfenicol. Por outro lado, foi sensível a todos os extratos, exceto o de mostarda. Por sua vez, *Escherichia coli* foi resistente a todos os antibióticos, exceto tetraciclina, amicacina, nitrofurantoína e colistina, enquanto apresentou sensibilidade intermediária à gentamicina, sendo sensível a todos os extratos vegetais, exceto o extrato de mostarda e o ácido acético. Staphylococcus aureus foi resistente à canamicina, co-trimoxazol, cloranfenicol, ampicilina e nitrofurantoína, apresentando sensibilidade intermediária à gentamicina e colistina, sendo sensível a todos os extratos vegetais, bem como ao ácido acético. **Discussão**: Pelos resultados, ficou evidente que os extratos mais eficazes foram hibisco e cravo, seguidos por canela, suco de limão e ácido acético, enquanto apenas S. aureus foi sensível ao extrato de mostarda. **Conclusões**: Os resultados do estudo mostraram que os extratos naturais das plantas foram eficazes contra diferentes bactérias patogênicas, com graus de efeito variados.

Palavras-chave: *Extratos vegetais, bactérias patogênicas, antimicrobiano, inibição, antibióticos.*

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ABSTRACT

Background: Pathogenic bacteria represent a major concern in that they can affect public health. Thus, an ongoing race between pharmaceutical manufacturers and the elevation of bacterial resistance to antibiotics is at its peak. In order to overcome this obstacle, recent studies are directed toward using natural alternatives that can replace antibiotics and have a significant impact on these pathogenic bacteria. **Aim:** the main aims of this study were (1) finding a natural and eco-friendly alternative for traditional chemical antibiotics that could give a good inhibition for the growth of some of the well-known pathogenic bacteria (2) testing the ability of five plant suspensions which are clove, hibiscus, mustard, cinnamon suspensions, and lemon juice as well as acetic acid (4%) to inhibit the growth of four pathogenic bacterial species which are *Klebsiella pneumonia*, *Enterobacter cloacae, Escherichia coli,* and *Staphylococcus aureus.* (3) compare the effects of the plant's suspension with the effect of several known antibiotics and explain their mode of action. **Methods:** the aqueous plant extract was prepared from the dried plant parts, and the purified bacteria colonies were obtained from hospital samples. Then, the ability of the plant extracts against these bacteria was tested using the agar-well diffusion method, and the inhibition zone for each bacteria was determined. **Results:** The results showed that *K. pneumoniae* was sensitive to the used antibiotics except chloramphenicol, nitrofurantoin, and ampicillin, and it was sensitive for all the extracts except for mustard. While *E. cloacae* was resistant to tetracycline, co-trimoxazole, ampicillin, and nitrofurantoin, it had an intermediate sensitivity to chloramphenicol. In contrast, it was sensitive to all extracts except for the mustard extract. However, *E. coli* was resistant to all antibiotics except for tetracycline, amikacin, nitrofurantoin, and colistin, while it has intermediate sensitivity to gentamycin, though it was sensitive to all plant extracts except mustard extract and acetic acid. *Staphylococcus aureus* was resistant to kanamycin, co-trimoxazole, chloramphenicol, ampicillin, and nitrofurantoin, though it has intermediate sensitivity to gentamycin and colistin, while it was sensitive to all plant extracts as well as acetic acid. **Discussion**: From the results, it was obvious that the most effective extracts were hibiscus and clove, followed by cinnamon, lemon juice, and acetic acid, while only S. aureus was sensitive to mustard extract.**Conclusions:** The results of the study showed that the natural plants' extracts were effective against different pathogenic bacteria with dissimilar degrees of effect.

Keywords: *Plant Extracts, Pathogenic Bacteria, Antimicrobial, Inhibition, Antibiotics*

الخلاصة

المعلومات الاساسية:تمثل البكتريا الممرضة خطرا أساسيا عل الصحة العامة. و نتيجة لذلك فان السباق المستمر بين شركات صناعة الادوية و الارتفاع في اعداد البكتريا المقاومة للمضادات قد وصل أوجه. و من اجل تلافي هذه المشكلة, فان الدراسات الحديثة متوجه باتجاه استخدام البدائل الطبيعية و التي يمكن ان تحل محل المضادات الحيوية و تكون ذات تاثير على البكتريا الممرضة. **اهداف الدراسة:**(1) تهدف هذه الدراسة الى إيجاد بدائل طبيعية و صديقة للبيئة بدلا من المضادات الحيوية الكيميائية التقليدية و التي يمكن تعطي تثبيطا جيدا ضد بعض البكتريا الممرضة المعروفة (2) دراسة قابلية خمس مستخلصات نباتية هي مستخلص نبات القرنفل و نبات الكركديه و الخردل و القرفة بالإضافة الى عصير الليمون وكذلك حامض الخليك بنسبة (4%) على تثبيط نمو اربع أنواع من البكتريا الممرضة هي الكليبسيلا الرئوية و بكتريا الامعائية المَذرَقِيَة و بكتريا الاشريكية القولونية و بكتريا المكورات العنقودية الذهبية (3) مقارنة تاثير هذه المستخلصات مع تاثير عدد من الأنواع المعروفة من المضادات الحيوية و شرح طرق تاثيرها على البكتريا. **طرق العمل:** تم عمل المستخلصات المائية من أجزاء النباتات المجففة, في حين تم جمع البكتريا الممرضة من المستشفى و تنقيتها و بعد ذلك تم اختبار كفائة هذه المستخلصات ضد البكتريا الممرضة باستخدام طريقة الانتشار بالاغار و تم تحديد منطقة التثبيط لكل بكتريا.**النتائج:**بينت النتائج ان بكتريا الكليبسيلا الرئوية كانت حساسة ضد المضادات المستخدمة في الدراسة ماعدا مضاد الكلورامفينيكول و نايتروفيورانتون و الامبسلين و كانت حساسة لكل المستخلصات ماعدا الخردل. بينما كانت بكتريا الامعائية المَذرَقِيَة كانت مقاومة لكل من لمضادات التراسيكلين والكوتريموكسازول و الامبسلين و نايتروفيورانتون و أظهرت حساسية متوسطة لمضاد الكلورامفينيكول و كانت حساسة لكل المستخلصات ماعدا الخردل. في حين كانت بكتريا الاشريكية القولونيةحساسة لمضادات التتراسيكلين و الاميكاسين و نايتروفيورانتون و الكولستين و متوسطة الحساسية لمضاد الجينتامايسين و كانت حساسة لكل المستخلصات ماعدا الخردل و حامض الخليك (4%). كذلك فان بكتريا المكورات العنقودية الذهبية كانت مقاومة لكل من مضادات الكانامايسين والكوتريموكسازول و الكلورامفينيكول و الامبسلين و نايتروفيورانتون و أظهرت حساسية متوسطة لمضاد الجينتامايسين و الكولستين الكلورامفينيكول و كانت البكتريا حساسة لكل المستخلصات و كذلك لحامض الخليك (4%). **المناقشة:** بينت النتائج بان اكثر المستخلصات تاثيرا كان مستخلص نبات الكركدية و من بعده نبات القرنفل و القرفة و من ثم عصير الليمون و حامض الخليك (4%) في حين ان مسنخلص نبات الخردل لم يؤثر الا على بكتريا المكورات العنقودية الذهبية.**الاستنتاجات:** أظهرت نتائج هذه الدراسة بان المستخلصات النتباتية كانت مؤثرة ضد الانواع المختلفة من البكتريا الممرضة و لكن بدرجات متفاوة من التاثير.

