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Evaluation of Antibacterial Effects of *Eucalyptus Camaldulensis* L., Extracts Against Some Pathogenic Bacteria in Vitro and HPLC-DAD

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Abstract

Our study was carried out in laboratory of Alnoor university –Iraq in (2025) to identify active compounds and evaluate in vitro antimicrobial which include effects of hexane and aquatic extracts with three concentration on two species of bacteria , the phenolic compounds for *Eucalyptus camaldulensis* tree identify active compounds extract was prepared by using Soxhlet hexane and Aqueous as a solvent HPLC High performers liquid Chromatography , was used to diagnose and identify phenolic compounds in Eucalyptus extract. Results showed identification of 5 active phenolic compounds, (Quercetin, Kaempferol, Ferulic, Gallic acid, Rutine).

These differences in chemical compounds could be used as an important indicator for plant identification.

The second aspect of study investigated inhibitory effect of two organic solvents on two bacterial species (*Staphylococcus aureus* and *Escherichia coli*). Research revealed that inhibition rates varied depending on solvent used. Furthermore, the *in vitro* antimicrobial potential of *E. Camaldulensis* extracts demonstrated varying degrees of inhibition at different concentrations, which also differed between Gram-positive and negative bacteria.

ضد بعض البكتيريا الممرضة في المختبر والمركبات الفينولية بواسطة تقنية كروماتوغرافيا السائل عالي الأداء - كاشف المصفوفة الثنائية (HPLC-DAD)

طلال طه علي

جامعة النور، كلية التقنيات الصحية والطبية، قسم التغذية، الموصل، العراق.

المستخلص

أُجريت هذه الدراسة في مختبر جامعة النور - العراق في عام (2025) لتحديد المركبات النشطة ومعرفة درجة تثبيطها للبكتيريا المرضية، والتي شملت تأثير مستخلصي الهكسان والمائي بثلاث تراكيز على نوعين من البكتيريا، والمركبات الفينولية في شجرة (اليوكالبتوس) نوع الكامالدولنيسيس (*Eucalyptus camaldulensis*). تم تحضير المستخلص لفصل ومعرفة المركبات الكيميائية النشطة باستخدام جهاز سوكسليت والمذيبين الهكسان والماء. واستخدمت تقنية الاستشراب السائل عالي الأداء (HPLC) لتحديد المركبات الكيميائية الفعالة في المستخلص. وأظهرت النتائج وجود 5 مركبات فينولية، وهي: الكيرسيتين، الكامفيرول، الفيروليك، حمض الجاليك، الروتين يمكن أن تُستخدم هذه الاختلافات في المركبات الكيميائية كمؤشر مهم لتحديد هوية النبات. تضمن الجانب الثاني من البحث تقييم التأثير التثبيطي باستخدام مذيبيين عضويين كمستخلصات على نوعين من البكتيريا) المكورة العنقودية الذهبية، الإشريكية القولونية(، وأظهر البحث اختلافًا في معدلات التثبيط اعتمادًا على المذيبات العضوية. أظهرت فعالية تثبيطية في المختبر لمستخلصات الكينا الكامالدولنيسيس درجات متفاوتة من التثبيط بتركيز مختلفة، حيث اختلفت في أنواع البكتيريا الموجبة والسالبة لصبغة غرام.

الكلمات المفتاحية: مستخلصات نباتية، مضاد للبكتيريا، اليوكالبتوس الكامالدولنيسيس، المكورة العنقودية الذهبية، الإشريكية القولونية، البكتيريا الممرضة، المركبات الفينولية.

1. Introduction

Eucalyptus trees are considered to be important medicinal, which is considered as one of the world most important and common medicinal species (Elansary *et al*, 2017), The active compounds of eucalyptus extracts were used as antibacterial, antifungal, and disinfecting agents and in aromatherapy (Sabo and Knezevic, 2019). In general research shows that there are over 900 different types would wide, most of which contain active compounds, specially volatile oils such as eucalyptol ,used as medications in traditional medicine (Sharifi- Rad *et al*, 2017).Numerous studies have shown that presence of several volatile oils in eucalyptus species (Benabdesslem *et al* , 2020).

