The Antibacterial and Antibiofilm Effect of some Medicinal Plant Extracts and their Synergistic Effect with Different Antibiotics

التأثير الضد بكتيري والضد بيوفيلمي لبعض المستخلصات النباتية وتأثيرها التآزر مع المضادات الحيوية

By

Yasmeen Mosa Ahmed Abu Madi

Supervisor:

Dr. Tarek El Bashiti
Assoc. Prof. of Biotechnology

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بناءً على موافقة عمادة البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيك لجنة الحكم على أطروحة الباحثة: باسمين موسى أحمد أبو ماضي لئيل درجة الماجستير في كلية العلوم/برنامج العلوم الحيوية/علم الحيوان، ووضعتها:

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The Antibacterial and Antifilm Effect of Some Medicinal Plant Extracts and their Synergistic Effect with different Antibiotics

وبعد المناقشة التي تمت اليوم الأحد 28 ذو الحجة 1439 هـ الموافق 09/09/2018م الساعة الحادية عشرة صبحًا، اجتمعت لجنة الحكم على الأطروحة والموكلة من:

د. طارق عبد القادر البيشتي

أ. د. عيسى بدر القضاوي

أ. د. محمد محمود أبو عودة

واعترفت لجنة الحكم بمراعاة هذه الأطروحة في كلية العلوم/برنامج العلوم الحيوية/علم الحيوان.

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توقيع الطالب

إدارة المكتبة المركزية
Abstract

Background: Antibiotics are important to inhibit bacterial activity, but some bacteria have the ability to adapt with environment and develop themselves, their ability to form biofilm, and their resistance to antibiotics. Therefore, alternative sources of antibiotics have to be found to inhibit bacterial activity.

Objective: The aim of the study was to assess the antibacterial and antibiofilm effect of some medicinal plant extracts and their synergistic with antibiotic against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and klebsiella pneumonia.

Materials: The medicinal plants that have been studying its effect against some clinically isolated bacteria is Glycyrrhiza glabra roots, Laurus nobili, Malus domestica peels, Melissa officinalis and Lagenaria siceraria peels. Clinical isolated bacteria used in the study are Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and klebsiella pneumonia.

Methods: The extract of medicinal plants were prepared using microwave method for ethanolic extract and aqueous extracts. The antibacterial activities of extracts were confirmed using the disk diffusion method and the well diffusion method; the inhibitory zones were recorded in millimeters. The minimal inhibitory concentration (MIC) of the plant extracts were confirmed using microdilution method. The synergistic effect between plants extracts and antibiotics was assessed using disk diffusion method. Antibiofilm activity was confirmed by using microtitre dish against S. aureus and P. aeruginosa.

Results: The results exhibit that there is a decrease in MIC of aquatic extract of M. domestica peels against E. coli (1.56mg/ml), and the ethanolic and aquatic extract of G. glabra roots, L. siceraria peels and M. officinalis against S. aureus 25 mg/ml, and the ethanol extract of G. glabra roots against P. aeruginosa varying from 12.5 to 6.25 mg/ml. The MIC for aquatic extract of Glycyrrhiza glabra roots and L. nobilis showed 25 mg/ml against K. pneumonia. The MIC for ethanolic extract of M. officinalis and M. domestica peels was 25 mg/ml against K. pneumonia. The MIC for the ethanolic and aquatic extract of L. siceraria peels was 25 mg/ml against k. pneumonia. The results showed that MBC of ethanolic and aquatic extract of G. glabra roots against E. coli at 200 mg/ml. The MBC of ethanolic extract of G. glabra roots against S. aureus at 200 mg/ml. The MBC of aquatic extract of M. officinalis against P. aeruginosa at 200 mg/ml. The MBC of ethanolic and aquatic extract of M. officinalis against K. pneumonia at 200 mg/ml. The results of this study have shown that L. nobili aquatic extract concentration of 12.5mg/mL is which showed the highest inhibition on S. aureus biofilm formation 86.7%. M. domestica peels ethanolic extract concentration of 25mg/mL is the highest inhibition (90%) on P. aeruginosa biofilm formation.

Conclusions: The plant extracts showed strong antibacterial and synergistic effect with most antibiotics against E. coli, S. aureus, P. aeruginosa and K. pneumonia. The antibiofilm effect against S. aureus and P. aeruginosa was clearly shown.

Key words: Anti-biofilm, plant extracts, antibacterial effect and synergistic effect.
المقدمة: المضادات الحيوية مهمة لتثبيط النشاط البكتيري لكن هناك بعض من البكتيريا لديها القدرة على التكيف مع البيئة وتطوير نفسها، وقدرتها على إدخال مضادات الحيوية لذلك لابد من ايجاد مصادر بديلة لتثبيط النشاط البكتيري، مثلéo: كان الهدف من هذه الدراسة هو تقييم التأثير المضاد للبكتيريا والبيوفيلم لبعض المستخلصات النباتية، وتآزرها مع المضادات الحيوية ضد الإشريكية القولونية، المكورات العنقودية الذهبية، الزائفة الزنجارية، الالتهاب الرئوي.

الأدوات: النباتات الطبية التي درس تأثيرها ضد بعض البكتيريا التي استخدمت في البحث هي جذور عرق السوس والغار، وقشور التفاح الأحمر، والبلسم الليمون، وقشور القرع الأخضر. البكتيريا المستخدمة في الدراسة هي الإشريكية القولونية، المكورات العنقودية الذهبية، الزائفة الزنجارية، الالتهاب الرئوي.

الطريق: تم إعداد مستخلص النباتات الطبية باستخدام طريقة الميكرلوف لمستخلص الإيثانول والمستخلصات المائية. تم تقييم الأنشطة المضادة للبكتيريا من مستخلصات باستخدام طريقة الانتشار عبر القرص بالإضافة إلى طريقة الانتشار عبر الصرف. تم تسجيل المناطق المثبطة في ملليمتر. التركز المثبط الأولي وقودة قيمة أقل تركيز مثبط من مستخلصات النباتية وحدها باستخدام طريقة الغلاف المثبطي، والنشاط ضد بيوفيلم باستخدام أطباقي المعايرة المائية ضد المكورات العنقودية الذهبية، الزائفة الزنجارية.

النتائج: أظهرت النتائج أن هناك انخفاض في قيمة أقل تركيز مثبط للبكتيريا (MIC) للمستخلص المائي لقشور التفاح الأحمر ضد الإشريكية القولونية 1.56 ملغ/مل، والمستخلص المائي والإيثانولي لجذور عرق السوس ولبسم الليمون وقشور الفرع الأخضر ضد البكتيريا المكونة عنقودية الذهبية 25 ملغ/مل. وتمت تقييم الاضرار الحيوية والنشاط ضد بيوفيلم باستخدام أطباقي المعايرة المائية. MIC للمستخلص المائي لجذور عرق السوس ونبات الغار ظهرت 25 ملغ/مل ضد بيوفيلم بالإضافة إلى تأثير ضعيف بقية البكتيريا المكونة عنقودية الذهبية، الزائفة الزنجارية، الالتهاب الرئوي.

ونتالي من التأثير الدائري بين مستخلصات النباتية والمضادات الحيوية باستخدام طريقة الانتشار عبر القرص.

الاستنتاج: أظهرت مستخلصات النباتات تأثير مضاد للبكتيريا والبيوفيلم مقابل مع معظم مضادات الحيوية ضد الإشريكية القولونية، المكورات العنقودية الذهبية، الزائفة الزنجارية، الالتهاب الرئوي، وقد تبين بوضوح التأثير المستحيل لتكوين بيوفيلم ضد المكورات العنقودية الذهبية، الزائفة الزنجارية.

الكلمات المفتاحية: بيوفيلم، مستخلصات نباتية، التأثير البكتيري، التأثير التآزري.
Dedication

To whom I carry his name with pride and pride, I ask God to extend your age to see the fruit of her harvest after waiting (my dear father).

To the light that illuminates my path of success, to who taught me to withstand, no matter how the circumstances change (my mother).

To my brothers and sisters who have been a great source of motivation and inspiration.

Yasmeen Abu Madi
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## List of Abbreviations

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<th>Full Form</th>
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<tbody>
<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant Staphylococcus aureus</td>
</tr>
<tr>
<td>MBC</td>
<td>Minimum Bactericide Concentration</td>
</tr>
<tr>
<td>BHIB</td>
<td>Brain Heart Infusion broth</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density</td>
</tr>
<tr>
<td>mg/ml</td>
<td>milligram/milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>millimeters</td>
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<tr>
<td>MHA</td>
<td>Muller-Hinton Agar</td>
</tr>
<tr>
<td>TTC</td>
<td>2, 3, 5-triphenyl tetrazolium chloride</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometre</td>
</tr>
<tr>
<td>NB</td>
<td>Nutrient broth</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>DW</td>
<td>Distilled water</td>
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Chapter 1 Introduction
Chapter 1
Introduction

1.1 Overview
The development of bacterial resistance to available antibiotics has stimulated researchers to find alternative antimicrobial agents, despite the role of antibiotics to prevent the growth of pathogens (Chanda, Sumitra & Rakholiya, 2011). Excessive use of antibiotics may enhance the resistance of bacterial species that cause human diseases (Abiramasundari, Priya, Jeyanthi, & Gayathri, 2011).

Medical plants are important natural resources that can be constantly renewed and have an effective role in protecting people from disease (Kannahi & Nadu, 2013). It is also an important source for containing huge amounts of antimicrobial agents (Raji & Raveendran, 2013).

Bacteria are considered to be the oldest and simplest living organisms and most commonly found on the Earth's surface when compared with other living organisms. They are also single-celled monoclonal organisms where the living genetic material is not surrounded by the nuclear membrane. Bacteria are simple life forms and appear in three basic structures: bacillus (plural, bacilli) straight and rodshaped, coccus (plural, cocci) spherical-shaped, and spirillus (plural, spirilla) long and helical-shaped, also called spirochetes. Bacteria reproduce by bilateral fission. Bacteria live in different places on Earth and are able to adapt and resist high-temperature environments such as hot springs, and in an atmosphere rich in toxic gases such as methane, which kills other species (Raven et al., 2011).

