SAWA UNIVERSITY

RESEARCH METHODS 1

CONTENTS OF SCIENTIFIC RESEARCH

Sawa University — جامعـــــة ســـــــاوة الاهلية

College of health and medical techniques — كليـــــة التقنيـــات الصحيـة والطبيـة

Department of Medical Laboratories — قسم تقنيات المختبرات الطبية

. Fourth Stage — المرحلـــــــــــة الرابعة

الجانب النظري — محاضرة رقم 11

Theoretical — Lecture No. 11

تدريسي المادة : م .د. قاسم عباس محمد

اهداف المحاضرة

- ماذا تتضمن الصفحات الأولى من البحث العلمي
- كيف تكتب ملخص البحث العلمي وما الذي لا يمكن ادراجه في الملخص
- ماذا تتضمن فصول البحث
- ماهي الصفحات الأخيرة من البحث

Republic of Iraq
Ministry of Higher Education
And Scientific Research
University of Sawa
College of Health and Medical Techniques
Department of Medical Laboratories

عنوان البحث باللغة الإنكليزية بخط غامق حجم 18 نوع (Time New Roman)

A study submitted by

أسماء الطلبة بالترتيب بخط غامق حجم 16

To college of health and medical technology-Sawa university As part of the requirements for obtaining a bachelor degree in the Department of Medical Laboratories

Supervisor

اللقب العلمي واسم المشرف بخط غامق وحجم 16

2026 A. D **1447 A.H**

أية قرأنية واحدة او أيات صغيرة من اختيار الطالب
ويفضل ان تكتب بنفس طريقة القرأن الكريم

Supervisor Certification

I certify that this project entitled (عنوان البحث باللغة الإنكليزية وبخط غامق) was prepared under my supervision in partial fulfilment of the requirements for the degree of Bachelor in (**Department of Medical Laboratories**).

Signature:

Name of supervisor

Signature:

Name of Head Department

Certification of Examining Committee

We, the members of the examining committee, have looked at the project (Comparative Study of Effect Antibiotics and Plants Extracts on (العنوان باللغة الإنكليزية) we have examined the students in its contents and what is related to it and we can decide that it is qualified for pursuing the degree of bachelor in (**Department of Medical Laboratories**).

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Name:
Member/ Supervisor:
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Signature:
Name:
Chairman:
Date:

Signature:
Name of the Dean:
Dean of Medical and Health Techniques College Date:

Dedication

I would like to dedicate this work to...

My gracious parents, may Allah keep protecting them.....

Those who helped and supported me, my brothers and sisters...

Those who accompanied me during my study – my friends...

Those who taught me even a single letter...

I give to you the humble fruition of my work.

نهدي بحثنا الى من نحب ان نهديهم

اسم او أسماء الطلبة

Acknowledgement

Praise be to God, praise be to Him greatly, and praise be to Him, and may peace and blessings be upon His most honorable creation, His light and purity, Muhammad, and upon his family and companions. Based on the principle that whoever does not thank people does not thank God, I extend my sincere thanks and appreciation to the Deanship of the College of Health and Medical Technologies for providing us with all the facilities to accomplish this work. Thanks also go to the Head of the Medical Laboratories Department. Many thanks to the supervising Assist. Prof. Dr. Hasan Raheem for his guidance which he never skimped on us. I also extend my sincere thanks and generosity to every hand that accompanied us in this work, whether from near or far and our thanks are also extended to our parents who made sure to provide all the appropriate conditions for us to accomplish this work.