Many species of Eucalyptus may number 900 kinds worldwide, with more number containing volatile oils used as medications in traditional medicine (Sharifi- Rad *et al.*, 2017).

several studies mentioned their presence in the essential oil of *Eucalyptus* genus (Benabdesslem *et al*, 2020). Research has found that common compounds detected in the petroleum ether (PEth) extract of *E. brevifolia*, such as spathulenol and eucalyptol, are known for their antiproliferative activity. Research has found that common compounds detected in the petroleum ether (PEth) extract of *E. brevifolia*, such as spathulenol and eucalyptol, are known for their antiproliferative activity. (Ivanov *et al*, 2021).

Recently, the use of Eucalyptus extracts has become common due to their being natural sources of fragrance compounds in cosmetics and cosmeceuticals. (Sharmeen *et al*, 2021).

Recent studies have confirmed that medicinal plants are a rich source of antioxidants and free radical scavengers, which serve as essential foundations for preventing the onset of diseases and promoting protection against them.(Famurewa *et al.* 2018). The World Health Organization recommended the necessity of continuing scientific research in the field of herbal medicine due to its distinct and positive role in treating numerous medical conditions. (Balogun *et al.* 2016)

plants, as medicine and food, are no less important than synthetic drugs. Humans have used them since ancient times and continue to use them as a source of food, and medicines(Bernabé. -Antonio *et al.*, 2015).

Eucalyptus plant extracts are used in the production of essential oils, antiseptic materials, cosmetic powders, and as a substitute for medications (Sabo and Knezevic, 2019). Currently, there are about 900 different *Eucalyptus* species over the world, of which about 300 containing volatile oils used as medications in traditional medicine (Sharifi-Rad et al., 2017). Surveys by the World Health Organization (WHO) have found that 80% of the population in developed countries worldwide prefer plant-based medicines for treating diseases. (Laghari *et al.*, 2023).

A number of essential oils in Eucalyptus have been identified, containing various chemical compounds such as phenols, alcohols, esters, flavonoids, ethers, aldehydes, ketones, and hydrocarbons like terpenes, as well as numerous organic compounds. (Al-Snafi, 2006)

Recently, the use of Medicinal plants in production of many pharmaceutical drugs to prepare various pharmaceutical has become widespread. Because of the presence of active compounds like flavonoids, coumarins, terpenes, alkaloids, flavones, polyacetylene .

The Eucalyptus tree, which belongs to the Myrtaceae family, has been experimentally proven to inhibit various types of pathogenic bacteria, in addition to its blood pressure-lowering properties. Also contain some chemical and medical compounds (Babayi m, *et al.*, 2004)

Natural products perform many biological activities such as growth, hormone and enzyme production, as well as the synthesis of secondary metabolites that play a protective role for the organism, like alkaloids, glycosides, terpenes, and others. These are generally used in the food, pharmaceutical, chemical, and cosmetic industries. (Luis *et al.*, 2012)

Acidic, basic, or enzymatic hydrolysis is performed to ensure the accuracy of results for total phenolic compounds extracted from the plant. (Sidhu *et al.*, 2022).

The secondary metabolites of medicinal plants have the ability to perform a defensive role against pathogenic organisms, acting as natural antibiotics. Among

these products are flavonoids, tannins, and alkaloids, which possess antimicrobial properties. (Attallah *et al*, 2021)

The active compounds in plant extracts have attracted the attention of many scientists, like Phenolic acids and flavonoids, which used as a therapeutic and health-beneficial properties. (Memon *et a l*, 2017)

Numerous studies have shown that the leaves of Eucalyptus species have antimicrobial activities against many pathogenic bacteria and fungi. (Wigmore, *et al*, 2016)

In recent years, *Eucalyptus* have been used extensively due to its presence of active compounds for cosmetics and cosmeceuticals (Sharmeen *et al*, 2021).