الكلمات المفتاحية: المستخلصات النباتية, البكتريا الممرضة,المضادات البكترية, التثبيط, المضادات الحيوية

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1. INTRODUCTION:

Diseases and infections caused by pathogenic bacteria are major health issues (Khan *et al.,* 2013). Many bacteria can cause severe disease and health problems. Among these pathogenic bacteria, *Klebsiella pneumoniae,* which is a widely spread, gram-negative, facultative anaerobic (Khan, 2022; Quintero *et al.,* 2022), encapsulated, immotile bacteria (Jasim and Farhan, 2020), usually attacks the gastrointestinal tract. It can also cause pneumoniae, sepsis, and meningitis in infants, as well as urinary tract infections in children, and it is associated with hospital infections (Piperaki*et al.,* 2017).

Another gram-negative pathogenic bacterium is *E. cloacae,* which is an opportunistic, facultatively anaerobic bacteria that cannot produce spores (Davin-Regli and Pagès, 2015). This bacterium is highly associated with nosocomial infections in intensive care patients, especially newborn babies (Ferry *et al.,* 2020). Also, *E. coli* is another widely spread gram-negative bacterium that is found naturally in the intestines of humans and other animals. However, some species can become pathogenic and cause some diseases, such as diarrhea and extraintestinal infections (Basavarajuand Gunashree, 2023; Okab *et al.,* 2020). Some species can produce a toxin (Shiga toxin) that can cause diseases (NCEZID, 2016).

On the other hand, *S. aureus* is a gram-positive, non-motile, facultative anaerobic bacteria that does not produce spores (Harris *et al.,* 2002). This bacterium is responsible for many infections and diseases, such as skin, nasal, urethra, and gastrointestinal tract infections, and even more serious diseases, including pneumonia, heart diseases, and septicemia (Strak, 2013). These pathogenic bacteria obtain many mechanisms and techniques that allow them to overcome the different mechanisms of their hosts and cause infections (Khan,2022).

Through the past decades, there has been an ongoing battle between antibiotics and pathogenic bacteria, represented by the emergence of new bacteria resistance strains and the development of new antibiotics (Yang *et al.*, 2021). What makes the situation even worse is the unsuitable and non-prescribed use of antibiotics, which increases the presence of resistant bacteria all around the world (Al-Hasani, 2018).

Due to the fact that the resistance of pathogenic bacteria to antibiotics is increasing at a high rate, other alternatives to treat pathogenic bacteria need to be explored. Plant extracts represent an appealing alternative since these extracts are available and contain many biologically active components that can hinder the growth of bacteria or even kill them (Al Sheikh *et al.*, 2020). In the present study, five plant suspensions were used. The first one is clove (*Syzygium aromaticum*), which belongs to the Myrtaceae family. The species is formed from dried flower buds. It has been used in many industries, such as the perfume industry, as a preservative in meat processing. It has been used widely as a medicinal plant due to its antioxidant activity (Batiha*et al.*, 2020). It also shows anticancer and antimutagenic activities (Pulikottil and Nath, 2015; Rabêlo *et al.,* 2024). It is an important spice that represents a main source of plant source phenolic compounds, and the main active compound is eugenol in the fresh plant (Cortés-Rojas *et al.*, 2014).

The second plant was *Hibiscus sabdariffa,* which belongs to the family Malyaceae and is frequently called "red sorrel" or "roselle" due to the red color of its calyxes, which is highly rich with anthocyanin, minerals, carotene, and vitamin C (Singh *et al.*, 2017). It's been traditionally known for its bioactive properties, especially as an antioxidant and antimicrobial agent, since it is rich in different Phyto-nutrients such as anthocyanin, phenolic acid, and organic acids (Etheridge and Derbyshire, 2020). Due to its distinctive color, it has been used in food production as a coloring agent, while flowers and fruits can be used to produce jams, and it is well-known for its use as herbal tea (Nguyen and Chuyen, 2020).

The third plant is cinnamon (*Cinnamomum zeylanicum*), which belongs to the Lauraceae family, and its parks are used as spices in many countries (Rawat *et al.*, 2020). Cinnamon contains many valuable components, such as cinnamaldehyde and trans-cinnamaldehyde, as well as catechins and procyanidins in their park. Cinnamon is a carminative, antiseptic, stimulating agent that has been used in flock medicine such as the maintenance of teeth and reducing their aching, cold, and coughing reduction, treating diarrhea, improving memory as well as relief arthritis pain (Rao and Gan, 2014).