Some types of bacteria have the ability to form biofilms and these species vary in terms of their speed in the production of biofilms. Biofilms allow microorganisms to capture nutrients and withstand environmental conditions (Al-refi, 2016). Bacteria within biofilms are more resistant to antibiotics and chemical agents than planktonic cells in suspension (Mohammadi & Rohloff, 2016).
1.2 Objectives
The general aim of this study is to assess the antibacterial and antbiofilm effect of some medicinal plant extracts and their synergistic effect with different antibiotic drugs.

1.2.1 Specific Objectives
1-To collect and to identify of medicinal plants.
2-To extract the selected medicinal plants by microwave method using ethanol and water as solvents.
3-To measure the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the selected plant extracts against pathogenic isolates of *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumonia*.

1.3 Significance of the study

Excessive use of antibiotics increases bacterial resistance. Antibiotics are known to be chemical compounds that may have side effects on humans, and may interact with other compounds in the case of human consumption of other drugs that treat other diseases, which reduces the effectiveness of each other. This has prompted researchers to find new antibacterial agents from natural sources.
Chapter 2 Literature review
Chapter 2
Literature review

2.1 Medicinal plants
Medicinal plants are a source of medicinal compounds that have played an important role in maintaining human health since ancient times. WHO confirmed that plant extracts are used as folk medicine in traditional treatments by 80% of the world's population. 50% of all modern clinical drugs are from Origin of natural product. The medicinal plants are characterized by their immune properties and antioxidants, which helps them act as anti-bacterial agents (Sulieman et al., 2017).

Palestine is characterized by the presence of many medicinal plants that are used as a treatment for many human diseases.
The medicinal plants which tested for their antibacterial and antibiofilm activity are the following:

2.1.1 Glycyrrhiza glabra roots

![Figure (2. 1) Roots of Glycyrrhiza glabra](image)

<table>
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<tr>
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<td>Order</td>
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</tr>
<tr>
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<td>Leguminosae</td>
</tr>
<tr>
<td>Genus</td>
<td>Glycyrrhiza</td>
</tr>
<tr>
<td>Species</td>
<td>glabra</td>
</tr>
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</table>

Glycyrrhiza glabra is a long-term sediment of 2.5 meters long and grows in the Mediterranean, Middle East and Asia Minor regions and is also cultivated widely in southern Russia and (Wittschier, Faller, & Hensel, 2009), belongs to the Leguminosae family. Roots are important parts with nutritional value and important medical properties. Licorice has been widely used as a medicine and seasoning. Japan has sought to use licorice as a major treatment for diseases. It has been used for more than 60 years as a preventive agent for duodenal ulcers and stomach (Zadeh, Kor, & Goftar, 2013). Glycyrrhiza roots are useful for
treating cough. *G. glabra* roots have contributed to the treatment of anemia, sore throat, tonsillitis, abdominal irritation and skin diseases. It is also used effectively in acidity, bleeding, jaundice, bronchitis, bronchitis. *G. glabra* root extract consists of saponin triterpenes (glycyrrhizin, glycyrrhetinic acid 10–25% and liquirit acid), flavonoids (liquirtin, isoflavonoids and formononetin) and other constituents such as coumarins, sugars, amino acids, tannins, starch (30%), choline, phytosterols and bitter principles (Damle, 2014). These active substances are essential to antiviral, antioxidant anticancer, anti-diabetic, anti-ulcer, antimalarial, anti-inflammatory, antifungal, anti-bacteria (Abbas, Zubair, Rasool, & Rizwan, 2016).

### 2.1.2 *Laurus nobilis*

![Leaves of *L. nobilis*](image)

**Figure (2. 2) Leaves of *L. nobilis***

<table>
<thead>
<tr>
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<tr>
<td>Species</td>
<td><em>L. nobilis</em></td>
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</table>

**Table (2. 2) Classification of *Laurus nobilis***

*L. nobilis* is a plant belonging to the Lauraceae family (Shokoohinia, Yegdaneh, Amin, & Ghannadi, 2014), which includes about 2500 species (Basak & Candan, 2013). The genus Laurus is found in Europe and consists of the two species *Laurus azorica* and *Laurus nobilis* (Basak & Candan, 2013). *L. nobilis* is also known as bay laurel, true bay, sweet bay, Grecian laurel and bay tree (Chahal, Kaur, Bhardwaj, Singla, & Kaur, 2017). *L. nobilis* is a small tree and the flowers are small and four lobed; male members consist of 8-12 stamens, and members of female consist of 2-4 staminodes (Chahal et al., 2017). The leaves range from 5-10 cm, 2-5 cm wide, and green in color (Chahal et al., 2017) and narrowly oblong-lanceolate leaves (Chahal et al., 2017). The most abundant component found in laurel essential oil is 1,8-cineole, also called eucalyptol. The leaves contain about 1.3% essential oils, consisting of 45% eucalyptol, 12% other terpenes, 8-12% terpinyl acetate, 3–4% sesquiterpenes, 3%
methyleugenol, and other α- and β-pinenes, phellandrene, linalool, geraniol, and terpineol (Kilic, Hafizoglu, Kollmannsberger, & Nitz, 2004). Dried leaves have been widely used in cooking, giving special flavors to foods (Basak & Candan, 2013). Essential oils derived from different parts such as leaves have been used in many industries such as medicine, food industries and cosmetics. Aromatic oils have useful functions as antibacterial, antifungal and antioxidant (Shokoohinia et al., 2014).

2.1.3 Malus domestica peels

Figure (2. 3) Peels of M. domestica

Table (2. 3) Classification of Malus domestica

<table>
<thead>
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<td>Malus</td>
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<tr>
<td>Species</td>
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</table>

M. domestica is a member of the family Rosaceae and sub-family Maloideae (Guadie, 2011). It is a small perennial tree with a height ranges between 5-12 meters. (Guadie, 2011). Malus domestica trees grow especially in Central Asia. The apple fruit contains phenolic compounds (procyanidins, polyphenolic acids) responsible for its nutrition value and medical properties (Yanrong Lv, 2016), and its is very good source of important phytochemicals like antioxidants, flavonoids (Guadie, 2011). Fruit is very important in treating chronic diseases such as asthma, cancer, diabetes and cardiovascular disease (CVD) (Yanrong Lv, 2016). Apple peel is of great biological benefit and more antioxidant than the apple flesh (Salgado, Curte, & Mansi, 2008). When analyzing apple peel oil, it was found to contain many important compounds such as α-phellandrene (20.6%), α-pinene (17.4%), β-pinene (17.1%), 8-diene (16.1%) and sabinene (9.5%); and some appreciable amounts of o-cymene (4.3%), β-cubebene (3.1%), and β-ocimene (2.5%) (Judžentienė, 2017).
2.1.4 *Melissa officinalis*

![Figure (2.4) *M. officinalis*](image)

<table>
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<td>Melissa</td>
</tr>
<tr>
<td>Species</td>
<td><em>M. officinalis</em></td>
</tr>
</tbody>
</table>

*M. officinalis* (lemon balm) is a plant belonging to the Lamiaceae family. A thick perennial plant with a height of about 1 meter. Its soft heart-shaped leaves are 2-8 centimeters long and the leaf edge is toothed (Moradkhani, Sargsyan, Bibak, Naseri, & Meftahizade, 2010), is a known herb that has long been used in traditional medicine to treat many disorders (Dabiri, Karbasizade, Club, & Branch, 2013), cultivated in different parts of the world, especially in Western Asia and South-Western Serbia (Abdellatif, Boudjella, Zitouni, & Hassani, 2014). It is found that the main component of *Melissa officinalis* is essential oil (Moradkhani et al., 2010). *Melissa officinalis* is a medicinal plant that plays an important role in the treatment of headaches, indigestion, and renal failure (Abdellatif et al., 2014) gastrointestinal diseases, neurological diseases (Dabiri et al., 2013). Essential oils of *Melissa officinalis* are used as a calming agent to reduce heart rate, antibacterial and to treat neurological disorders, anti-inflammatory, antivirus, antispasmodic, antioxidant, to a neurotherapeutic agent, peripheral analgesic (Dabiri et al., 2013). Essential oils of *M. officinalis* are used as an antitumoral agent as a potential for cancer or for prevention (Moradkhani et al., 2010).
2.1.5 *Lagenaria siceraria* peels

![Image of Lagenaria siceraria](image)

**Figure (2.5) L. siceraria**

<table>
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</table>

**Table (2.5) Classification of *Lagenaria siceraria***

*L. siceraria* (bottle gourd) is a plant belonging to the Cucurbitaceae family. Its length is between 7.9-15.5 cm, elliptical shaped, dark green, bitter taste. These plants originate in India because of the wet climate that helped them grow. The fruits have many benefits used as a heart tonic, diuretic, and are also used to treat diabetes, high blood pressure and flatulence and liver diseases (Payal, Pankti, Manodeep, & Jagadish., 2012). The seeds are used as treatment for headaches by mixing seed oil with castor oil (Upaganlawar & Balaraman, 2009). Fruits are a good source of vitamin B, vitamin C and β-caroten. Also it was found that the fruits of *L. siceraria* have immunomodulatory activity, anticancer activity, antimicrobial activity, cytotoxic activity and antioxidant activity. Studies have indicated that *L. siceraria* fruits are a good source of glucose, fructose and amino acids such as leucines, phenylalanine, valin, tyrosine, alanine, threonine, glutamic acid, serine, aspartic acid (Payal et al., 2012).
2.2 Bacteria The isolated bacteria used in our study are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

2.2.1 *Escherichia coli*

![Figure (2.6) Gram stain of E. coli](image)

*E. coli* is the most common species of the family Enterobacteriaceae and the leading cause of opportunistic infection (Kenneth & Ray, 2004). All members of the family Enterobacteriaceae are facultative, all ferment glucose and reduce nitrates to nitrites and all are oxidase negative (Kenneth & Ray, 2004).

*E. coli* is characterized by gram negative "appear red or pink under microscope", non-sporing bacilli, rod-shaped bacterium (Mahon and Manuselis, 1995). Some of the *E. coli* strains live naturally between aerobic commensal bacteria present in the intestine (Gillespie & Bamford, 2012).