Performing acid hydrolysis is typically required in extraction research to obtain accurate and scientific results regarding the nature of the active compounds. (Sidhu *et al*, 2022)

Medicinal plants are an important source for combating free radicals through the presence of antioxidants, which can play a significant role in treating pathological conditions. (Famurewa *et al*. 2018)

2. Work Material

2.1. Plant material collection and authentication

2.2. Sample collection

Eucalyptus leaves were collected from Mosul city north part of Iraq, in May2025

2.3. Sample preparation

The leaves of *E. camamdulensis* aerial parts were collected in May 2025, from Mosul city forests (North part of Iraq). The plant samples were cleaned, foreign materials were isolated, and the samples were washed and sub sequently drird in the shade at room temperature. The samples were

ground by using an electric grinder the dried leaves, which were then stored in plastic bags and preserved in a refrigerator. Until used for analyses

2.4. Methods

This study was conducted in May 2025 at the laboratories of Alnoor university in Mosul, Iraq.

The present study was carried active components of *Eucalyptus* leaves on two species of bacteria (*Staphylococcus aureus* & *Escherichia coli*) free extract was prepared by using soxhlet and extracts using two solvents divided in two aquatic and hexane extracts

Which include the extract effects of *Eucalyptus camaldulensis* have been selected, which are important medicinal plants based on many previous researches

2.5. Plant taxonomy:

The plant classification was based on morphology anatomy depending on numerous scientific sources, and was carried out by authentication was performed depending on morphology, anatomy and equipment, drawing on numerous scientific sources, and was carried out by Dr .Talal Taha .Ali from the Alnoor university of Mosul-Iraq.

2.6. Preparation of samples:

by solvation extraction using soxhlet apparatus, fifty grams (50 gr) of *Eucalyptus* leaves air dried dissolve in 400ml of hexane , the extraction done by soxhlet apparatus , the extraction process using the soxhlet device took seven hours, number of Soxhlet cycles are 18 cycle , also the same quantity dissolved in water collect the extract and using steror and rotary evaporator for the purpose of concentrating the volume to 25 cm³ of the concentrated extract.

The extraction of active compounds from the samples were carried out with the help of soxhlet apparatus (ALChaabawi and Satmbekova, 2023).

2.7. Sterilization of alcoholic extract

The stock solution of hexane plant extract was prepared at a concentration of 100 mg per 1 mL using (Dimethyl Sulfoxide DMSO) as a solvent. Three dilutions of the extract were then prepared in order to obtain concentrations of 50, 100%, and 200% relative to the stock concentration (100 mg/mL). and then the mixture was subjected to pasteurization after sterilizing by a temperature of (62 C°) for 10 minutes.

2.8 investigating plant extracts' effectiveness to inhibit bacteria

2.8.1. Culture preparation:

The bacteria were collected from many cases from Mosul General Hospital and Al-Salam hospital Mosul city and the types of bacteria were classified in the college of Science / Department of Biology / University of Mosul.

Bacterial species were identified using the central health laboratory at mosul general hospital as *Staphylococcus aureus* & *Escherichia coli*, based on established diagnostic criteria. (*Staphylococcus aureus*) (**Staphylococcus aureus USA300-FPR3757**, *Escherichia coli* (EHEC- O157:H7)

All samples were maintained by subculture in Mueller-Hinton agar for up to seven days (Cruz *et al*, 2007).

At 37 °C, the liquid Brain Heart Infusion medium was used to cultivate the bacterial cultures. Each bacterium was spread into Mueller-Hinton agar plates at a concentration of 10⁶ cells/mL following a 6-hour growth period. The surface of each infected plate was next covered with filter paper discs (6 mm in diameter) that had been soaked with extract or phytochemicals (50 µL). Each extract was placed simultaneously into a hole formed (50 µL) in brand-new plates to assess the effectiveness of the process. The plates were incubated for 24 hours at 37 °C. At such time, the inhibition zone could be seen. Overall, grown bacteria having halos of 7 mm or more were thought to be vulnerable to the extract under examination. The extracts were dissolved in the culture medium using DMSO Indifferent concentrations showing different inhibition rates (Nascimento *et al* ,2000).