نشكر أصحاب الفضل في تقديم المساعدة لأعداد البحث

اسم او أسماء الطلبة

Abstract

This experimental study was conducted for the purpose of comparing the effect of antibiotics and plant extracts on Gram-negative bacteria *Pseudomonas aeruginosa* and Gram-positive bacteria *Staphylococcus aureus* isolation from urine of patients suffering from urinary tract infections (UTI), Plant extracts were extracted of each of the (*Syzygium aromaticum* , *Eucalyptus globulus*, and *Trigonella foenum-graecum*) were extracted using ethanol (70%) once and using distilled water again, the active compounds in each extract were identified and diagnosed by using a gas chromatograph device integrated with a GC/MS mass spectrometer. Concentrations of plant extracts that used in study was(30%,50%,70%) the results of the study appeared on *Pseudomonas aeruginosa* the aqueous *Eucalyptus globulus* extract (70% and 50%) had an inhibition effect (1.1/1.0 cm) respectively ($p \leq 0.05$), alcoholic *Eucalyptus globulus* extract (70%, 50% and 30%) had an inhibition affect (0.9/0.7/0.6 cm) respectively ($p \leq 0.05$) while *Syzygium aromaticum* (70%, 50%, and 30%) , with inhibition effect reached (1.2, 1.1, and 0.9 cm) respectively ($p \leq 0.05$), as well as the aqueous *Syzygium aromaticum* (70% , 50%, 30%) which had an inhibition effect (1.0, 1.0 and 0.8 cm) respectively ($p \leq 0.05$) ,which was highest effect on bacterial growth compared to the effect of all antibiotics used in study except Tobramycin 30, which had a inhibition effect (2.6 cm),

هنا يكتب ملخص مختصر للبحث ويشمل:

1. مقدمة بسيطة تتحدث عن مشكلة الدراسة وسبب اجرائها.
2. مختصر لطريقة العمل (عدد العينات ومكان اجراء البحث وكتابة طريقة اجراء التجربة باختصار).
3. نكتب النتائج (ارقام ونسب وغيرها) ولا ندرج الجداول او الرسوم او الصور في الخلاصة.
4. نكتب مختصر للتوصيات في اخر الخلاصة
5. يفضل عدم ادراج المختصرات في الخلاصة.

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Abbreviations

Symbols	Meaning
ADP	Adenosine diphosphate
aGM1	Asialo -ganglioside M1
C.Alc	Alcoholic *Syzygium aromaticum* extract
C.w	Aqueous *Syzygium aromaticum* extract
C°	Degree Celsius
CA-MRSA	Community acquired- Methicillin resistant *S. aureus*
CEO	Clove essential oil
CF	Cystic fibrosis
CGD	Chronic granulomatous diseas
CoNS	Coagulase-negative *staphylococci*
E.Alc	Alcoholic *Eucalyptus globulus* extract
E.W	Aqueous *Eucalyptus globulus* extract
et al	Et alia
EtOH	Ethanol alcohol
ETA	Exotoxin A
F.Alc	Alcoholic *Trigonella foenum-graecum* extract
F.W	Aqueous *Trigonella foenum-graecum* extract
g	grama
GC-MC	Gas chromatograph mass spectrometry
HCA-MRSA	Health care - Methicillin resistant *S. aureus*
IL-1	Interleukin-1

Chapter One

Introduction

Chapter One:
Introduction

1.1. Introduction

In recent years, many plants have been found to have inhibitory activity against pathogens, due to the compounds and active elements they contain after extraction and purification, in additional their lack of side effects and the inability of germs to develop resistance to them (Kalaivani *et al.*, 2012).

Plants are the main and alternative source of treatment Infectious diseases ,thus plant extracts provide continuous efforts to find new effective compounds against many resistant bacteria including *staphylococcus spp* , *pseudomonas spp* (Anand *et al.*, 2022).

There are many studies on the use of plant extracts to inhibit and kill microorganisms when scientific progress, with all its capabilities, fails to cure some diseases as it is an economical, safe, and highly efficient treatment , the efficiency of these extracts varies depending on the method of extraction, the type of solvent used for extraction, and the test microorganism (Aziz *et al.*, 2018).

Plant extracts showed an inhibitory effect on *Pseudomonas aeruginosa* , which is ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses hospital acquired infections such as ventilator associated pneumonia and various sepsis syndromes, (Behzadi *et al.*, 2021).