The fourth plant is Mustard (*Sinapes arvensis*), which belongs to the Brassicaceae family and the spice is made by grinding the seeds of the plant, which can be mixed with

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various liquids such as water or vinegar (Bukhari *et al.*, 2021). It is a medical plant that has been reported to be used for treating stomach cramps and respiratory congestion, enhancing intestine activity, lowering blood pressure, and treating ringed worms, as well as acting as an antioxidant, anticancer, antifungal agent (Khan,2019).

The fifth plant is lemon (*Citrus limon*), which is an edible fruit that contains highly available components such as ascorbic acid, citric acid vitamins, minerals, essential oils, and flavonoids (Rafique *et al.*, 2020). In the past few decades, there has been a trend for substituting medicine with natural plants and herbs and their remedy. Among these plants, lemon is used for treating a variety of diseases, such as UTI and kidney stones (Irfan *et al.*, 2019).

This study was conducted to test the effectiveness of the aforementioned plant suspension as well as acetic acid (4%) against four pathogenic bacteria (*K. pneumoniae, E. cloacae, E. coli, and S. aureus*) and in comparison with some of the well-known antibiotics.

2. MATERIALS AND METHODS:

2.1. Material

The materials used in this study are listed in Table 1.

2.2. Methods

2.2.1. Preparation of natural suspension

The four spices (clove, hibiscus tea, cinnamon, and mustard) were collected (as dried herbs) from the local market in Hillah City/ Iraq. These dried herbs were grinded by an electrical grinder to obtain a fine powder, then 30 g of each herb was taken, and 90 ml of sterilized distilled water was added to each plant powder at a ratio of 1:3 (W/V). It was left to soak in water for two hours, after which it was quickly mixed for one hour in a mixer to prepare an aqueous suspension for experiments without filtration (Al-Defiery *et al.*, 2021). Also, lemon juice was prepared by squeezing fresh lemons to obtain one liter of the juice that was kept in the refrigerator at 4 °C to be used later, as well as acetic acid (4% concentration) was collected from the local market (1 liter container). The aqueous suspensions of each natural material were kept in sterilized polyethylene bottles at 4 °C until use.

2.2.2. Pathogenic Bacteria Strains

The bacterial isolates addressed in this study were *K. pneumoniae*, *E. coli*, *E. cloacae,* and *S. aureus*. These bacterial species were collected as purified and fully identified cultures of each bacterial species from the hospitals of Babylon Province. Bacterial isolates were diagnosed based on morphology and microscopic, physiological, and biochemical characteristics in the laboratories of the Department of Biology - College of Science for Women - University of Babylon.

2.2.3. Inoculum preparation

At first, nutrient agar was prepared by completely suspending 28 gm of nutrient agar powder in 1000 mL of distilled water. The media was sterilized by autoclave and then poured into a Petri dish in sterilized condition to prevent contamination of the media. After that, the medium was left to solidify in a sterilized hood. The isolates of bacteria were prepared by streaking on sterile nutrient agar and incubation at 35°C for 24 hours (Amankwah *et al.*, 2022). After obtaining Well-isolated colonies of each isolate, they were cultivated into the nutrient broth in sterilized test tubes by sterilized loop and incubated for 18 hours at 35 °C. Then, from each cultivated nutrient broth of every isolate, 0.2 ml was taken and added into 20 ml of sterilized nutrient broth and kept in the incubator at 35°C for three to five hours to standardize the culture to 106 cfu/ml (Collins *et al.*, 2004) after adjusting the turbidity of the suspensions to 0.5 McFarland standard.

2.2.4. Determination of plant suspensions antimicrobial activity

The plant suspensions were studied for their antibacterial activity by using the agar-well diffusion method (NCCLS, 1993). Agar medium (Muller Hinton) was prepared by suspending 38 g of the media in 1000 ml distilled water. Then, it was transferred to an autoclave to sterilize the media. After that, the media was allowed to cool to 45 - 50 °C, and then media was poured and cooled in Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm in sterilized conditions. The agar medium was allowed to cool at room temperature (30 ± 2 °C) in a sterilized illuminated hood. The following step was to swab the media with the suspension of each bacteria (approximately 106 cfu /mL) using a sterilized swab of cotton. The wells were

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made by making holes with a sterile cork borer (six millimeters diameter) made of stainless steel. The aqueous suspensions of each natural material, lemon juice, and acetic acid (50 μL) were put in the wells in triplicate. The plates were incubated for 20 - 24 hours at a temperature of 35 °C. The antimicrobial activity is calculated in millimeters of the zone of inhibition and then averaged.

2.2.5. Determining the activity of antibiotics

The activity of eleven antibiotics: Tetracycline (TE), Gentamycin (GEN), Streptomycin (S), Kanamycin (K), Co-Trimoxazole (Sulpha/trimethoprim) (COT), Amikacin (AM), Chloramphenicol (C), Ampicillin (AMP), Nitrofurantoin (NIT), Ciprofloxacin (CIP), and Colistin (Methane Sulphonate) (CL) were tested against the isolated pathogenic bacteria. All the antibiotics were manufactured by Himedia Laboratories Pvt. Ltd. India. The Kirby-Bauer disc diffusion method was used to examine the susceptibility of antibiotics against four bacteria used in this study (CLSI, 2018). The volume of 0.1 ml of the bacterial inoculum (106 cfu/ml) was distributed by sterile cotton swab on a sterile agar medium (Muller Hinton). The multidisc of antibiotics, as a standard, were located by forceps in contact with the agar according to the NCCLS method (Ortez, 2005; NCCLS, 2012). Next, the medium was sealed and incubated for 20-24 hours at a temperature of 35 °C in triplicate. The antibiotics activity was estimated upon obviously visible zones of inhibition around discs that were measured with a diameter average (millimeters) of three replicates.