2.2.1.1 *E. coli* infections

*E. coli* is initially considered to be harmless to the colon, and most strains do not cause disease to humans, but may be associated with many diseases of the digestive system, meninges, wounds, urinary tract (Mahon and Manuselis, 1995). Other infections caused by *E. coli* include cholecystitis, peritonitis, septic wounds and bedsores. They may also infect the lower respiratory passages or cause bacteraemia (Gillespie & Bamford, 2012).

2.2.1.2 Antimicrobial Susceptibility

*E. coli* is one of the least likely species of active agents against Enterobacteriaceae due to the frequent occurrence of R plasmids, The strains acquired in hospitals have the ability to resist antibiotics such as tetracycline, erythromycin and amoxicillin. Nitrofurantoin, norflaxacin,
gentamicin and ciprofloxacin were considered suitable for the treatment of *E. coli* (Kibret & Abera, 2011).

### 2.2.2 *Staphylococcus aureus*

![Gram stain of *S. aureus*](image)

**Figure (2.7) Gram stain of *S. aureus***

<table>
<thead>
<tr>
<th>Domain</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>Class</td>
<td>Bacilli</td>
</tr>
<tr>
<td>Order</td>
<td>Bacillales</td>
</tr>
<tr>
<td>Family</td>
<td>Staphylococcaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Staphylococcus</td>
</tr>
<tr>
<td>Species</td>
<td><em>aureus</em></td>
</tr>
</tbody>
</table>

**Table (2.7) Classification of *S. aureus***

*S. aureus* is gram positive bacteria. Its cells are characterized as spherical. The diameter ranging from 0.5 to 1.7 µm, unable to move (non-motile) (Al-refi, 2016) can not form spores (Harris et al., 2002). *S. aureus* is a facultative anaerobe that grows temperature of 37ºC and and pH of 7.5 (Harris et al., 2002). *S. aureus* produces white colonies that tend to shift to a buff-golden color over time (Harris et al., 2002).

*S. aureus* is found mainly on the skin especially in wet areas such as the nose, axillae and thighs (Irving et al., 2006).

#### 2.2.2.1 Infections

*S. aureus* infection ranges from mild to life-threatening, and this type tends to injure the skin and often cause abscesses. *S. aureus* can migrate through the blood stream, infect the heart valves cause endocarditis. The bacteria also affects soft tissue and lung (pneumonia) (Larry M. Bush, 2018).

#### 2.2.2.2 Antimicrobial Susceptibility

Approximately 90% of *S. aureus* resistant to beta-lactam compounds including methicillin, penicillin, oxacillin, and amoxicillin (Kelman et al., 2011).
2.2.3 *Pseudomonas aeruginosa*

*P. aeruginosa* is the type species of the genus *Pseudomonas* (Gillespie & Bamford, 2012).

![Figure (2.8)](image)

**Figure (2.8)** Gram stain of *P. aeruginosa*

<table>
<thead>
<tr>
<th>Domain</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Proteobacteria</td>
</tr>
<tr>
<td>Class</td>
<td>Pseudomonadales</td>
</tr>
<tr>
<td>Order</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>Family</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>Genus</td>
<td>aeruginosa</td>
</tr>
</tbody>
</table>

**Table (2.8)** Classification of *P. aeruginosa*

*P. aeruginosa* is gram-negative bacteria, aerobic bacteria, rod shape and motile have pili (Al-refi, 2016). It has the ability to produce blue and yellow pigments that distinguish them from most other gram negative bacteria (Mohammed, Yossef, & Mohammad, 2014). It has the ability to produce the blue pigment called pyocyanin (Mohammed et al., 2014).

### 2.2.3.1 Epidemiology

*P. aeruginosa* is widely distributed in soil, water, vegetation and can be isolated from the skin, throat, and stool of healthy persons (Baron, 1996).

### 2.2.3.2 Infections

*P. aeruginosa* causes many infections in individuals who have weak immunity, and some types of human infections may be associated with these bacteria such as Urinary tract infections, Ventilator-associated pneumonia, Surgical site infection, Respiratory infections, Ocular infections, Ear infections (external otitis, malignant external otitis). Individuals infected with HIV, diabetes and cystic fibrosis are the most likely to be infected with *P. aeruginosa* (Trautmann et al., 2008).

### 2.2.3.3 Antimicrobial Susceptibility

*P. aeruginosa* is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed, making susceptibility testing essential (Baron, 1996).
2.2.4 *Klebsiella pneumoniae*

![Figure (2.9) Gram stain of *K. pneumoniae*](image)

*K. pneumoniae* is gram-negative, facultative anaerobic, rod-shaped bacterium, encapsulated, lactose-fermenting (Tsai et al., 2010) and belong to the family Enterobacteriaceae (Li, Zhao, Liu, Chen, & Zhou, 2014). *K. pneumoniae* is an opportunistic pathogen that is found widely distributed in mouth and intestines plants and skin, as it is found in medical devices (Tsai et al., 2010).

### 2.2.4.1 Infections

*K. pneumoniae* is the second most common cause of gram-negative bacteremia after *E. coli*. Infection appears in people with weak immune system, the disease affects middle-aged men and older people with diabetes, alcoholism, liver disease, pulmonary disease, renal failure (Tsai et al., 2010).

*K. pneumoniae* is major nosocomial pathogens, which can cause bronchitis, urinary tract, pneumonia, wound infections and soft tissue infection, especially in diabetics, tumor patients, infants (Dong et al., 2015).

### 2.2.4.2 Antimicrobial Susceptibility

*K. pneumoniae* resistant to drugs has become an emerging problem in most countries of the world especially extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase-producing strains (Lin et al., 2016).

### 2.3 Antibiotic resistance

The discovery of antibiotics in the mid-twentieth century led to the possibility of controlling, managing and treating infectious diseases caused by bacteria. Injuries that may be fatal are treatable. From that time on, microbial antibiotics have become important factors to save lives and alleviate the suffering of millions of people. They have also played a role in helping...
organ transplantation and chemotherapy for cancer. But despite these benefits, however, microbes have become more resistant to various antibiotics over time. Antibiotics have become ineffective. All major bacterial infections worldwide have become resistant (Jouda, 2013).

2.4 Biofilm

2.4.1 Biofilm definition
Biofilms are very different in construction and specifications from one peripheral location to another so they are difficult to define clearly. Biofilms are microbial clusters, a matrix of extracellular polymeric materials randomly distributed in a shaped matrix or glycocalyx. Surface free energy (SFE) and surface roughness may contribute to increase rate of microbial adhesion (Al-refi, 2016). Biofilm is characterized by resistance to host defense mechanisms and antibiotic resistance because matrix protects biofilm bacteria (Singh, Parsek, Greenberg, & Welsh, 2002).

2.4.2 Biofilm development
Biofilm formation is a dynamic and complex process influenced by several bacterial and/or environmental factors (Vogeleer, Tremblay, Mafu, Jacques, & Harel, 2014). Biofilms form on biotic and abiotic surfaces both in the environment (Kostakioti, Hadjifrangiskou, & Hultgren, 2013). The formation of a biofilm begins with the attachment of free-floating microorganisms to a surface (O’Toole & Kolter, 1998). Planktonic bacteria reversibly attach to surfaces (Donlan & Costerton, 2002).

*P. aeruginosa* required flagellar-mediated motility to swim toward a surface and transient attachment (O’Toole & Kolter, 1998). *S. aureus* is non-motile and forms a biofilm on the bottom of the well (O’Toole, 2011).
2.5 Previous Studies

In 2017, Iqbal et al. evaluated the antibacterial activity of ethanolic extract of Glycyrrhiza glabra roots against S. aureus and E.coli. It was found that the ethanolic extracts showed mild antibacterial activity against S. aureus and E. coli, respectively (Iqbal et al., 2017).

In 2017, Iqbal et al. evaluated the Antioxidant activity of ethanolic extract of Glycyrrhiza glabra roots against S. aureus and E. coli. It was found that the ethanolic extracts showed highest antioxidant activity of 92% (Iqbal et al., 2017).

In 2010, Fatima Khattak et al. studied the minimal inhibitory concentration (MIC) of crude methanolic extract of Glycyrrhiza glabra roots against different strains of S.aureus and E.coli. It was found that the methanolic extracts un-irradiated (control) samples showed antibacterial effects against these microorganisms (Fatima Khattak & James Simpson, 2010).

In 2010, Nitalikar et al. evaluated the antibacterial potential of ethanolic extract of Glycyrrhiza glabra roots against some pathogenic bacteria. The least activity in terms of zones of growth inhibition was shown by chloroform extract against E. coli (11 mm), P. aeruginosa (14 mm), Bacillus subtilis (16 mm) and S. aureus (18 mm). While the highest level was inhibited by acetone extract against E. coli (15 mm), P. aeruginosa (22 mm), Bacillus subtilis (22 mm) and S. aureus (32 mm) (Nitalikar, Munde, Dhore, & Shikalgar, 2010).

In 2016, Chakotiya et al. evaluated effects of Glycyrrhiza glabra extract on inhibition biofilm formation against P. aeruginosa. The results indicated that Glycyrrhiza glabra extract inhibited the biofilm formation in a range of 70-81% in the concentration range of 50-200 µg/ml-1 (Chakotiya, Tanwar, Narula, & Sharma, 2016).

In 2008, Gupta et al. evaluated the antimicrobial potential of ethanolic Glycyrrhiza glabra root extract against Mycobacterium tuberculosis H37Ra and H37Rv strains. It was found that the ethanolic extracts showed antymycobacterial activity at 500 (mg/ml) (Gupta et al., 2008).

In 2016, Abbas et al. evaluated antimicrobial activity of Glycyrrhiza glabra roots against some pathogenic microorganisms. It was found that 100% methanolic extract showed good activity against E. coli and Bacillus subtilis, showing the highest inhibition zones (33 and 27.5 mm) and the lowest MIC values (9.28 and 30.2 mg/mL), respectively. Least activity was exhibited against Aspergillus niger and Rhizoctonia solani with the smallest inhibition zones (16.5 and 16 mm) and the highest MIC values (150 and 152 mg/mL). 80% methanolic extract showed strong activity against B. subtilis and E. coli with inhibition zones (30 and 28.5 mm) and the lowest MIC values (12.2 and 20.1 mg/ml), respectively. Least activity was exhibited
against *S. aureus* with inhibition zone (19 mm) and the highest MIC value (110 mg/ml) (Abbas et al., 2016).