. Previous study showed that effect of plant extrat on *S. aureus* bacteria, which is the main cause of hospital infections or infections associated with contamination of medical devices, as well as causing pyogenic infections to humans and poisonings, including superficial skin injuries such as blisters and boils, and many other serious injuries to organs and tissues of the body (Goel *et al.*, 2023)

In present study, alcoholic and aqueous extracts of *Trigonella foenum-graecum* , *Syzygium aromaticum* and *Eucalyptus globulus* were used (which are among the most famous and widely used medicinal plants) (Anand *et al.*, 2022).

1.2. Aim of the Study:

1. Isolation and diagnosis of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from urinary tract infection (UTI).
2. Determination of the sensitivity of *Pseudomonas aeruginosa* and *Staphylococcus aureus* towards some antibiotics belonging to different groups and search for alternative treatment using plant extracts .
3. Determination of the sensitivity of *Pseudomonas aeruginosa* and *Staphylococcus aureus* towards alcoholic and aqueous extracts of *Trigonella foenum-graecum* , *Syzygium aromaticum* and *Eucalyptus globulus* .
4. Evaluating the effect of antibiotics on bacteria and comparing it to the effect of plant extracts on these bacteria .

Chapter Two

Literatures Review

Chapter Two
Literatures Review

2.1. Bacteria causing urinary tract infections

Urine does not normally contain bacteria, and a small, harmless number may be present in some healthy people ,in general, the presence of bacteria and a high number of bacteria may indicate a urinary tract infection (Brunzel, 2021).

Bladder infection (Cystitis) or pyelonephritis is attributed to two main types of bacteria:They are aerobic(Gram-negative bacteria, which are considered the most common example *Klebsiella spp* ,*Proteus spp, Mycoplasma spp* and *Pseudomonas spp*), and (Gram-positive bacteria, which are considered the least common example *Streptococcus spp*, *Staphylococcus spp* and *Enterococcus spp*) (Chou *et al.*, 2022).

2.1.1. *Staphylococcus aureus*

Staphylococci are gram-positive spherical cells, usually arranged in grapelike irregular clusters , they grow readily on Many types of media and are active metabolically, fermenting Carbohydrates and producing pigments that vary from white to deep yellow (Salih, 2022).

Some are members of the normal microbiota of the skin and mucous membranes of humans , others cause -Suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia (Fink and Smith, 2020).

The pathogenic *staphylococci* often hemolyze blood, coagulate plasma, and produce variety of extracellular enzymes and toxins ,the most common type of food poisoning is caused by a heat-stable Staphylococcal enterotoxin , *Staphylococci* rapidly develop Resistance to many antimicrobial agents ,which consequently ,presents difficult therapeutic problems (Ibrahim, 2020).

5. The peptidoglycan of *S. aureus* has endotoxin-like properties (i.e., it can stimulate macrophages to produce cytokines and can activate the complement and coagulation cascades) , this explains the ability of *S. aureus* to cause the clinical findings of septic shock yet not possess endotoxin (Duza, 2021).

2.1.1.3. Pathogenesis of *S. aureus*

Staphylococcus aureus causes disease both by producing toxins and by inducing pyogenic inflammation ,the typical lesion of *S. aureus* infection is an abscess ,abscesses undergo central necrosis and usually drain to the outside (e.g., furuncles and boils), but organisms may disseminate via the bloodstream as well ,foreign bodies, such as sutures and intravenous catheters , are important predisposing factors to infection by *S. aureus* (Ansari, 2019).

Two other characteristics further distinguish these species, namely, *S. aureus* usually ferments mannitol and hemolyzes red blood cells, whereas *S. epidermidis* and *S. saprophyticus* do not (Chalise, 2021).

Hemolysis of red cells by hemolysins produced by *S. aureus* is the source of iron required for growth of the organism , the iron in hemoglobin is recovered by the bacteria and utilized in the synthesis of cytochrome enzymes used to produce energy (Marquart, 2020).

Several important toxins and enzymes are produced by *S. aureus* , the three clinically important exotoxins are enterotoxin, toxic shock syndrome toxin, and exfoliatin (G. Abril *et al.,* 2020).