2.8.2. Antimicrobial Activities:

Eucalyptus leaves possess a distinctive aroma resulting from the presence of active compounds, which can play a positive role in combating pathogenic bacteria.

An inhibitory study was conducted on the pathogenic bacteria *Agrobacterium* using three different concentrations (200, 100, 50 mg) to identify the inhibitory capacity of the active compounds against some pathogenic bacteria.

Table (1): Result statistical analysis of aquatic *E. camaldolensis* extracts on two types of epidemic bacteria.

Average	<i>E. coli</i>	<i>S. aureus</i>	concentration
9.75 b	11.50 b	8.0 b	50
7.0 c	10.0 b	4.0 c	100
13.25 a	17.0 a	9.50 b	200
	12.83	7.17 b	Average

Table (2): Result statistical analysis of Hexane *E. camaldolensis* extracts on two types of epidemic bacteria.

Average	<i>E. coli</i>	<i>S. aureus</i>	Concentration
13.50 b	11.0 cd	16.0 ab	50
10.75 c	9.0 d	12.50 bcd	100
16.0 a	13.50 bc	18.50 a	200
	11.17 b	15.67 a	Average

3.3. HPLC assessment of phenolic compounds:

Table 3 shows that the number of standards for active compounds is 6 standards. Each standard was run three times by using the reverse-phase liquid chromatography coupled with a diode array detector (HPLC-DAD) instrument examined at 325 nm, 310 nm, and 270 nm, data included the retention time (Rt), regression coefficient (R^2), the linear equation between concentration and peak area, and maximum absorption wavelength of 6 phenolic.

HPLC technology shows the presence of 6 different phenolic compounds, which included 5 phenolic compounds while one not dignose because no standard, the active compounds are (Quercetin, Kaempferol, Ferulic, Gallic acid, Rutine) (Table 3).

The emergence of differences in the proportions and quantities of active compounds in plants is associated with many factors, including the species, environment, harvest season, soil nature and composition, and the age of the plant.

Table (3): Shows the retention time for standards prepared in the laboratory.

Retention time	Standard vehicles	Type sequence
3.95	Kaempferol	1
4.49	Quercetin	2
5.84	Ferulic	3
6.48	Gallic acid	4
10.42	Luteolin	5
8.36	Rutine	6

Table (4): shows the values of the Rt rate of chemical compounds diagnosed in HPLC technology for Hexane *Eucalyptus camaldulensis* extract.

Retention time	Standard vehicles	Type sequence
3.90	Kaempferol	1
4.48	Quercetin	2
5.89	Ferulic	3
6.46	Gallic acid	4
8.32	Rutine	5