2.2.6 Statstical analysis

The statistical analysis was done by using the SPSS program (Tukey Test) to measure the significance of the results.

3. RESULTS AND DISCUSSION:

3.1. Results

In the present study, the plant suspensions, as well as some of the well-known antibiotics, were tested against four pathogenic bacteria, which were *K. pneumoniae, E. cloacae, E. coli,* and *S. aureus* by measuring the zone of inhibition (ZOI) of each, and comparing it with the standard ZOI for each antibiotic as shown in Table 2.

The effectiveness of each antibiotic was determined by measuring the ZOI. The results show that *K. pneumonia* was susceptible for TE, GEN, K, CIP, S, COT, CL, and AM with ZOI of 30, 28, 25, 22, 20, and 20, 20 mm, respectively. At the same time, the bacteria were resistant to C, AMP, and NIT (Table 2, Figure 1); for the plant extracts, the highest efficiency was for clove with ZOI of 18 mm, followed by hibiscus tea with ZOI of 16 mm. By lemon juice and acetic acid, both had a ZOI of 14 mm, and the lowest was with cinnamon with a ZOI of 12 mm, and the bacteria was resistant to mustard extract, as shown in (Table 3, Figure 5).

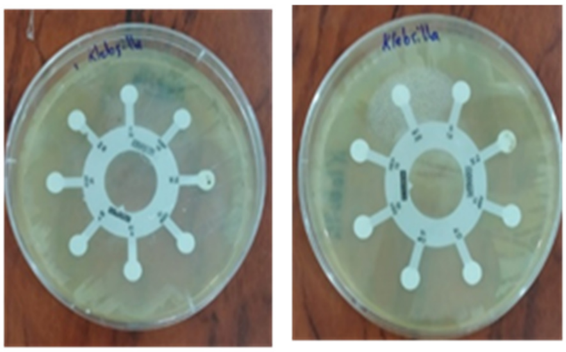

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Figure 1: the inhibition zone of *K. pneumoniae* by antibiotics

E. cloacae was highly sensitive for K, followed by CIP, GEN, AK, S, CL with ZOI of 25, 23,23, 22, 18mm, respectively, as well as it was intermediately sensitive for C with ZOI of 14 mm and was resistant for TE, COT, AMP, NIT (Table 2, Figure 3), while for the plant extracts the bacteria was sensitive in the following order: clove, lemon juice, hibiscus tea, acetic acid, cinnamon extract with ZOI of 18, 16, 15, 14, 13 mm, respectively, whereas it was resistant to mustard extract (Table 3, Figure 5).

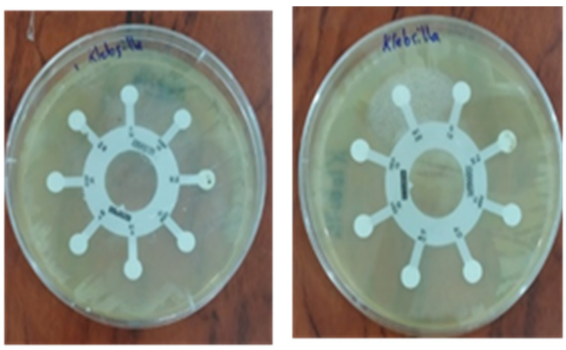

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Figure 2: The inhibition Zone of *E. Cloacae* caused by different antibiotics

E. coli was highly sensitive for NIT, AK, CL, and TE with ZOI of 22, 21, 17, 15 mm,

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respectively, and intermediately sensitive for GEN with ZOI of 13 mm and resistant for S, K, COT, C, AMP and CIP (Table 2, Figure4), On the other hand, the bacteria were sensitive for clove and hibiscus extracts followed by cinnamon extract and lemon juice with ZOI of 20, 18, 13, 13 mm, respectively, while it was resistant for mustard extract and acetic acid (Table 3, Figure5).

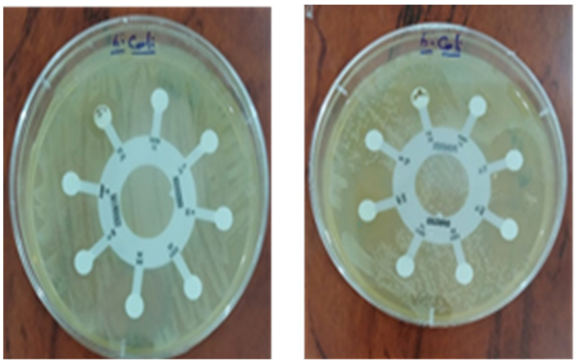

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Figure 3: the inhibition zone of *E. Coli* by different antibiotics

S. aureus was sensitive for AK, CIP, TE, S with ZOI of 22, 21, 21, 20, 15 mm, respectively, and intermediately sensitive for both CL and GEN with ZOI 15 and 14 mm, respectively, though it was resistant for K, COT, C, AMP, NIT (Table 2, Figure 5). While the bacteria were sensitive for hibiscus tea, clove, lemon juice, and mustard extracts, and the lowest was for cinnamon extract and acetic acid with ZOI of 21, 18, 14, 14, 12, and 12 mm, respectively (Table 3, Figure 5).

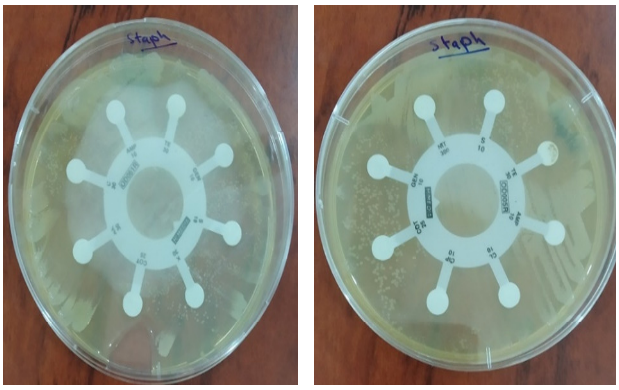

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Figure 4: The inhibition zone of S. aureus by different antibiotics.