1. Enterotoxin causes food poisoning characterized by prominent vomiting and watery, non bloody diarrhea , it acts as a superantigen within the gastrointestinal tract to stimulate the release of large amounts of IL-1 and IL-2 from macrophages and helper T cells, respectively (Patel *et al.,* 2023).

Chapter Three

Materials and Methods

Chapter Three
Materials and Methods

3.1 Materials

3.1.1 Instruments and Equipments:

The necessary instruments and apparatuses used for preparing the appropriate experiments in present study, table(3.1).

Table 3.1 Instruments and Equipments

No.	Instruments and Equipments	Company	Origin
1.	Autoclave	Hirayama	Japan
2.	Blender	Newal	China
3.	Burner	Amal	Turkey
4.	Compound light Microscope	Novel	France
5.	Cover slips	ALS	China
6.	Electric balance	Sartorius	Germany
7.	Eppendrof tube 2 ml	Hamburg	Germany
8.	Filter papar	GEMA	Spain
9.	Funnel	GEMA	Spain
10.	Forceps	GEMA	Spain
11.	Gauza	GEMA	Spain
12.	Hood	Medilab	Korea
13.	Hot plate	GEMA	Spain
14.	Incubator	Memmert	Germany
15.	Micropipette	Dragon lab	China
16.	Measuring cylinder	GEMA	Spain

3.2 Methods

3.2.1 Preparing the Culture Media

The culture media were prepared according to the instructions of the manufacturing company stated on the containers and it was autoclaved at 121° C for 15 minutes, in 15 bar/in², and incubated at 37 ° C for 24 hours to be sure from their sterility and then kept at 4° C until they were used as shown in table (3-5) (Orekan *et al.*, 2021).

Table 3.5 Standard Culture Media.

No.	Medium	Purpose
1.	Blood agar base	Determining the ability of bacteria to hemolysis human blood cell
2.	MacConkey agar	To cultivation of Gram- negative bacteria.
3.	Mannitol Salt agar	Selective media for the isolation of pathogenic *Staphylococci.*
4.	Muller Hinton agar	Antimicrobial susceptibility testing.
5.	Nutrient agar	Growth and subculture of bacterial isolates
6.	CHROMagar™ *Pseudomonas*	CHROMagar™ Pseudomonas Selective media for *Pseudomonas* isolates.

No=Number

3.2.3 Chemical Solutions and Reagents

A. Gram stain solutions

It consists of four solutions which included (crystal violet, iodine, ethanol and Sufranine), this stain was used to study bacterial cells morphology and its arrangement (Mohamed, 2018).

B. Coagulase test reagent

The Tube Coagulase Test consists of putting approximately 1 ml of coagulase reagent (rabbit plasma) in a labeled test tube and incubating it at 34 to 37 degrees Celsius from 4 to 24 hours , if the tube has a thick, solid clot within 24 hours, it is *Staphylococcus aureus*(Deka *et al.*, 2020).

Statistical analysis

Statistical analysis was conducted by using Chi-square (χ^2) test to determine the statistical differences among different groups by using design statistical package for social science (SPSS 23) ,probability of($P \leq 0.05$) was considered to be statistically significant (Obialigwe *et al.*, 2023).

Chapter Four

Results and Discussion

Chapter Four:
Results and Discussion

4.1. Phenotypic tests for pathogenic bacteria

4.1.1. Phenotypic tests for *P.aeruginosa*

Pseudomonas aeruginosa isolates appear small in size, fruity or earthy odor , the colonies appear pigmented , smooth with flat edge and an elevated appearance on nutrient agar (figure 4.2A) , this isolate produce pyocyanin (blue green pigment) specially on CHROMagar™ *Pseudomonas* (figure 4.2B) , on blood medium was streaked with a pure colony of bacteria and incubated at 37°c for 24—48 hours , the appearance of a clear zone around the colony indicates for ß—hemolysis (figure 4.2C) , the colonies of *P.aeruginosa* isolate does not ferment lactose on macConkey agar and is differentiated from lactose fermenting bacteria (Enterobacteriaceae) (figure 4.2D), the bacterial cells from smear preparation are gram negative, rod-shaped, and occur as single, in pairs, or in short chains (figure 4.1) , presumptively regards *P. aeruginosa,* the bacterial colonies were able to grows well at 37—42 °C , its growth at 42 °C helps differentiate it from other *Pseudomonas* species in the fluorescent which in accordance with previous data to (Knutelski *et al.,* 2021) (Alshammari, 2022).