In 2014, Ghadiri *et al.* evaluated Antimicrobial Activity of *Laurus nobilis* ethanolic extract on *S. aureus* by agar well diffusion method. It found that the Laurus nobilis extract showed antibacterial activity against *S. aureus*. Zone diameter of the inhibition of *Laurus nobilis* extract was similar to the zone diameter of the inhibition of tetracycline (Ghadiri, Ahmadi, Moridikyia, Mahdavi, & Tavakoli, 2014).

In 2014, Chmit *et al.* evaluated Antibacterial activities of essential oil and fatty oil extracted from *Laurus nobilis* against three Gram-positive bacteria (*Staphylococcus epidermidis CIP 444, S. aureus ATCC 25923* and *Enterococcus faecalis ATCC 29212*) and two Gram-negative strains (*E.coli* and *P.aeruginosa*) by minimal inhibitory concentration (MIC). It found that the extracts showed different inhibitory capabilities toward the tested bacterial strains with both Gram-positive bacteria and Gram-negative bacteria being sensitive. Among Gram-negative bacteria, *P.aeruginosa* was the most sensitive; whereas *S. epidermidis* was the most sensitive among the Gram-positive bacterial strains. Fatty oil was more efficient against *S. epidermidis, E. faecalis*, and *P. aeruginosa* than *S. aureus* and *E. coli*. Essential oil was given similar bacterial effects on all gram negative bacteria and gram-positive (Chmit et al., 2014).

In 2013, Ouibrahim *et al.* evaluated Antibacterial activities of essential oil extracted from *Laurus nobilis* against Gram-positive *Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, S. aureus* and *Staphylococcus epidermidis* and Gram-negative *E.coli ATCC 25922, E. coli, Klebsiella oxytoca, K. pneumoniae, P. aeruginosa and Enterobacter sp*). It found that the highest sensitivity was in *Enterobacter sp* of essential oil of *L. nobilis* (22.4 mm of zone inhibition), *K. pneumoniae* (17.6 mm), *S. aureus* (15.6 mm), *S. aureus ATCC 25923* (15 mm) but essential oil of *L. nobilis* no effect was shown against *P. aeruginosa* (Ouibrahim et al., 2013).

In 2012, Naseer unnisa *et al.* evaluated the antibacterial potential of *Malus domestica* against some pathogenic bacteria by agar well diffusion method. It found that *M. domestica* exhibited high activity on *S. aureus* with a zone of inhibition (20 mm), *P. aeruginosa* with a zone of inhibition (16 mm), *E.coli* with a zone of inhibition (15 mm) and *K. pneumoniae* with a zone of inhibition (10 mm), but aqueous extracts of *M. domestica* showed high antibacterial effect on *S. aureus* with a zone of inhibition (18 mm) followed by *E. coli* with a zone of inhibition (13 mm), *P. aeruginosa* with a zone of inhibition (12 mm) and lowest activity was recorded for *K. pneumoniae* (9 mm) (Naseer unnisa, Hajera tabassum, 2012).
In 2006, Abu-shanab et al. evaluated the antibacterial activity of _Melissa officinalis_ by the well diffusion method against _Methicillin-Resistant Staphylococcus aureus_. The result showed high antibacterial effect of ethanolic extracts of _M. officinalis_ on MRSA with a zone of inhibition (15 mm) but less effect of Aquatic extracts on MRSA with a zone of inhibition (12 mm). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the ethanolic extract of _M. officinalis_ were in the range of 3.125 to 12.50 mg/ml and 12.50 to 25.00 mg/ml, respectively (Abu-shanab, Adwan, Jarrar, Abu-hijleh, & Adwan, 2006).

In 2010, Chanda et al. evaluated the antimicrobial activity of methanolic extract of _L. siceraria_ by agar well diffusion method. The result showed that methanolic _L. siceraria_ extract gave the antibacterial activity against _K. pneumonia NCIM2719_ with a zone of inhibition (9 mm) but no antibacterial activity was observed against _S. aureus ATCC29737_ (Chanda, Baravalia, & Kaneria, 2010).

In 2013, Ullah et al. evaluated the antimicrobial activity of methanolic extract of _L. siceraria_ by agar disc diffusion method. The result showed that methanolic _L. siceraria_ extract gave the antibacterial activity against _E. coli_ with a zone of inhibition (6 mm) but no antibacterial activity was observed against _S. aureus_ and _P. aeruginosa_ (Ullah et al., 2013).
Chapter 3 Materials and Methods
Chapter 3
Materials and Methods

3.1 Materials

3.1.1 Bacteria
Pathogenic strains of *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and were obtained from microbiology department of Al-Shifa hospital, and were maintained on Brain Heart Infusion (BHI) agar medium (HiMedia) at 4 °C for further experiments.

3.1.2 Medicinal plants
*Glycyrrhiza glabra*, *L. nobilis, M. officinalis*, *M. domestica* and *L. siceraria* were used in this study and obtained from the local markets as shown in Table (3.1).

Table (3.1) List of medicinal plants tested for their anti-microbial and anti-biofilm activity

<table>
<thead>
<tr>
<th>Binomial Name</th>
<th>Arabic name</th>
<th>Family</th>
<th>Part Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glabra</em></td>
<td>عرق السوس</td>
<td>Leguminosae</td>
<td>roots</td>
</tr>
<tr>
<td><em>L. nobilis</em></td>
<td>الفار</td>
<td>Lauraceae</td>
<td>leaves</td>
</tr>
<tr>
<td><em>M. domestica</em></td>
<td>التفاح الأحمر</td>
<td>Rosaceae</td>
<td>peels</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>بلسم الليمون</td>
<td>Lamiaceae</td>
<td>leaves</td>
</tr>
<tr>
<td><em>L. siceraria</em></td>
<td>القرع الأخضر</td>
<td>Cucurbitaceae</td>
<td>peels</td>
</tr>
</tbody>
</table>

3.1.3 Media and Chemicals
Different of media were required for carrying out this study, Brain Heart Infusion Broth (BHIB) with 1% glucose, Nutrient broth (NB) and Mueller-Hinton agar (HiMedia). Also 70% ethanol was used for extraction process and Dimethyl sulfoxide (DMSO) for dissolving the extracts. 2% crystal violet and 95% methanol for biofilm assay. These media and the solvents were bought from different import companies in Gaza shown in Table (3.2,3.3).

Table (3.2) shows Media that used in this study.

<table>
<thead>
<tr>
<th>Media</th>
<th>Manufacture country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mueller-Hinton agar media</td>
<td>Himedia, Indian</td>
</tr>
<tr>
<td>Nutrients Broth</td>
<td>Himedia, Indian</td>
</tr>
<tr>
<td>Brain Heart Infusion Broth</td>
<td>Himedia, Indian</td>
</tr>
</tbody>
</table>
Table (3.3) shows Chemicals that used in this study.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Manufacture country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrazolium chloride (TTC)</td>
<td>Sigma-Aldrich, USA</td>
</tr>
<tr>
<td>Dimethyl sulfoxide (DMSO)</td>
<td>AppliChem- Germany</td>
</tr>
<tr>
<td>70% Ethanol</td>
<td>Medline- USA</td>
</tr>
<tr>
<td>2% Crystal violet</td>
<td>Sigma-Aldrich, USA</td>
</tr>
<tr>
<td>95% Methanol</td>
<td>Medline- USA</td>
</tr>
</tbody>
</table>

3.1.4 Antibiotics

Antibiotics used include: Aztreonam, Cefazolin, Doxycycline, Rifampicin, Ceftazidime, Tetracyclines, Neomycin, Ciprofloxacin, Amikacin shown in table (3.4).

Table (3.4) shows antibiotics potency that used in this study.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Antibiotics potency</th>
<th>Manufactured by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aztreonam</td>
<td>30 μg</td>
<td>Himedia, Indian</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>30 μg</td>
<td>Himedia, Indian</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10 IU</td>
<td>Liofilchem, Italy</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>30 μg</td>
<td>Liofilchem, Italy</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>30 μg</td>
<td>Himedia, Indian</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>30 μg</td>
<td>Bioanalyse, Turkey</td>
</tr>
<tr>
<td>Neomycin</td>
<td>30 μg</td>
<td>Himedia, Indian</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>30 mcg</td>
<td>Himedia, Indian</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30 mcg</td>
<td>Himedia, Indian</td>
</tr>
</tbody>
</table>

3.2 Methods

3.2.1 Preparation of plant extract

The plant were extracted using microwave assisted method using water or ethanol as solvent.

Ten g of air-dried plant parts powder was taken in 150 ml of distilled water and ten g was taken in 150 ml of 80% ethanol. Then placed inside the microwave for one minute at a temperature of 40 °C and left for a minute to cool. This was repeat for 12 times and then the extract was filtered and allowed to evaporate in oven at 45 °C. The dried extract is dissolved in Dimethyl sulfoxide (DMSO) and stored at -4°C until use (Jameela et al., 2011).
3.2.1.2 Preparation of plant extracts standard concentration

One gram of each extract was dissolved in 5 ml of DMSO. Thus 200 mg / ml of stock was obtained as a standard concentration of extracts. Then extracts were sterilized using 0.22 μm membrane filters (Sulieman et al., 2017).

3.2.2 Plant extracts activity assay

3.2.2.1 Agar disc-diffusion method: Agar disc-diffusion method was followed to determine the antibacterial activity. A suspension of inoculum was introduced to MHA (cooled to 40 - 45°C) swirl gently to mix well. After solidification, sterile filter paper discs approximately 6mm in diameter were impregnated with stock extracts and placed on the surface agar plate. Incubation period of 24h at 37°C for bacteria, and (24-48)h at 37°C for fungi. The antibacterial activity was evaluated by measuring zones of inhibition of microbial growth surrounding the plant extracts (Manoj Kumar, 2010).