The statistical analysis showed that the antibiotics have a significant effect on the pathogenic bacteria at P<0.01, with a p-value of 0.00318.

On the other hand, the effect of the plant suspension on the bacteria was significant at P<0.01, with a p-value of 0.00125.

3.2. Discussion

In general, antibiotics have been used for the treatment of different bacterial infections for a long time, and these antibiotics have various modes of action in order to pass bacterial growth super; thus, it is crucial to understand how these antibiotics work on bacterial cells. Some of these antibiotics prevent the production of bacteria proteins, such as tetracycline (TE), which blocks bacterial protein production through the binding with the bacterial ribosomes (Speer *et al.,* 1993), so as amikacin (AK) and gentamicin (GEN), which inhibit bacterial protein biosynthesis (Adaszyńska-Skwirzyńska*et al.,* 2023), and Streptomycin (S) which inhibits protein synthesis in bacteria.

Through binding to the 30S ribosomal subunit [II], also Chloramphenicol (C) inhibits protein synthesis in bacteria by binding to peptidyl transferase enzyme (Alobaidallah*et al.,* 2024), while other antibiotics can damage the bacteria cell wall, such as Kanamycin (K) that disrupts the bacterial membrane (John *et al.,* 2017), and Ampicillin (AMP) that binding to penicillin-binding proteins which responsible for the formation of the cell wall (Gudisa, 2022), as well as, colistin (CL) which cause the bacterial outer membrane disturb by selectively targeting lipopolysaccharide (Sabnis*et al.,* 2021).On the other hand, some antibiotics affect the bacterial genetic material, such as ciprofloxacin (CIP), which inhibits bacterial DNA gyrase/topoisomerase II and DNA topoisomerase IV and subsequently blocks bacteria DNA unwinding and duplicating (Masadeh *et al.,* 2015), or it could hinder the detachment of gyrase from DNA (Sharma *et al.,* 2010) along with nitrofurantoin (NIT) which inhibits bacterial enzymes that are required for carbohydrate synthesis and total protein synthesis by the nonspecific attack on bacterial ribosomal proteins (Shakti and Veeraraghavan, 2015), although, co-trimoxazole (COT) can hinder the microbial synthesis of folic acid (James *et al.,* 2015).

But that does not mean that these antibiotics will be optimum for overcoming the bacteria growth since some antibiotics have side effects ranging from simple side effects such as nausea and headache caused by nitrofurantoin (Shakti and Veeraraghavan, 2015) up to more drastic side effects such that caused by chloramphenicol including damage to bone

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marrow and the cause of aplastic anemia (Alobaidallah *et al.,* 2024), that coupled with the increase bacteria resistant to antibiotics that lead to the test for alternative method to get red of bacterial infection which can be done through the use of natural plant materials (AlSheikh *et al.,* 2020).

The results of this study show that *K. Pneumonia* was susceptible to some antibiotics and resistant to others; the results were analogous to the findings of (Younus, 2024) in that the bacteria were sensitive to GEN, CIP, and S, while they differed from their findings in that they found K. pneumonia were resistant to TE, C, and NIT. On the other hand, the results also cohere with the findings of (Ayatollahi *et al.* 2020) in that more than 67 of the bacteria were sensitive to CIP. More than 56 of the bacteria were sensitive to COT, although they found that the bacteria was sensitive to AM, which disagrees with their findings in that the bacteria was sensitive to AMP. The results of this study disagree with the findings of (Adeosun *et al.,* 2019), who found that more than 70 % of the bacteria were immediately sensitive to CL, and the increase in the resistance of the bacteria to the antibiotics could be attributed to the formation of biofilm or due to the presence of multi-drug resistance plasmids as well as the other different genes that can encode for the resistance other than ß-lactams (Sweedan,2018).

For the *K. Pneumonia,* when it was tested against the plant suspension, it showed that it was most sensitive to the clove and less to the hibiscus tea, lemon juice, acetic acid, and the lowest to cinnamon, while it was resistant to mustard. The cloves contain variable content that possess antimicrobial activity, and the main compound is eugenol, which has a damaging effect on the cell membrane and can cause the death of bacterial cells, which gives the antibacterial effect of the clove (Ginting *et al.,* 2021) The results show a higher ZOI caused by clove extract (18 mm) than the results found by Bisht *et al.* (2020) who found that ZOI caused by clove extracts range from 9 mm to 15 and 16mm for extract concentrations range from 6.25 to 100 mg/ml for *k. pneumoniae* AmpC-producing strains and non-ESBI, AmpC MBL strains, respectively, and from 10 to 16 mm for ESBL-producing strains.

This study results disagree with the findings of Mak *et al.* (2013), who found that aqueous extracts of hibiscus at 50 and 100 mg/ml were not effective against *K. pneumoniae* and that the bacteria was intermediately sensitive to chloramphenicol. The present study results were lower than the results recorded by Hindi and Chabuck (2013), who found that lemon juice was effective against *K. pneumonia* with ZOI 30 mm. and disagreed with the findings of Ewansiha (2020), who found that *K. pneumoniae* was resistant to lemon juice extract. It was also lower than the results of Pangprasit *et al.* (2020), who found that acetic acid was effective against *Klebsiella* spp. with ZOI 31.7 mm. The results of this study were much higher than the findings of Al-Zubaidy (2017), who found that the cinnamon extract was against *k. pneumoniae* causing a ZOI of 4.2 and 2.5 mm when treated with the bark and powder aqueous cinnamon extracts, respectively. The inhibitory effect of the cinnamon extract can be attributed to its high content of phenolic compounds, which can affect the cell wall integrity of *K. pneumoniae* and subsequently prevent their growth (AL Mjalawi *et al.,* 2019). The results also agree with the findings of Rahman *et al.* (2010), who found that mustard extract was unable to inhibit the growth of *K. pneumoniae*.