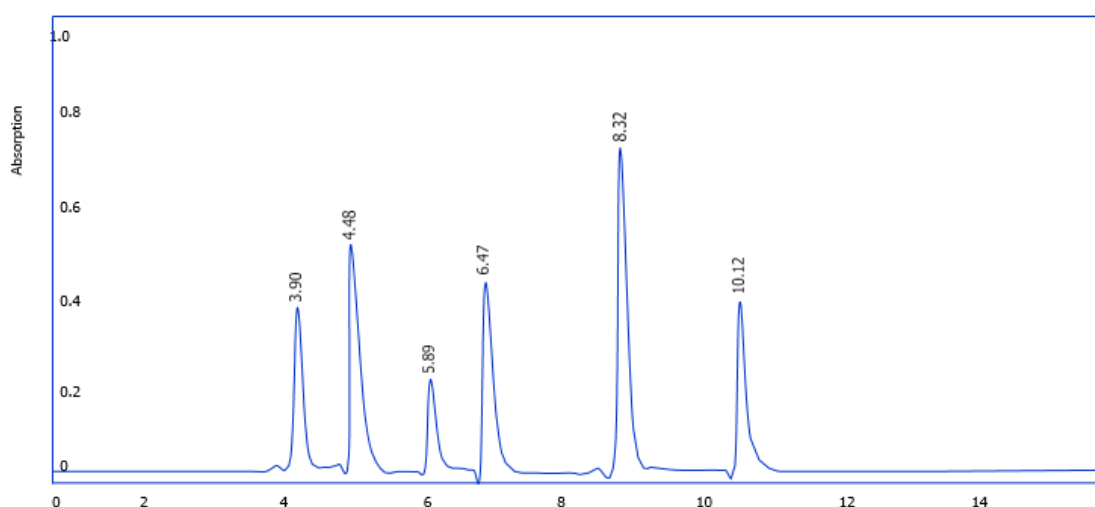


Figure (1) Curve of *Eucalyptus camaldulensis* phenolic compounds diagnosed using HPLC technique

Result chromatography Table (Uncal - F:\ sample 2)

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.90	1895.07	400.11	13.00	13.00	0.05	
2	4.48	3014.45	600.74	18.00	18.00	0.08	
3	5.89	1320.11	200.14	10.00	10.00	0.03	
4	6.47	2011.41	558.74	15.00	15.00	0.05	
5	8.32	6521.00	879.88	29.00	29.00	0.10	
6	10.12	1985.66	425.98	15.00	15.00	0.06	
	Total	16746.90	3054.74	100.00	100.00		

2.9. HPLC analysis:

The analysis of various Eucalyptus extracts was performed using analytical HPLC-DAD (Rahmani et al., 2020). Water separation solution: ethanol 60:40 and temperature 30 °C and pressure 3.5-3.7 MPa to obtain retention time R_t and a number of peaks and curves to compare them with the retention time that will be obtained in the compounds to be separated and diagnosed 5 μ (μ L) of each sample of the plant extract were injected into the H P L C device for the purpose of detecting the expected compounds to appear, and the carrier phase (acetonitrile: Water (90:10) with a flow velocity of 1.3 ml/min and chromatographic responses at a wavelength of (320) nm based on Al-Jubouri (2007

1.10. Statistical analysis:

A completely randomized design (C R D) and Duncan's multiple testing were used

3.Results and discussion

3.1Bacterial inhibition rates:

The *in vitro* antimicrobial potential of *Eucalyptus camaldulensis* extracts (aqueous and Hexane) extract, showed varying degrees of inhibition with different concentrations, as they differed in the two types of bacteria positive and negative.

The results of antimicrobial aquatic *E. camaldulensis* activity are shown in Table 1.

The antibacterial activity of *Eucalyptus camaldulensis* aqueous extracts are shown in Table 1.

Table (1) shows the effect of the inhibitory effectiveness of *E. camaldulensis* extracts (diameter of the circle of inhibition measured in mm) in the two types of pathological bacteria. The results revealed that inhibitory effects on the *E. coli*, (17mm) surpassing. While the extract of the *Staphylococcus aureus* bacteria showed the highest inhibition of all three concentrations at concentration of 200 mg (9.50). Table (2), which pertains to the hexane extract shows a significant superiority of *Staphylococcus aureus* bacteria at a concentration of 200 mg (18.50) mm While the extract of the *E.*

coli bacteria showed the highest inhibition of all three concentrations at concentration of 200 mg (13.50).

3.2. Statistical analysis:

A completely randomized design and Duncan's multiple testing were used statistical analysis in general it is a factorial experiment with duplicates for each transaction that includes two factors, the first is the type of bacteria and the second is the three concentrations of the plant extract. Table 1 aqueous extract experiment included the interaction between the two factors, bacteria and concentrations, and the other side included the comparison between the concentrations in terms of finding significant differences. The table(1) also included searching for the presence of significant differences between the two types of bacteria, as the negative bacteria *Escherichia coli* showed a significant superiority over the *Staph* bacteria at a concentration of 200 mg , while no significant differences were observed between 50 and 200mg concentrations for *E. coli*, similarly m no significant differences were observed between 50 and 200 mg concentrations for *Staphylococcus aureusm*, regarding the interaction between species and concentrations in table (1) the 200 mg concentration showed the highest significant effect . followed by the 50 mg concentration, with the least Signiant effect at100mg, also *E. coli* showed the greatest significant interaction effect between concentrations and species.