3.2.2.2 Well diffusion method assay

An inoculum suspension was swabbed uniformly to solidified 20 mL Mueller-Hinton Agar (MHA) for bacteria. The inoculum was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using Glass Pasteur pipettes. Aliquot of 20 μl from each plant crude extract (200 mg/ml) was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37°C for 24 h. The resulting inhibition zones were measured in millimeters (mm) (Shohayeb & Halawani, 2012).

3.2.2.3 Determination of MIC of plant extracts by Microdilution Method

The extracts were diluted a number of times through a sterile diluent (Mueller-Hinton broth) after were diluted the obtained concentration range was from (100 to 0.1953) mg/ml. 50 μl of the inocula were added to each well except positive control. 50 μl of the inocula is used as a negative control agent. The test plates were incubated at 37°C for 18 h. After 18 h 50 μl of a 0.01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the wells and the plate was incubated for another one hour. Tetrazolium salt is known to be colorless and turns red when biologically active bacteria are grown, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC (Shohayeb & Halawani, 2012).

3.2.2.4 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal activity is determined by the semi-inhibitory concentrations of the extracts used after reading their MICs. Five micron of the semi-inhibitory concentrations and
are placed on the surface of the agar plate. The plates were incubated at 37°C for 24 h (Shohayeb & Halawani, 2012). We determine the concentrations of the extracts where the bacteria grew again and the concentrations that killed the bacteria and did not regenerate again.

3.2.2.5 Microtiter Dish Biofilm Formation Assay.

3.2.2.5.1 Tube method

Biofilms can form in test tubes. For this reason, 0.1 mL of bacterial culture (obtained by adjusting turbidity to 0.5 McFarland standards) was transferred to glass test tubes containing 10 mL NB test tubes which were incubated at 37°C for 72 hours. The medium was then removed and the tubes were washed with distilled water, air dried and biofilm formation were assayed by crystal violet (Al-refi, 2016).

3.2.2.5.2 Tissue culture plate method

Three wells of sterile 96-microtiter U-bottomed plate were filled with 200 μl of bacterial suspension. Negative control contained broth only. After incubation for 24 hrs at 37°C, wells were washed three times with 250 μl of DW. After 15 min, plates were stained for 10 min with 200 μl of 0.1% crystal violet per well (Chmit et al., 2014),(Merghni, Marzouki, Hentati, Aouni, & Mastouri, 2015). Excess stain was removed and rinsed off by placing the plates under running tap water. The plates were air-dried. The adherent cells were resolubilized with 160 μl of 95% (V/V) methanol per well. The optical density (OD) of each was measured at 570 nm (Al-reefi, 2018). Results interpreted according to the followings criteria; OD <0.005 (-), OD 0.500-1.500 (+), OD >1.500 (++)(Al-refi, 2016).

3.2.2.6 The in vitro determination of the effect of plant extract on biofilm formation.

3.2.2.6.1 Biofilm inhibition assay

Organism from fresh agar were inoculated in BHI broth with 1% glucose and incubated for overnight at 37°C in stationary condition. To test plant extracts to inhibit plankton growth, and to determine the concentration that does not affect bacterial growth, (sub-PMIC50) for anti-biofilm assay were used. After were diluted the obtained sub-PMIC50 concentration. 96 well U bottom tissue culture plates were filled with sub-PMIC50 concentration of plant extract and 100μ suspension bacteria plates were incubated for overnight at 37°C. After incubation period well was removed by tapping the plate, washed with DW to remove planktonic bacteria (Al-refi, 2016).
Adherent organisms in plates were stained with crystal violet (0.1% W/V) for 10 min, excess stain was rinsed off by DW and plates were kept for drying, then resuspended each well for 200μL 95% (v/v) methanol, transferred to 96 well-flat bottom plates. Optical density of stained adherent bacteria were determined with a micro-ELISA reader at wavelength of 620 nm (Al-refi, 2016). The percentage inhibition was then compared with the positive control (Mohammadi & Rohloff, 2016).

\[
\text{Percentage inhibition} = \frac{(\text{OD})_{\text{Negative control}} - (\text{OD})_{\text{Experimental}}}{(\text{OD})_{\text{Negative control}}} \times 100%
\]

### 3.2.3 Antibiotic sensitivity test

Antibiotics sensitivity test was performed by placing the specific antibiotic on the surface of the agar plate after culturing the bacteria using sterile forceps. The plates were incubated at 37°C for 18 h. Then the zones of inhibition were measured in millimeter by using ruler.

### 3.2.4 Synergism between plant extract and antibiotics

The bacterial cultures were grown in NB at 37°C. After 4 h of growth, each bacteria was inoculated on the surface of Mueller-Hinton agar plates. The antibiotic discs saturated with (10-20) μl of extracts (at a concentration of 200mg/ml) and are placed on the surface of the agar plate after bacterial culture. The plates were incubated at 37°C for 24 h. Measuring the diameter of cleared zones and comparing them with antibiotics alone (Sulieman et al., 2017).
Chapter 4 Result
Chapter 4

Result

4.1 Evaluation of antibiotics activity

4.1.1 Against *Escherichia coli*

By disc plate method the effectiveness of antibiotics was determined against *E. coli* (Table 4.1). Amikacin was showed the strongest inhibition zone against *E. coli* (18mm) followed by Doxycycline (8mm). While it was resistance against cefazolin, ceftazidime and Rifampicin as shown in (Table 4.1).

4.1.2 Against *Staphylococcus aureus*

As shown in (Table 4.1), Ciprofloxacin was showed the highest inhibition zone (30 mm) followed by tetracycline (20 mm) against *S. aureus*. While no effect was shown of Cefazolin, Aztreonam, and Cefazolin against *S. aureus* as shown in (Table 4.1).

4.1.3 Against *Pseudomonas aeruginosa*

Ciprofloxacin was had the strongest activity against *P. aeruginosa* (31mm) followed by Amikacin (17mm) followed by Aztreonam (15 mm). Neomycin (11mm), Doxycycline (8 mm) as shown in (Table 4.1).

4.1.4 Against *klebsiella pneumonia*

Doxycycline was showed the highest activity against *K. pneumonia* (32 mm) followed by Ciprofloxacin (24mm) followed by Amikacin (19mm). Tetracycline (16mm). Neomycin (14mm) but Aztreonam, Rifampicin, Cefazolin were showed only inhibition zone (7mm). It was found that *K. pneumonia* resistance to ceftazidime as shown in (Table 4.1).
Table (4.1) Evaluation of antibiotics activity

<table>
<thead>
<tr>
<th>Microorganis</th>
<th>Escherichia coli</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas aeruginosa</th>
<th>klebsiella pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td>Inhibition zone (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>8 mm</td>
<td>25 mm</td>
<td>8 mm</td>
<td>32 mm</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Not used</td>
<td>30 mm</td>
<td>31 mm</td>
<td>24 mm</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>7 mm</td>
<td>0 mm</td>
<td>15 mm</td>
<td>7 mm</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0 mm</td>
<td>6 mm</td>
<td>7 mm</td>
<td>7 mm</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0 mm</td>
<td>0 mm</td>
<td>7 mm</td>
<td>0 mm</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>0 mm</td>
<td>0 mm</td>
<td>7 mm</td>
<td>7 mm</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7 mm</td>
<td>20 mm</td>
<td>7 mm</td>
<td>16 mm</td>
</tr>
<tr>
<td>Neomycin</td>
<td>7 mm</td>
<td>19 mm</td>
<td>11 mm</td>
<td>14 mm</td>
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<tr>
<td>Amikacin</td>
<td>18 mm</td>
<td>19 mm</td>
<td>17 mm</td>
<td>19 mm</td>
</tr>
</tbody>
</table>

4.2 Evaluation of plant extracts activity

4.2.1 Against Escherichia coli

4.2.1.1 Well Diffusion Method

Ethanolic extract of G. glabra roots was exhibited the highest effect against E. coli with a zone of inhibition = 25.3 mm, but aquatic extract of G. glabra roots was showed less effect against E. coli with a zone of inhibition = 15.3 mm compared to ethanolic extract of G. glabra roots.

Ethanolic extract of L. nobilis showed a greater effect against E. coli with a zone of inhibition = 22.3 mm than L. nobilis (extracted by water) against E. coli with a zone of inhibition = 20 mm at a concentration of 200 mg/ml as shown in Table 4.2 and Figure (4.2).

Aquatic extract of M. domestica peels showed a strong effect against E. coli with a zone of inhibition = 20 mm than ethanolic extract of M. domestica peels against E. coli with a zone of inhibition = 18.3 mm at a concentration of 200 mg/ml as shown in Table (4.2) and Figure (4.2). M. officinalis did not exhibit any antibacterial effect at a concentration of 200 mg/ml as shown in Table (4.2) and Figure (4.4).
Aquatic extract of *L. siceraria* peels was showed a zone of inhibition = 5.3 mm against *E. coli* as shown in Table (4.2) and Figure (4.3). Ethanolic extract of *L. siceraria* peels did not exhibit any antibacterial effect at a concentration of 200 mg/ml as shown in (Table 4.2) and Figure (4.3).

### 4.1.1.2 Disc Diffusion Method

Aquatic extract of *M. officinalis* was showed less effect against *E. coli* with a zone of inhibition = 8 mm, but *G. glabra* roots, *Lagenaria siceraria* peels, *M. domestica* peels, *L. nobilis* and *M. officinalis* (extracted by water and ethanol) were showed less effect against *E. coli* with a zone of inhibition = 7.3 mm at a concentration of 200 mg/ml as shown in (Table 4.2).

**Table (4. 2)** Antimicrobial activity of plant extracts on *E.coli* by well diffusion and disc diffusion method

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>well diffusion method</th>
<th>disc diffusion method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>W</td>
</tr>
<tr>
<td><em>G. glabra</em> roots</td>
<td>25.3±0.577</td>
<td>15.3±0.577</td>
</tr>
<tr>
<td><em>L. nobilis</em></td>
<td>22.3±0.577</td>
<td>20±0.0</td>
</tr>
<tr>
<td><em>M. domestica</em> peels</td>
<td>18.3±0.577</td>
<td>20±0.0</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L. siceraria</em> peels</td>
<td>0</td>
<td>5.3±0.577</td>
</tr>
<tr>
<td></td>
<td>7.3±0.577</td>
<td>7.3±0.577</td>
</tr>
<tr>
<td></td>
<td>7.3±0.577</td>
<td>7.3±0.577</td>
</tr>
<tr>
<td></td>
<td>7.3±0.577</td>
<td>7.3±0.577</td>
</tr>
<tr>
<td></td>
<td>8±0.0</td>
<td>7.3±0.577</td>
</tr>
</tbody>
</table>

* Antimicrobial Activity Assays.