For the effect of the different antibiotics on *E. cloacae,* the results were similar to the findings of (Dehkordi *et al.,* 2022), who found that more than *E. cloacae* strains were resistant to TE, more than 84% of the bacterial strains were sensitive to COT, and disagree with their findings in that 76% of the bacteria strain were resistant to K and 61 of the bacterial strain were resistant to C. on the other hand this study results disagree with the findings of (Mulinganya *et al.,* 2021) in that they found that the bacteria was resistant to GEN and sensitive to NIT, and agree with their findings in that the bacteria was sensitive to CL. The results also agree with the findings of (Nyenje *et al.,* 2012), who found that the bacteria were sensitive to S., also agree with the findings of (Al-Niaeem *et al.,* 2021), who found the bacteria were resistant to AMP. Djeussi *et al.* (2013) Found that *E. cloacae* was sensitive to GEN and immediately sensitive to AK, CIP, and resistance AMP.

When *E. cloacae* were tested against different plant suspensions, the bacteria were susceptible to clove, followed by lemon juice, hibiscus tea, acetic acid, and cinnamon extracts, respectively, and it was resistant to mustard extract.

Rajaram (2012) found that *Enterobacter* spp. When treated with garlic clove extract, the inhibition zone was increased from 8.5- to 18.5

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mm, and the concentration increased from 25 ug/ml to 200 ug/ml. It disagrees with the findings of (Berthold-Pluta *et al.,* 2019), who found that lemon juice has a feeble antimicrobial effect against *E. cloacae.* It also agrees with the findings of Al-Niaeem *et al.* (2021), who found that hibiscus extracts were effective against *E. cloacae* BM67. The results of this study disagree with those of Marasini *et al*. (2015), who found that the cinnamon extracts were not effective against *E. cloacae*. At the same time, it agrees with the findings of Miceli *et al.* (2014), who found that mustard aqueous extracts were not effective against different Enterobacter species.

The different antibiotics were tested against *E. coli,* and the results of the present study go along with the findings of (Raheema and Mahood, 2016) in that *E. coli* were sensitive to NIT and resistant to CIP and disagreed with them in that the bacteria were sensitive to AMP and resistant to GEN. Furthermore, the results agree with the findings of (Mohammadpour *et al.,* 2011) in that the bacteria was sensitive to AK and resistant to C and disagree with their findings in that the bacteria were R. To TE. However, the results agree with the findings of (Hassan *et al.,* 2023) in that the bacteria were sensitive to CL. It also agrees with the findings of (de Paula and Marin, 2008), who found that more than 81% of the *E. coli* isolates were resistant to S. On the contrary, the results disagree with the findings of (Dib *et al.,* 2019) in that they found that only 10.9 percent of the *E. coli* isolates were resistant to K. In addition, the results also agree with the findings of (Christopher *et al.,* 2013) in that more than 78 % of the bacterial isolates were resistant to COT.

On the other hand, *E. coli* were sensitive to clove and hibiscus extracts, followed by cinnamon extract and lemon juice, and were resistant to mustard extract and acetic acid. This study result found that cinnamon extracts cause a ZOI of 13 mm which was higher than the results of Mukhtar and Ghori (2012) who found that the cinnamon aqueous suspension range from 60% to 100% cause an inhibition zone range from 9.3 to 10.3 mm in *E. coli* , and also much higher than the results of Saliem and Abedsalih (2018) who found that the cinnamon extracts both chloroform and methanol ranging from 100 ug/ml to 200 ug/ml have an inhibition zone range from 0 to 4.3 mm and 4 to 6.2 mm. on the other hand, the present study results state that *E. coli* was resistant to mustard extract which disagree with the findings of Akkoyun *et al.* (2007) who found that the black mastered cause a ZOI with 10 mm, and the results of Krishnan *et al.* (2013) who found that the water extract of mustard cause a ZOI with 2.4 mm. the results agree with the findings of Atwaa *et al.* (2022) who found both lemon and hibiscus tea water extract were effective on *E. coli* with inhibition zone 19 and 22 mm respectively, though Ekawati and Darmanto (2019) found that lemon juice extracts ZOI ranged from zero to 19 mm as the extracts concentration increased from 100 mg/ml to 1000 mg/ml. The results of this study also disagree with the findings of Hoque *et al.* (2008), who found that clove extract was not effective against six strains of *E. coli, while agreeing with the findings of Rabelo et al. (2024), who found that the cloves were effective in the inhibition of E. coli*.

When testing the effects of the antibiotics against different antibiotics, the results were in agreement with the results of Hemeg *et al.* (2020), who found that *S. aureus* was resistant to AMP and disagreed with their findings in that the bacteria were resistant to both S and GEN. While it shows results that are affiliated with the conclusions of Arsene *et al.* (2021), who found that *S. aureus* was sensitive to TE and CIP, it also agreed that the bacteria were intermediately sensitive to NIT. The results found a lower ZOI in S. aureus when treated with AK, and they disagreed with them in that the bacteria were sensitive to both K and COT. As well as disagree with (Wahaab *et al.,* 2016) in that the bacteria were sensitive to C.

S. aureus was sensitive to hibiscus tea, clove, lemon, and mustard suspensions, and the lowest was for cinnamon extract and acetic acid. Our results show a lower inhibition zone (12 mm) of *S. aureus* when treated with cinnamon extract than the results of Fernandez-Soto *et al.*(2023), who found that the inhibition zone of *S. aureus* was 17 mm, as well as lower than the results of Hussein (2017) who found that the aqueous extracts of cinnamon were effective against *S. aureus* and show an inhibition zone with 15 mm. The present study results agree with the findings of Ogidi *et al.* (2019), who found that mustard extract was effective against *S. aureus* with ZOI 5, 9, and 12 mm when treated with methanolic, ethanolic and ethyl acetate extract, respectively. At the same time, the results of the present study agree with the findings of Atwaa *et al.*(2022), who found that hibiscus extracts were highly effective against *S. aureus*. However, the results of this study show a higher inhibition zone for *S. aureus* when treated with clove extracts than the results of Mostafa *et al.* (2018) who found that clove

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extracts show an inhibition zone of 15.8 mm. Our results were much lower than those of Patel *et al.*(2022), who found that lemon juice was effective against *S. aureus* with ZOI 26 mm.