Regarding Table 2, that includes two factors, the first is the type of bacteria and the second is the three concentrations of the plant extract. Include hexan extract experiment included the interaction between the two factors, bacteria and concentrations, and the other side included the comparison between the concentrations in terms of finding significant differences. which is concerned with the statistical analysis of the Hexane extract, includes the same factors and results presented in Table 2.

The results were performed in two replicates by using the simple experimental data analysis design was using Duncan multiple range completely randomized design. About the result and details of the results are in tables (2).

Table (2) showed a significant advantage for *staphylococcus aureus* bacteria at a concentration of 200 mg m while no significant differences were found between 50 mg and 100mg concentration for the same species.

For *Escherichia coli*, the 200 concentration also showed significant effect, while, the 50 mg and 200 mg concentration showed no significant differences.

Regarding the interaction between the two species of bacteria and concentration, the 200 mg concentration showed significant effect followed by the 50 mg concentration, with the least significant effect at 100mg, also *S. aureus* species showed the greatest significant interaction effect between concentrations and species.

4. Conclusion and Recommendation:

4.1. Conclusion:

We conclude that the phenolic active compounds of leaves of *Eucalyptus camaldulensis* shows significant antibacterial on hexane extract on Gram+, while shows significant antibacterial on Gram- in Aquatic extract.

Almost of the world's population relies on medicinal plants, and there is a long history of utilizing herbal therapy to treat a variety of many diseases, like inflammations, wounds, and many illnesses. Traditional treatment methods using medicinal plants are more efficient, safer, and more economical than the use of modern synthetic drugs in treating some conditions, which are always associated with synthetic antimicrobials (Dahiya, 2012).

(Dahiya, 2012). It has been proven that acetone and ethanol extracts from 15 plants used as folk medicine by tribal people in the Mandla region showed strong efficacy against bacteria that cause urinary tract infections (UTIs) (Sharma *et al*, 2009)

Recent scientific research has been conducted to evaluate pathogenic bacteria and the extent of their impact been conducted to evaluate natural treatments for bacterial infections. Here we demonstrate the antibacterial activity of phenolic active compounds extracted from *Eucalyptus camaldulensis* prevalent in Iraq.

"Bacterial infectious diseases pose a major obstacle to individual health. From the past to the present, research continues to develop new drugs, such as antibiotics, with minimal side effects.

"Medicinal plants play a significant role in treating various infections through multiple methods, with some minor side effects."

In this study, the results showed that eucalyptus tree extract has multiple significant inhibitory effects, varying among species and strains, on both Gram-positive and Gram-negative bacteria.

It was found that Gram-positive bacteria were less sensitive than Gram-negative bacteria to the aqueous extract, as shown in Table 1, where the highest inhibition was 17 mm against *Escherichia coli*, while the lowest inhibition was 4 mm against *Staphylococcus* species.

As for the hexane extract, the highest inhibition was 18.5 mm against *Staphylococcus aureus*, while the lowest inhibition was 9 mm against *Escherichia coli*. Numerous research results have confirmed that Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria. (Bibi Y.& Nisa S. m., 2011).

Consequently, it was found that different types of bacteria exhibited varying resistance towards phenolic compounds, and the effect of the berry extract was similar to that achieved in our research. (Yik *et al*, 2011)

Through our research, it was found that the most prevalent compounds in the eucalyptus extract were phenolic compounds and flavonoids (Nascimento *et al*, 2000)

The activity of phenolic compounds might be due to the fact of the differences in the cell surface components between Gram positive and negative bacteria, the outer membrane of Gram negative bacteria functions as a barrier against hydrophobic compounds (Bauer, 1966) . Many studies showed that the antimicrobial effect of phenolic may be due to the potential mutagenic activity of these compounds. also, other mechanisms that might involve in the antimicrobial action of phenolic, which is genotoxicity (Cruz *et al*, 2007). The phenolic substances have also an important role to discourage the growth of bacteria through inhibition of the enzymes responsible for the metamorphosis of the protein (Dahiya P& Purkayastha, 2012)