Method of extraction: E= ethanol, W= water

Values are mean ± SD of three separate experiments.
**Figure (4.1)** The effect of *G. glabra* roots extract (By well diffusion method) against *E. coli*

**Figure (4.2)** The effect of *L. nobilis* and *M. domestica* peels extract (By well diffusion method) against *E. coli*

**Figure (4.3)** The effect of *L. siceraria* peels extract (By Well diffusion method) against *E. coli*

**Figure (4.4)** The effect of *M. officinalis* Extract (By Well diffusion method) against *E. coli*

**Figure (4.5)** The effect of *M. officinalis* extract (By disc diffusion method) against *E. coli*

**Figure (4.6)** The effect of *G. glabra* roots and *L. siceraria peels* extract (By disc diffusion method) against *E. coli*

**Figure (4.7)** The effect of *L. nobilis* and *M. domestica* peels extract (By disc diffusion method) against *E. coli*
4.2.2 Against *Staphylococcus aureus*

4.2.2.1 Well Diffusion Method

Aquatic extract of *M. domestica* peels showed the highest effect against *S. aureus* with a zone of inhibition = 25.3 mm but ethanolic extract of *M. domestica* peels was showed less effect against *S. aureus* with a zone of inhibition = 20 mm compared to aquatic extract of *M. domestica* peels as shown in Table (4.3) and Figure (4.9). *L. siceraria* peels did not exhibit any antibacterial effect at a concentration of 200 mg/ml as shown in Table (4.3) and Figure (4.10).

Ethanol extract of *G. glabra* roots was showed a greater effect against *S. aureus* with a zone of inhibition = 24 mm, but aquatic extract of *G. glabra* roots was showed less effect against *S. aureus* with a zone of inhibition = 23.3 mm compared to ethanolic extract of *G. glabra* roots at a concentration of 200 mg/ml as shown in Table (4.3) and Figure (4.8).

Ethanol extract of *L. nobilis* showed a greater effect against *S. aureus* with a zone of inhibition = 20 mm than aquatic extract of *L. nobilis* against *S. aureus* with a zone of inhibition = 18.3 mm at a concentration of 200 mg/ml as shown in (Table 4.3) and Figure (4.9).

Aquatic extract of *M. officinalis* showed a greater effect against *S. aureus* with a zone of inhibition = 14 mm than ethanolic extract of *M. officinalis* with a zone of inhibition = 12 mm at a concentration of 200 mg/ml as shown in Table (4.3) and Figure (4.11).

4.2.2.2 Disc Diffusion Method

*L. nobilis* (extracted by ethanol and water) showed the highest effect against *S. aureus* with a zone of inhibition = 11 mm as shown in Table (4.3) and Figure (4.13). *M. officinalis* (extracted by ethanol and water) showed low effect against *S. aureus* with a zone of inhibition = 7.3 mm as shown in Table (4.3) and Figure (4.14). *G. glabra* roots (extracted by ethanol and water) was showed low effect against *S. aureus* with a zone of inhibition = 7.3 mm as shown in Table (4.3) and Figure (4.12). *L. siceraria* peels (extracted by ethanol and water) showed very low effect against *S. aureus* with a zone of inhibition = 6.3 mm as shown in Figure (4.12). *M. domestica* peels was shown effect against *S. aureus* with a zone of inhibition = 7.3 mm at a concentration of 200 mg/ml as shown in (Table 4.3) and Figure (4.13).
Table (4. 3) Antimicrobial activity of plant extracts in *S. aureus* by well and disc diffusion method

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>well diffusion method</th>
<th>disc diffusion method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>W</td>
</tr>
<tr>
<td><em>G. glabra</em> roots</td>
<td>24±0.0</td>
<td>23.3±0.577</td>
</tr>
<tr>
<td><em>L. nobilis</em></td>
<td>20±0.0</td>
<td>18.3±0.577</td>
</tr>
<tr>
<td><em>M. domestica</em> peels</td>
<td>20±0.0</td>
<td>25.3±0.577</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>12±0.0</td>
<td>14±0.0</td>
</tr>
<tr>
<td><em>L. siceraria</em> peels</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Antimicrobial Activity Assays.

Method of extraction: E= ethanol, W= water

Values are mean ± SD of three separate experiments
Figure (4.8) The effect of *G. glabra* roots extract (By Well diffusion method) against *S. aureus*

Figure (4.9) The effect of *L. nobilis* and *M. domestica* peels extract (By Well diffusion method) against *S. aureus*

Figure (4.10) The effect of *L. siceraria* peels extract (By Well diffusion method) against *S. aureus*

Figure (4.11) The effect of *M. officinalis* extract (By Well diffusion method) against *S. aureus*

Figure (4.12) The effect of *G. glabra* roots and *L. siceraria* peels extract (By disc diffusion method) against *S. aureus*

Figure (4.13) The effect of *L. nobilis* and *M. domestica* peels extract (By disc diffusion method) against *S. aureus*

Figure (4.14) The effect of *M. officinalis* extract (By disc diffusion method) against *S. aureus*
4.2.3 Against *Pseudomonas aeruginosa*

4.2.3.1 Well Diffusion Method

Aquatic extract of *G. glabra* roots was showed the highest effect against with a *P. aeruginosa* with zone of inhibition = 13.3 mm as shown in (Table 4.4) and Figure (4.15), then aquatic extract of *L. siceraria* peels with zone of inhibition = 9 mm but ehanolic extract of *L. siceraria* peels showed low effect with a zone of inhibition = 6.3 mm Figure (4.17). *M. officinalis, L. nobilis and M. domestica* peels did not show antibacterial activity against *P. aeruginosa* as shown in (Table 4.4).

4.2.3.2 Disc Diffusion Method

Aquatic extract of *G. glabra* roots and *M. officinalis* were showed low effect against *P. aeruginosa* with zone of inhibition = 8 mm as shown in Figure (4.18, 4.21), then aquatic extract of *L. siceraria* peels with zone of inhibition = 7.3 mm as shown in Figure (4.18). *L. nobilis and M. domestica* peels were showed very low effect against *P. aeruginosa* with zone of inhibition = 6.3 mm as shown in Table (4.4) and Figure (4.20).

Ethanolic extract of *G. glabra* roots, *L. siceraria* peels and *M. officinalis* showed a weak effect against *P. aeruginosa* with zone of inhibition = 7.3 mm then Ethanolic extract of *L. nobilis and M. domestica* peels with zone of inhibition = 6.3 mm at a concentration of 200 mg/ml as shown in Table (4.4) and Figure (4.20).

Table (4. 4) Antimicrobial activity of plant extracts on *P. aeruginosa* by well and disc diffusion method

<table>
<thead>
<tr>
<th>plant extracts</th>
<th>Well diffusion method</th>
<th>disc diffusion method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>W</td>
</tr>
<tr>
<td><em>G. glabra</em> roots</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>13.3±0.577</td>
</tr>
<tr>
<td><em>L. nobilis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. domestica</em> peels</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L. siceraria</em> peels</td>
<td>6.3±0.577</td>
<td>9±0.0</td>
</tr>
</tbody>
</table>

* Antimicrobial Activity Assays.

Method of extraction: E= ethanol, W= water

Values are mean ± SD of three separate experiments
Figure (4.15) The effect of *G. glabra* roots extract (By Well diffusion method) against *P. aeruginosa*

Figure (4.16) The effect of *L. nobilis* and *M. domestica* extract (By Well diffusion method) against *P. aeruginosa*

Figure (4.17) The effect of *L. siceraria* peels extract (By Well diffusion method) against *P. aeruginosa*

Figure (4.18) The effect of *G. glabra* roots and *L. siceraria* peels extract (By disc diffusion method) against *P. aeruginosa*

Figure (4.19) The effect of *M. officinalis* extract (By Well diffusion method) against *P. aeruginosa*

Figure (4.20) The effect of *L. nobilis* and *M. domestica* peels extract (By disc diffusion method) against *P. aeruginosa*

Figure (4.21) The effect of *M. officinalis* extract (By disc diffusion method) against *P. aeruginosa*
4.2.4 Against *klebsiella pneumonia*

4.2.4.1 Well Diffusion Method

Ethanolic extract of *G. glabra* roots was showed the highest effect against *K. pneumonia* with a zone of inhibition = 23.3 mm, but *G. glabra* roots (extracted by water) was showed less effect with a zone of inhibition = 9 mm compared to ethanolic extract of *G. glabra* roots as shown in (Table 4.5) and Figure (4.22). *M. officinalis* did not show antibacterial activity at a concentration of 200 mg/ml as shown in (Table 4.5) and Figure (4.24).

Ethanolic extract of *L. nobilis* showed a greater effect against *K. pneumonia* with a zone of inhibition = 17.3 mm than aquatic extract of *L. nobilis* with a zone of inhibition = 15.3 mm at a concentration of 200 mg/ml as shown in (Table 4.5) and Figure (4.23).

Aquatic extract of *M. domestica* peels showed intermediate effect against *K. pneumonia* with a zone of inhibition = 13.3 mm but ethanolic extract of *M. domestica* peels was showed less effect with a zone of inhibition = 10.3 mm at a concentration of 200 mg/ml as shown in (Table 4.5) and Figure (4.23).

Aquatic extract of *L. siceraria* peels showed mild effect against *K. pneumonia* with a zone of inhibition = 11 mm but ethanolic extract of *L. siceraria* peels was showed less effect with a zone of inhibition = 9 mm at a concentration of 200 mg/ml as shown in (Table 4.5) and Figure (4.25).

4.2.4.2 Disc Diffusion Method

Ethanolic extract of *M. domestica* peels and *L. nobilis* were showed the highest effect against *K. pneumonia* with a zone of inhibition = 10.3 mm but ethanolic extract of *L. siceraria* peels, *G. glabra* roots and *M. officinalis* showed mild effect with a zone of inhibition = 9 at a concentration of 200 mg/ml as shown in (Table 4.5).