Furthermore, biochemical tests were performed to support the results Vitek device (El Sherif *et al.,* 2022) ,biochemical which is a rapid accurate technique for the differentiation of *P.aeruginosa* (Capatina *et al.,* 2022*) P. aeruginosa* reacted positively to catalase test (figure 4.3B) , oxidase test (figure 4.3B) and Simmon"s citrate tests ,while it was negative for methyl red, Coagulase test , it the biochemical properties of the organism recorded in this study are the same as obtained by to (Alshammari, 2022).

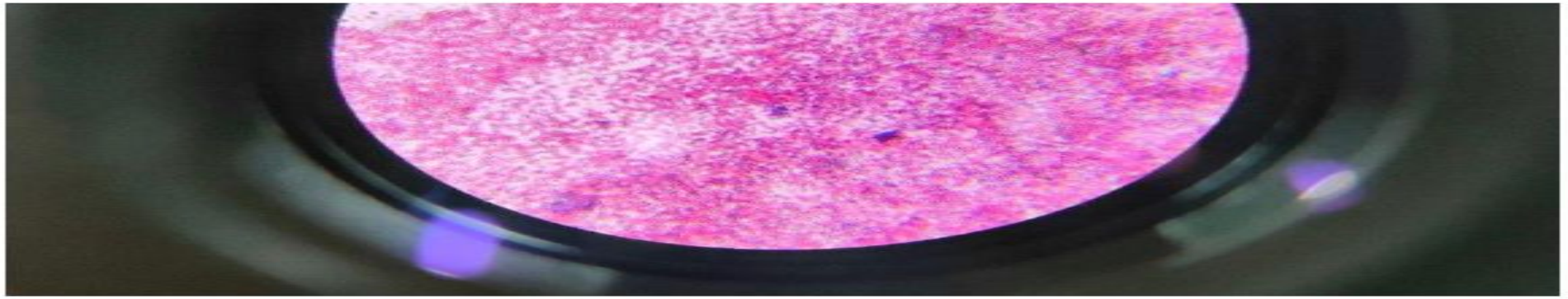


Figure 4.1 Microscopic image of gram-negative *Pseudomonas aeruginosa* bacteria (pink-red rods).