In general, the results show that Hibiscus extract was the most effective extract (Figure 9), and that could be attributed to their contents, such as phenolic compounds, organic acids, polysaccharides as well as anthocyanins, or it could be attributed to the effect of the hibiscus extracts on the cell wall integrity of the bacteria (Prasetyoputri *et al.,*2021), followed by clove extracts, The antimicrobial activity of the clove extract is credited to eugenol, which is considered the main active component of the clove extract (Nascimento *et al*., 2000). While the antimicrobial activity of lemon juice is correlated to the high content of flavonoids that can desaturate the bacteria cell wall proteins and the saponin that can affect the cell wall membrane (Ekawati and Darmanto, 2019), on the other hand, the cinnamon extract antimicrobial activity is related to its active components such as flavonoids, saponins, quinones, steroids and tannins (Parisa *et al*., 2019). Acetic acid's antimicrobial activity could be related to its effect on the cell wall (Ryssel *et al.,* 2019), and it prevents the formation of biofilm (Halstead *et al*., 2019).

The main aim of this study was to show the real unfixed antimicrobial effect of these plant suspensions and that using them in daily diet could improve health, and further study on extracting the active component of these plants and using them as antibiotics in singular matter or in a synergistic way should be conducted.

4. CONCLUSIONS:

It could be concluded from the results of this study that plant suspensions can act as a strong antimicrobial agent that can suppress the growth of different bacteria. The hibiscus extract shows the best ability to inhibit the growth of tall tested bacteria, both positive and negative bacteria. The plant suspension could be, in some cases, more effective than the traditional chemical antibiotics that are used on a daily basis.

5. DECLARATIONS

5.1. Study Limitations

There were some limitations of this study, including the following:

1-the difficulty of obtaining a pure culture of each of the used pathogenic bacteria from the hospital.

2-the difficulty of obtaining a sterilized condition at each stage of the study since any contamination will interfere with results.

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5.3. Funding source

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5.4. Competing Interests

The authors declare that there are no conflicts of interest.

5.5. Open Access

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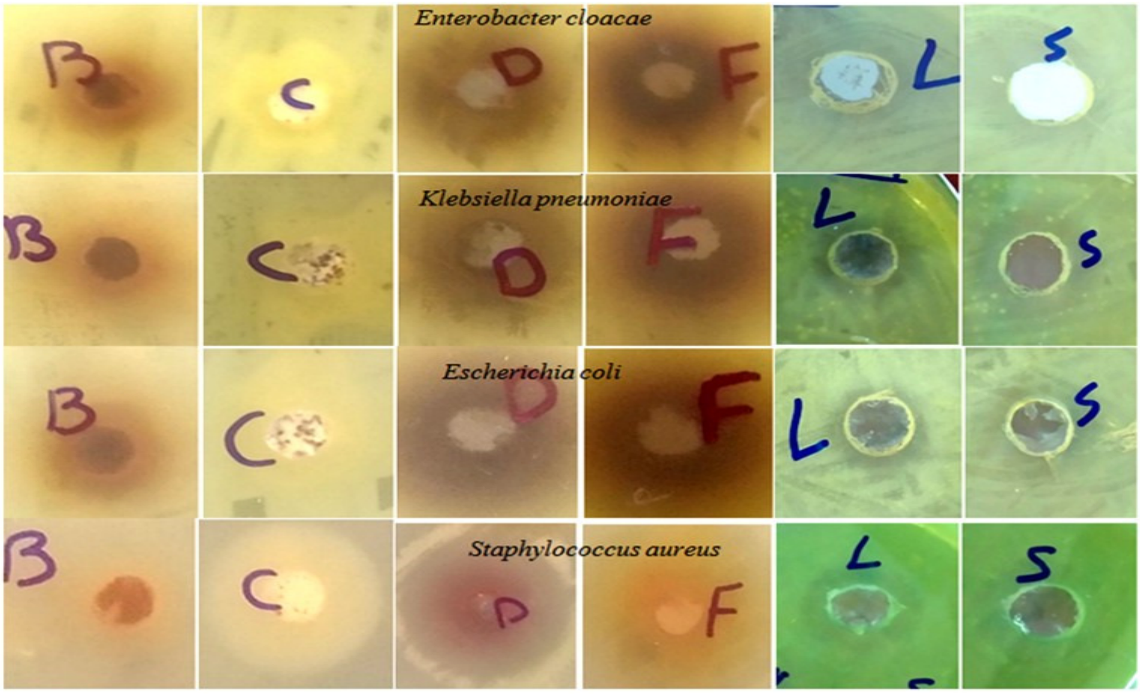

Figure 2: The inhibition zone caused by plant suspensions and acetic acid on the different bacterial species

S: acetic acid, **L**: lemon juice, **B**: cinnamon extract, **D**: Hibiscus extract, **C**: Mustard extract, **F**: clove extract.