Aquatic extract of *M. domestica* peels and *L. nobilis* were showed the highest effect against *K. pneumonia* with a zone of inhibition = 10.3 mm as shown in (Table 4.5) and Figure (4.27). then aquatic extract of *G. glabra* roots showed mild effect against *K. pneumonia* with a zone of inhibition = 9 mm as shown in Figure (4.26). Aquatic extract of *M. officinalis* showed low effect against *K. pneumonia* with a zone of inhibition = 8 m as shown in Figure (4.28). Aquatic extract of *L. siceraria peels* showed low effect against *K. pneumonia* with a zone of inhibition = 7.3 mm at a concentration of 200 mg/ml as shown in (Table 4.5) and Figure (4.26).
Table (4. 5) Antimicrobial activity of plant extracts on *K. pneumonia* by well and disc diffusion method

<table>
<thead>
<tr>
<th>plant extracts</th>
<th>Well diffusion method</th>
<th>Disc diffusion method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>W</td>
</tr>
<tr>
<td><em>G. glabra</em> roots</td>
<td>23.3±0.577</td>
<td>9±0.0</td>
</tr>
<tr>
<td><em>L. nobilis</em></td>
<td>17.3±0.577</td>
<td>15.3±0.577</td>
</tr>
<tr>
<td><em>M. domestica</em> peels</td>
<td>10.3±0.577</td>
<td>13.3±0.577</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>L. siceraria</em> peels</td>
<td>9±0.0</td>
<td>11±0.0</td>
</tr>
</tbody>
</table>

* Antimicrobial Activity Assays.
Method of extraction: E= ethanol, W= water
Values are mean ± SD of three separate experiments
Figure (4.22) The effect of *G. glabra* roots extract (By Well diffusion method) against *K. pneumonia*

Figure (4.23) The effect of *L. nobilis* and *M. domestica* peels extract (By Well diffusion method) against *K. pneumonia*

Figure (4.24) The effect of *M. officinalis* extract (By Well diffusion method) against *K. pneumonia*

Figure (4.25) The effect of *L. siceraria* peels extract (By Well diffusion method) against *K. pneumonia*

Figure (4.26) The effect of *G. glabra* roots and *Lagenaria siceraria* Peels extract (By disc diffusion method) against *K. pneumonia*

Figure (4.27) The effect of *L. nobilis* and *M. domestica* peels extract (By disc diffusion method) against *K. pneumonia*

Figure (4.28) The effect of *M. officinalis* extract (By disc diffusion method) against *K. pneumonia*
4.3 Minimum inhibitory concentration of plant extracts alone using Microdilution method

The minimum inhibitory concentration (MIC) results showed that all tested plant extracts were showed antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumonia* with MIC values ranging from 0.19 to 100 mg/ml. The tested extracts showed different levels of antimicrobial activity depending on tested species.

4.3.1 Against *Escherichia coli*

MIC values of all tested plant extracts against *E. coli* are summarized in Table (4.6). The MIC of the ethanolic extracts of *G. glabra* roots and *M. officinalis* was 12.5 mg/ml as shown in Figures (4.29 and 4.32). While *M. domestica* peels and *L. siceraria* peels against *E. coli* was 6.25 mg/ml as shown in Figures (4.31 and 4.33). The MIC of the ethanol extract of *L. nobilis* was the least concentration 3.125 mg/ml against *E. coli* as shown in Table (4.6) and Figure (4.30).

MIC of the water extract of *G. glabra* roots was 12.5 mg/ml as shown in Figure (4.29). While *L. nobilis*, *M. officinalis* and *L. siceraria* peels were 6.25 mg/ml as shown in Table (4.6) and Figures (4.30, 4.32 and 4.33). The MIC for *M. domestica* peels against *E. coli* was the least concentration (1.56 mg/ml) as shown in Table (4.6) and Figure (4.31).

Table (4.6) Minimal inhibitory concentrations (MIC) of the plant extracts on *E. coli*.

<table>
<thead>
<tr>
<th>Plant Solv.</th>
<th>G. glabra roots</th>
<th>L. nobilis</th>
<th>M. domestica peels</th>
<th>M. officinalis</th>
<th>L. siceraria peels</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>12.5±0.0</td>
<td>3.125±0.0</td>
<td>6.25±0.0</td>
<td>12.5±0.0</td>
<td>6.25±0.0</td>
</tr>
<tr>
<td>W</td>
<td>12.5±0.0</td>
<td>6.25±0.0</td>
<td>1.56±0.0</td>
<td>6.25±0.0</td>
<td>6.25±0.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD of two separate experiments
Figure (4.29) The MIC of *G. glabra* roots extract against *E. coli*

Figure (4.30) The MIC of *L. nobilis* extract against *E. coli*

Figure (4.31) The MIC of *M. domestica* peels extract against *E. coli*

Figure (4.32) The MIC of *M. officinalis* extract against *E. coli*

Figure (4.33) The MIC of *L. siceraria* peels extract against *E. coli*
4.3.2 Against *Staphylococcus aureus*

MIC values of all tested plant extracts against *S. aureus* are summarized in Table (4.7). The MIC of the ethanol extract of *M. domestica* peels was 50 mg/ml as shown in Figure (4.36), while *G. glabra* roots, *L. nobilis*, *M. officinalis* and *L. siceraria* peels against *S. aureus* was 25 mg/ml as shown in Figures (4.34, 4.35, 4.37 and 3.38).

MIC of the water extract of *M. domestica* peels was from 100-50 mg/ml as shown in Figure (4.36). *L. nobilis* was 50 mg/ml as shown in Figure (4.35). The MIC for *G. glabra* roots *M. officinalis* and *L. siceraria* peels against *S. aureus* was 25 mg/ml as shown in Table (4.7) and Figures (4.34, 4.37 and 3.38).

Table (4.7) Minimal inhibitory concentrations (MIC) of the plant extracts on *S. aureus*.

<table>
<thead>
<tr>
<th>PLANTS SOLV.</th>
<th><em>G. glabra</em> roots</th>
<th><em>L. nobilis</em></th>
<th><em>M. domestica</em> peels</th>
<th><em>M. officinalis</em></th>
<th><em>L. siceraria</em> peels</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>25±0.0</td>
<td>25±0.0</td>
<td>50±0.0</td>
<td>25±0.0</td>
<td>25±0.0</td>
</tr>
<tr>
<td>W</td>
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<td>100-50</td>
<td>25±0.0</td>
<td>25±0.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD of two separate experiments

**Figure (4.34)** The MIC of *G. glabra* roots extract against *S. aureus*.

**Figure (4.35)** The MIC of *L. nobilis* extract against *S. aureus*.
4.3.3 Against *Pseudomonas aeruginosa*

MIC values of all tested plant extracts against *P. aeruginosa* are summarized in Table (4.8). The MIC of the ethanolic extracts of *L. nobilis, M. domestica* peels and *M. officinalis* was 25 mg/ml as shown in Figures (4.40, 4.41 and 4.42). The MIC for *L. siceraria* peels was from 25-12.5 mg/ml as shown in Figure (4.43). The MIC for *G. glabra* roots against *P. aeruginosa* was the least concentration (12.5-6.25 mg/ml) as shown in Figure (4.39).

MIC of the water extract of *M. domestica* peels was from 50-25 mg/ml. While for *L. nobilis* was 50 mg/ml as shown in Figure (4.41). The MIC for *L. siceraria* peels and *M. officinalis* against *P. aeruginosa* was 25 mg/ml as shown in Figures (4.42 and 4.43). The MIC for *G. glabra* roots against *P. aeruginosa* was the least concentration (12.5mg/ml) as shown in Table (4.8) and Figure (4.39).
Table (4.8) Minimal inhibitory concentrations (MIC) of the plant extracts on *P. aeruginosa*

<table>
<thead>
<tr>
<th>PLANTS SOLV.</th>
<th><em>G. glabra</em> roots</th>
<th><em>L. nobilis</em> peels</th>
<th><em>M. domestica</em> peels</th>
<th><em>M. officinalis</em></th>
<th><em>L. siceraria</em> peels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E</strong></td>
<td>12.5-6.25 (9.37±4.42)</td>
<td>25±0.0</td>
<td>25±0.0</td>
<td>25±0.0</td>
<td>25-12.5 (18.75±8.84)</td>
</tr>
<tr>
<td><strong>W</strong></td>
<td>12.5±0.0</td>
<td>50±0.0</td>
<td>50-25 (37.5±17.7)</td>
<td>25±0.0</td>
<td>25±0.0</td>
</tr>
</tbody>
</table>

Values are mean ± SD of two separate experiments.

Figure (4.39) The MIC of *G. glabra* roots extract against *P. aeruginosa*

Figure (4.40) The MIC of *L. nobilis* extract against *P. aeruginosa*

Figure (4.41) The MIC of *M. domestica* peels extract against *P. aeruginosa*.
4.3.4 Against *klebsiella pneumonia*

MIC values of all tested plant extracts against *K. pneumonia* are summarized in Table (4.9). The MIC of the ethanolic extracts of *G. glabra* roots and *L. nobilis* was 50 mg/ml as shown in Figures (4.44 and 4.45). The MIC for *M. domestica* peels, *M. officinalis* and *L. siceraria* peels was 25 mg/ml as shown in Figures (4.46, 4.47 and 4.48).

MIC of the water extracts of *M. domestica* peels and *M. officinalis* was 50 mg/ml as shown in Figures (4.46 and 4.47). The MIC for *G. glabra* roots, *L. nobilis* and *L. siceraria* peels was 25 mg/ml as shown in Table (4.9) and Figures (4.44, 4.45 and 4.48).