The active activity of certain type of *E. camaldulensis* against *Staphylococcus aureus*, bacteria have been proven experimentally, and this is attributed to the presences of compounds that play a positive role in killing these bacteria. The in vitro results of our study provide evidence that *Eucalyptus camaldulensis* active compounds represents a potentially rich source of antibacterial drugs against known resistant bacteria, *S. aureus* and *E. coli*, we conclude that the active compounds of leaves of the *Eucalyptos*

camaldulensis shows significant antibacterial activities. against gram positive bacteria, (Cruz *et al*, 2007)

We need more researches of *E. camaldulensis* active compounds to determine the extent of the effect of these compounds on pathogens in vitro and in vivo experiments

We conclude that the essential oil and active compounds of leaves of the Eucalyptus tree shows significant antibacterial activities.

4.2. Recommendation:

Given the medicinal importance of Eucalyptus trees m, we recommend continuing research on the same species against other types of bacteria and at different concentrations.

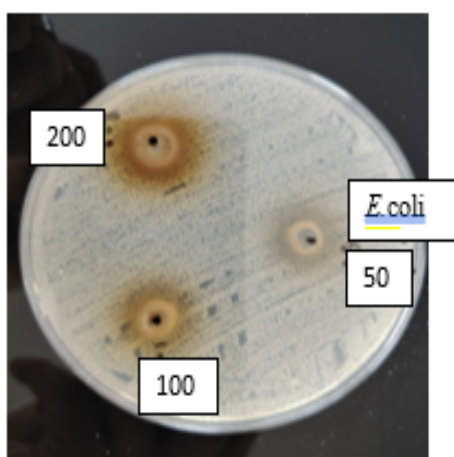


Figure (3) shows the inhibition rate of Hexane extract against gram-negative

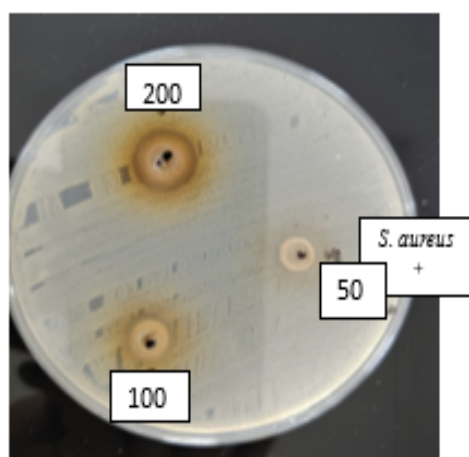


Figure (2) shows the inhibition rate of Hexane extract against gram-positive

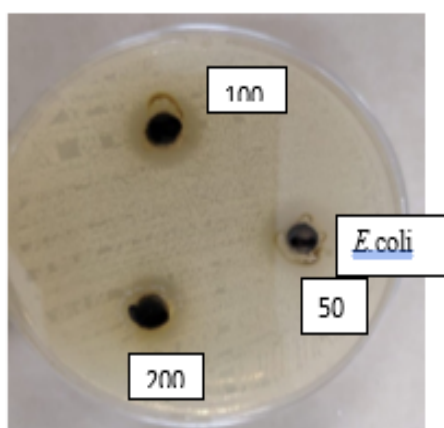


Figure (5) shows the inhibition percentage of the aqueous extract of gram-negative bacteria.

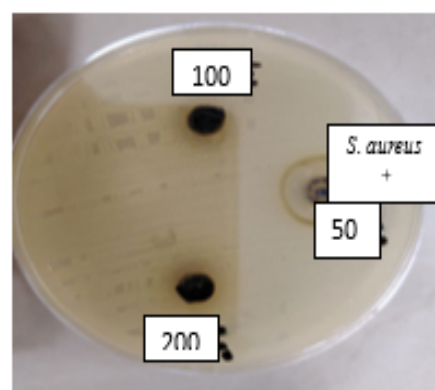


Figure (4) shows the inhibition rate of the aqueous extract of gram-positive.

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