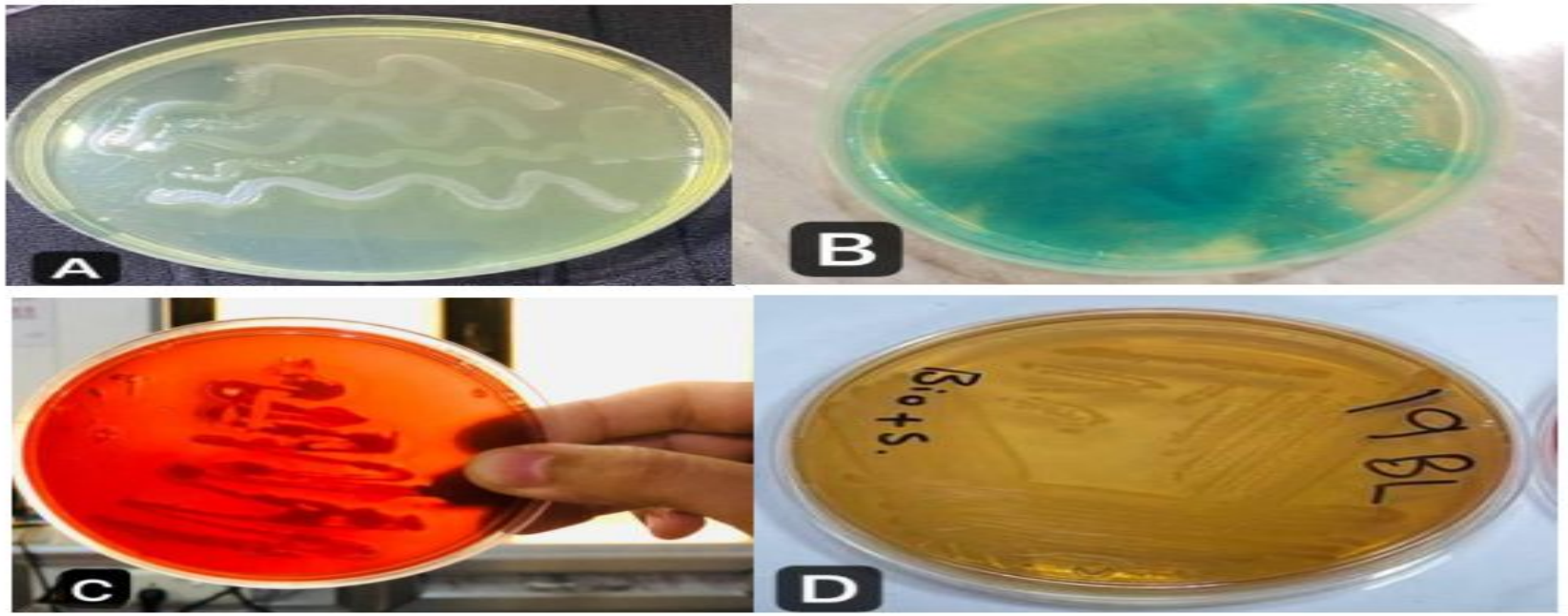


Figure 4.2 A. *P. aeruginosa* on nutrient agar B. CHROMagar Pseudomona for the isolation and detection of *P.* aeruginosa C. *P. aeruginosa* beta hemolysis on blood agar D. *P. aeruginosa* on

4.2. Results of screening compounds of plant extracts by mass spectrometry

The current study showed GC-MS results for *Eucalyptus globulus* Aqueous that were about 46 compounds, of which there are five highest compounds (table 4.1),where the (7-octen-2-ol,2-methyl-6-methylen) compound was the highest concentration (16,8313) while (1-hexen-3-ol) was the less concentration (5,042).

Table 4.1 showing the percentage of the top five compounds in *Eucalyptus globulus* Aqueous extract .