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Table 1: The materials used in this study

Plant materials	Clove (powder)
	Hibscus tea(powder)
	Cinnamon (powder)
	Mustard (powder)
	Lemon juice
Chemicals	Distilled water
	Acetic acid (4% w/w)
	Nutrient broth
	Muller Hinton agar
Bacterial Strains	*K. pneumoniae*
	E. cloacae
	E. coli
	S. aureus
Antibiotics	Tetracycline (30µg)
	Gentamycin (10 µg)
	Streptomycin (10 µg)
	Kanamycin (30 µg)
	Co-Trimoxazole (25 µg) (Sulpha/ trimethoprim)
	Amikacin (30 µg)
	Chloramphenicol (30 µg)
	Ampicillin (10µg)
	Nitrofurantoin (300 µg)
	Ciprofloxacin (5µg)
	Colistin (10 µg) (Methane Sulphonate).
Equipment and Supplies	Blender
	Sterilized polyethylene bottles
	Incubator
	Petri dishes
	Sterile cork borer (6mm)
	Sterile cotton swabs
	Forceps
	Micropipettes and tips
	McFarland

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Table 2: *Standard zone of inhibition (ZOI) for the different antibiotics used in this study*

Antibiotic	symbol	Dose (µg)	Zone of inhibition (mm)			Reference
			R	**I**	**S**	
Tetracycline	TE	30	≥11	12-14	≤15	(NCCLS, 2012)
Gentamycin	GEN	10	≥12	13-14	≤ 15	(NCCLS, 2000)
Streptomycin	S	10	≥11	12-14	≤15	(NCCLS, 2012)
Kanamycin	K	30	≥13	14-17	≤18	(NCCLS, 2012)
Co-Trimoxazole (Sulpha/ trimethoprim)	COT	25	≥10	11-15	≤16	(NCCLS, 2012)
Amikacin	AM	30	≥13	15-16	≤17	(NCCLS, 2000)
Chloramphenicol	C	30	≥12	13-17	≤ 18	(NCCLS, 2012)
Ampicillin	AMP	10	≥ 11	12-13	≤14	(NCCLS, 2000)
Nitrofurantoin	NIT	300	≥14	15-16	≤17	(Kemal *et al.*, 2016)
Ciprofloxacin	CIP	5	≥ 15	16-20	≤21	(NCCLS, 2000)
Colistin (Methane Sulphonate).	CL	10	≥11	12-16	≤17	(CLSI, 1993)

R: Resistant, I: Intermediate Sensitive, S: Sensitive

Table 3: Zone of Inhibition (mm) of antibiotic on the bacterial species used in this study

Antibiotic	Concentration (mcg)	Bacteria Isolates			
		S.aureus	***E. coli***	***E. cloacae***	***K. pneumoniae***
TE	30	20	15	10	30
GEN	10	14	13	23	28
S	10	15	R	22	20
K	30	R	R	30	25
COT	25	R	R	R	20
AK	30	22	21	23	22
C	30	R	11	14	R
AMP	10	R	R	R	R
NIT	300	R	22	R	R
CIP	10	21	15	25	22
CL	10	15	17	18	20

TE : Tetracycline, GEN: Gentamycin, S: Streptomycin, K: Kanamycin, COT: Co-Trimoxazole (Sulpha/ Trimethoprim, AK: Amikacin, C: Chloramphenicol, AMP: Ampicillin, NIT: Nitrofurantoin, CIP: Ciprofloxacin, CL: Colistin (Methane Sulphonate)

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Table 4. Zone of inhibition (mm) of plant suspensions and acetic acid against different bacteria used in this study

Plant Extracts	Bacteria isolate			
	K. pneumonia	*S.*aureus	*E. coli*	*E. cloacae*
Cinnamomum Zylanicum(cinnamon)	12	12	13	13
Sinapes arvensis(Mustard)	R	14	R	R
Hibiscus sabdariffa (Hibscus Tea)	16	21	18	15
Syzygium aromaticum (clove)	18	18	20	18
Acetic acid (4% w/w of CH_3COOH)	14	12	R	14
Citrus limon	14	14	13	16

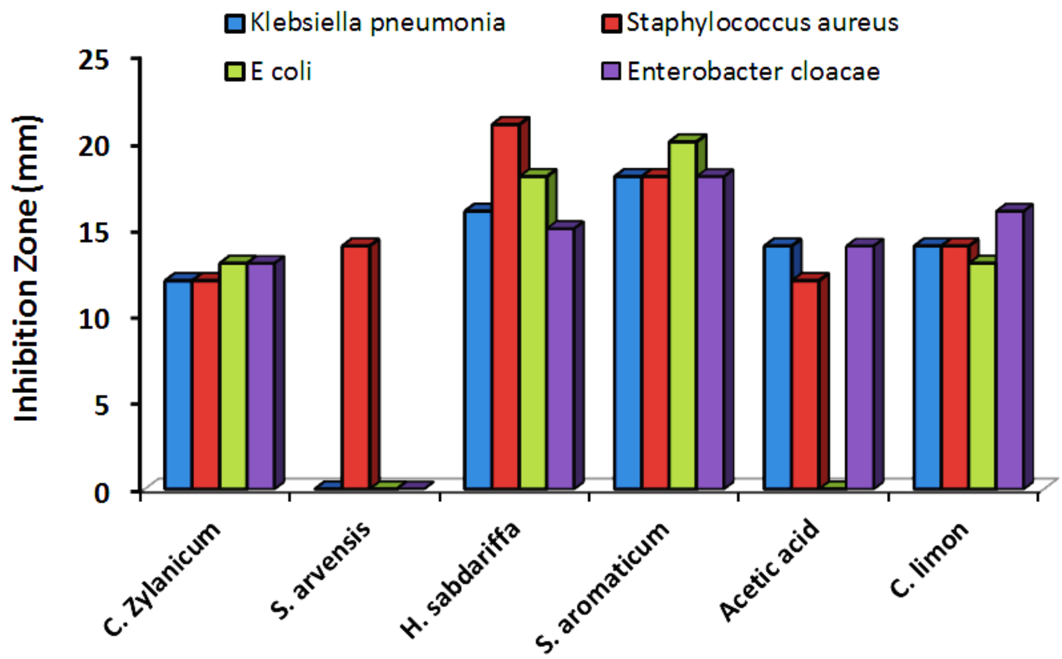

Figure 6: A comparison between the different plant suspensions and acetic acid against different bacterial species used in this study

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