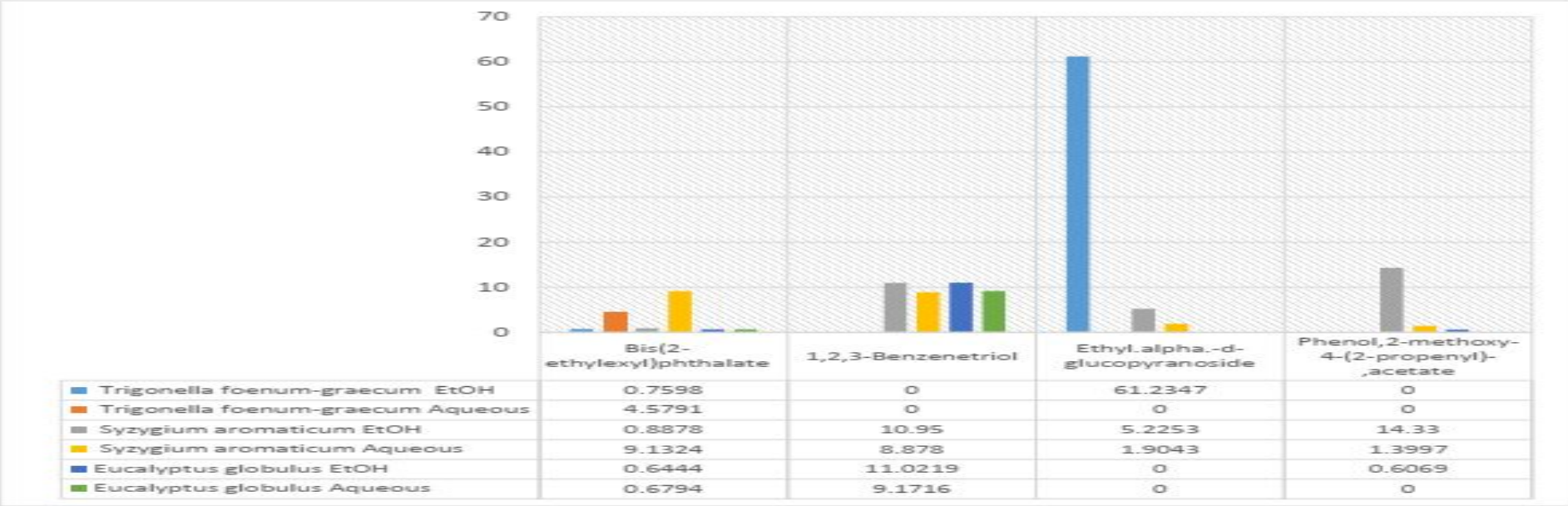
No.	Compound Name	Molecular formula	R.T	Area Pct
1	7-octen-2-ol,2-methyl-6-methylen	$C_{10}H_{18}O$	18,196	16,8313
2	1,2,3-Benzenetriol	$C_6H_6O_3$	15,989	9,1716
3	Methoxyacetic acid ,2-ethylhexyl ester	$C_{17}H_{34}O_3$	19,139	6,4689
4	Isoaromadendrene epoxide	$C_{15}H_{24}O$	20,084	5,8703
5	1-hexen-3-ol	$C_6H_{12}O$	5,042	5,2441

No. =Number

R.T=Retention time

While current study showed GC-MS results for *Eucalyptus globulus* EtOH extract that were about 58 compounds, of which there are five highest compounds in (table 4.2) ,where the (1,2,3-Benzenetriol) compound was the highest concentration (11.0219) while (Z,Z-3,6-Nonadienal) compound was the less concentration (3,737) .

As found that both the aqueous and alcoholic *Syzygium aromaticum* extracts and *Eucalyptus globulus* extracts contain (1,2,3-Benzenetriol) compound in different proportions, while the aqueous and alcoholic *Trigonella foenum-graecum* extracts don't contain (1,2,3-Benzenetriol) compound.



	Bis(2-ethylexyl)phthalate	1,2,3-Benzenetriol	Ethyl.alpha.-d-glucopyranoside	Phenol,2-methoxy-4-(2-propenyl)-,acetate
Trigonella foenum-graecum EtOH	0.7598	0	61.2347	0
Trigonella foenum-graecum Aqueous	4.5791	0	0	0
Syzygium aromaticum EtOH	0.8878	10.95	5.2253	14.33
Syzygium aromaticum Aqueous	9.1324	8.878	1.9043	1.3997
Eucalyptus globulus EtOH	0.6444	11.0219	0	0.6069
Eucalyptus globulus Aqueous	0.6794	9.1716	0	0

Figure 4.7 Common Compound in all plant extracts

Chapter Five:

Conclusions and

Recommendations

Chapter Five:
Conclusions and Recommendations

5.1. Conclusions

1. The (7-octen-2-ol, 2-methyl-6-methylen),(2-[2-[2-(2-Butoxyethoxy)ethoxy]ethoxy]ethyl acetate) and Bis(2-ethylhexyl)phthalate compounds have the highest concentration in *Eucalyptus globulus* Aqueous extract , *Trigonella foenum-graecum* Aqueous extract and *Syzygium aromaticum* Aqueous extract respectively.
2. The (1,2,3-Benzenetriol) ,(Ethyl.alpha-d-glucopyranoside) and (Bis(2-ethylhexyl)phthalate) compounds have the highest concentration in *Eucalyptus globulus* EtOH extract , *Trigonella foenum-graecum* EtOH extract and *Syzygium aromaticum* EtOH extract respectively.
3. All the extracts contained the Bis(2-ethylexyl)phthalate compound in different proportions .
4. All the extracts containing (Bis(2-ethylhexyl)phthalate) as well as (1,2,3-Benzenetriol) compounds were inhibition effect against bacteria more than extracts did not contain them .
5. The effect of EtOH extracts are highest than Aqueuos extracts , shown that the diameters of the bacterial colonies decreased with an increase in the concentration of the extract used, at a time when the percentages of inhibition were directly proportional with an increase the concentration of the extract .
6. *Trigonella foenum-graecum* EtOH at all concentration , *Trigonella foenum-graecum* Aqueous at all concentration and *Eucalyptus globulus* Aqueous 30% were non effect on *P.aeruginosa* isolate .
7. *P.aeruginosa* isolate showed sensitive to Tobramycin 30
8. *S.aurus* isolate showed highly sensitive to Erythromycin 15 and resistant to pencillin 10.

5.2. Recommendations

1- Conduct an HPLC test to evaluate and identify the active compounds in each extract.
2- Increase the search for alternative plant extracts to antibiotics.
3- Reducing the indiscriminate use of antibiotics, which has made bacteria resistant to many of them.
4- Paying attention to medicinal plants because of their many benefits, both medically and environmentally.

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الخلاصة

أجريت هذه الدراسة التجريبية لغرض مقارنة تأثير المضادات الحيوية والمستخلصات النباتية على البكتيريا سالبة الجرام الزانفة الزنجارية و البكتيريا موجبة الجرام المكورات العنقودية الذهبية المعزولة من بول المرضى الذين يعانون من التهابات المسالك البولية، وتم استخلاص المستخلصات النباتية لكل من نباتات (القرنفل و اوراق الكاليتوس و الحلبة) بأستخدام كحول الايثانول ٧٠٪مرة و بالماء المقطر مرة أخرى، وتم تحديد وتشخيص المركبات النشطة في كل مستخلص بأستخدام جهاز الكروماتوجراف الغاز المدمج بمطياف الكتلة GC/MS. وكانت تراكيز المستخلصات النباتية المستخدمة في الدراسة(٧٠٪،٥٠٪،٣٠٪)، وأظهرت نتائج الدراسة على بكتريا الزانفة الزنجارية المستخلص الماني لنبات الكاليتوس (٧٠٪) و (٥٠٪) كان لها تأثير تثبيطي (١.١,١.٠سم) على التوالي($p<0.05$) ، وكان لمستخلص الكاليتوس الكحولي (٧٠٪،٥٠٪،٣٠٪) تأثير تثبيطي (٠.٦,٠.٧,٠.٩سم)على التوالي($p<0.05$) ، في حين كان لمستخلص القرنفل الكحولي (٧٠٪،٥٠٪،٣٠٪) بلغ التأثير التثبيطي(١.٢,١.١,٠.٩سم) على التوالي($p<0.05$) ، وكذلك مستخلص القرنفل الماني (٧٠٪،٥٠٪،٣٠٪) الذي كان له تأثير تثبيطي (١.٠,١.٠,٠.٨سم) على التوالي($p<0.05$) ، وهو أعلى تأثير على نمو البكتيريا مقارنة بتأثير جميع المضادات الحيوية المستخدمة في الدراسة

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة ساوة
كلية التقنيات الطبية والصحية
قسم المختبرات الطبية

العنوان باللغة العربية

دراسة تقدم بها كل من

اسم او أسماء الطلبة

لمجلس كلية التقنيات الصحية والطبية /جامعة ساوة الاهلية وزارة التعليم العالي والبحث العلمي
كجزء من متطلبات نيل شهادة البكالوريوس في قسم المختبرات الطبية

المشرف

اسم المشرف مع لقبه العلمي العلمية

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Any questions?