**Table (4.9)** Minimal inhibitory concentrations (MIC) of the plant extracts on *K. pneumonia*

<table>
<thead>
<tr>
<th>PLANTS SOLV.</th>
<th><em>G. glabra</em> roots</th>
<th><em>L. nobilis</em></th>
<th><em>M. domestica</em> peels</th>
<th><em>M. officinalis</em></th>
<th><em>L. siceraria</em> peels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E</strong></td>
<td>50±0.0</td>
<td>50±0.0</td>
<td>25±0.0</td>
<td>25±0.0</td>
<td>25±0.0</td>
</tr>
<tr>
<td><strong>W</strong></td>
<td>25±0.0</td>
<td>25±0.0</td>
<td>50±0.0</td>
<td>50±0.0</td>
<td>25±0.0</td>
</tr>
</tbody>
</table>
Figure (4.44) The MIC of *G. glabra* roots extract against *K. pneumonia*.

Figure (4.45) The MIC of *L. nobilis* extract against *K. pneumonia*.

Figure (4.46) The MIC of *M. domestica* peels extract against *K. pneumonia*.

Figure (4.47) The MIC of *M. officinalis* extract against *K. pneumonia*.

Figure (4.48) The MIC of *L. siceraria* peels extract against *K. pneumonia*. 
4.4 Minimum bactericidal concentration (MBC)
Tested extracts showed different Minimum bactericidal concentration to kill bacteria depending on the tested species.

4.4.1 Against *Escherichia coli*
MBC values of all tested plant extracts against *E. coli* are summarized in Table (4.10). Minimum bactericidal concentration of ethanol and water extract of *M. domestica* peels, *M. officinalis*, *L. siceraria* peels and *L. nobilis* to kill *E. coli* was greater than 200 mg/ml. While the MBC of ethanol and water extract of *G. glabra* roots to kill *E. coli* was 200 mg/ml.

**Table (4.10)** Minimal bactericidal concentrations (MBC) of the plant extracts on *E. coli*

<table>
<thead>
<tr>
<th>plant Solv.</th>
<th>G. glabra roots</th>
<th>L. nobilis</th>
<th>M. domestica Peels</th>
<th>M. officinalis</th>
<th>L. siceraria Peels</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
</tr>
<tr>
<td>W</td>
<td>200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
</tr>
</tbody>
</table>

4.4.2 Against *Staphylococcus aureus*
MBC values of all tested plant extracts against *S. aureus* are summarized in Table (4.11). Minimum bactericidal concentration of ethanol and water extract of *M. domestica* peels, *M. officinalis*, *L. siceraria* peels and *L. nobilis* to kill *S. aureus* was greater than 200 mg/ml as shown in (Table 4.11). MBC of ethanol extract of *G. glabra* roots to kill *S. aureus* was 200 mg/ml as shown in (Table 4.11) and Figure (4.49). While MBC of water extract of *G. glabra* roots to kill *S. aureus* greater than 200 mg/ml.

**Table (4.11)** Minimal bactericidal concentrations (MBC) of the plant extracts on *S. aureus*

<table>
<thead>
<tr>
<th>plants Solv.</th>
<th>G. glabra roots</th>
<th>L. nobilis</th>
<th>M. domestica peels</th>
<th>M. officinalis</th>
<th>L. siceraria peels</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
</tr>
<tr>
<td>W</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
</tr>
</tbody>
</table>
**Figure (4.49)** MBC for ethanolic extract of *G. glabra* roots against *S. aureus*

### 4.4.3 Against *Pseudomonas aeruginosa*

MBC values of all tested plant extracts against *P. aeruginosa* are summarized in Table (4.12). Minimum bactericidal concentration of ethanolic and water extract of *M. domestica* peels, *L. siceraria* peels, *G. glabra* roots and *L. nobilis* to kill *P. aeruginosa* was greater than 200 mg/ml. MBC of aquatic extract of *M. officinalis* to kill *P. aeruginosa* was 200 mg/ml as shown in (Table 4.12) and Figure (4.50). While MBC of ethanolic extract of *M. officinalis* to kill *P. aeruginosa* greater than 200 mg/ml as shown in (Table 4.12).

**Table (4.12)** Minimal bactericidal concentrations (MBC) of the plant extracts on *P. aeruginosa*

<table>
<thead>
<tr>
<th>Solv.</th>
<th><em>G. glabra</em> roots</th>
<th><em>L. nobilis</em> peels</th>
<th><em>M. domestica</em> peels</th>
<th><em>M. officinalis</em></th>
<th><em>L. siceraria</em> peels</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
</tr>
<tr>
<td>W</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>200 mg/ml</td>
<td>&gt;200 mg/ml</td>
</tr>
</tbody>
</table>

**Figure (4.50)** MBC of aquatic extract of *M. officinalis* against *P. aeruginosa*

### 4.4.4 Against *klebsiella pneumonia*

MBC values of all tested plant extracts against *K. pneumonia* are summarized in Table (4.13). Minimum bactericidal concentration of ethanol and water extract of *M. domestica* peels, *L. nobilis* and *G. glabra* roots to kill *K. pneumonia* was greater than 200 mg/ml. MBC
of ethanolic and aquatic extract of *M. officinalis* to kill *K. pneumonia* was 200 mg/ml as shown in (Table 4.13) and Figure (4.53). MBC of ethanol extract of *L. siceraria* peels to kill *K. pneumonia* was 200 mg/ml as shown in Figure (4.51), while MBC of water extract was 100 mg/ml as shown in Figure (4.52).

**Table (4.13)** Minimal bactericidal concentrations (MBC) of the plant extracts on *K. pneumonia*

<table>
<thead>
<tr>
<th>Solv.</th>
<th>plants</th>
<th><em>G. glabra</em> roots</th>
<th><em>L. nobilis</em> peels</th>
<th><em>M. domestica</em> peels</th>
<th><em>M. officinalis</em></th>
<th><em>L. siceraria</em> peels</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>200 mg/ml</td>
<td>200 mg/ml</td>
<td>200 mg/ml</td>
</tr>
<tr>
<td>W</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>&gt;200 mg/ml</td>
<td>200 mg/ml</td>
<td>200 mg/ml</td>
<td>100 mg/ml</td>
</tr>
</tbody>
</table>

**Figure (4.51)** MBC of ethanolic extract of *L. siceraria* peels against *K. pneumonia*

**Figure (4.52)** MBC of aquatic extract of *L. siceraria* peels against *K. pneumonia*

**Figure (4.53)** MBC of ethanolic extract of *M. officinalis* against *K. pneumonia*
4.5 Assessment of biofilm formation.

4.5.1 Tube method
The formation of clear biofilm by *P. aeruginosa* and *S. aureus* was shown to be pigmented with crystal violate as shown in Figure (4.54).

![Figure (4.54) (A) formation of biofilm by *S. aureus* (B) formation of biofilm by *P. aeruginosa*](image)

4.5.2 Screening of the *P. aeruginosa* and *S. aureus* for biofilm formation by tissue culture plate.
Is showing the quantitative measurements of adherent biofilm stained by crystal violate dye using the 96 microtiter plates which is read by an ELISA reader as shown in Figure (4.55).

![Figure (4.55) (A)Anti-biofilm activity of *P. aeruginosa* (B) Anti-biofilm activity of *S. aureus* using the crystal violet assay](image)

4.5.3 Effect of crude extract of Plants on Biofilm formation of *P. aeruginosa* and in vitro.
In addition to testing of the plant extracts for inhibition of planktonic growth, their effect on biofilm formation was also investigated. To ensure a concentration that is not affecting the microbial growth, extract concentrations below (sub-PMIC50) for anti-biofilm assay were used. Crystal violate staining method is easily and widely used to measure both the formation and inhibition of biofilms. *M. domestica* peels ethanolic extract concentration of 25mg/mL is showed 90% inhibition on *P. aeruginosa* biofilm formation.
The ethanolic extracts of *G. glabra* roots, *L. nobili*, *M. officinalis* and and *L. siceraria* peels inhibited *P. aeruginosa* biofilm by 89.6%, 86.8%, 84.3% and 81.5%, respectively as shown in Table (4.14).

The Aquatic extracts of *G. glabra* roots, *L. nobili*, *M. domestica* peels, *M. officinalis* and *Lagenaria siceraria* peels inhibited *P. aeruginosa* biofilm by 87.5%, 88.5%, 86.7%, 83.2% and 89%, respectively as shown in Table (4.14).

**Table (4.14)** Effects of ethanolic and aquatic extracts on biofilm formation and biofilm inhibition of *P. aeruginosa*.

<table>
<thead>
<tr>
<th>Plant Ethanolic extracts</th>
<th>Sub-PMIC50 in mg/Ml</th>
<th>(MBIC50) in mg/mL</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glabra</em> roots</td>
<td>3.123</td>
<td>1.56</td>
<td>89.6%</td>
</tr>
<tr>
<td><em>L. nobili</em></td>
<td>12.5</td>
<td>6.25</td>
<td>86.8%</td>
</tr>
<tr>
<td><em>M. domestica</em> peels</td>
<td>50</td>
<td>25</td>
<td>90%</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>3.123</td>
<td>1.56</td>
<td>84.3%</td>
</tr>
<tr>
<td><em>L. siceraria</em> peels</td>
<td>6.25</td>
<td>3.123</td>
<td>81.5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant Aquatic extracts</th>
<th>Sub-PMIC50 in mg/Ml</th>
<th>(MBIC50) in mg/mL</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glabra</em> roots</td>
<td>6.25</td>
<td>3.123</td>
<td>87.5%</td>
</tr>
<tr>
<td><em>L. nobili</em></td>
<td>25</td>
<td>12.5</td>
<td>88.5%</td>
</tr>
<tr>
<td><em>M. domestica</em> peels</td>
<td>50</td>
<td>25</td>
<td>86.7%</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>25</td>
<td>12.5</td>
<td>83.2%</td>
</tr>
<tr>
<td><em>L. siceraria</em> peels</td>
<td>25</td>
<td>12.5</td>
<td>89%</td>
</tr>
</tbody>
</table>

4.5.4 **Effect of crude extract of Plants on Biofilm formation of *S. aureus* and in vitro.**

In addition to testing of the plant extracts for inhibition of planktonic growth, their effect on biofilm formation was also investigated. To ensure a concentration that is not affecting the microbial growth, extract concentrations below (sub-PMIC50) for anti-biofilm assay were used. Crystal violate staining method is easily and widely used to measure both the formation and inhibition of biofilms. *L. nobili* aquatic extract concentration of 12.5 mg/mL is showed the highest inhibition on *S. aureus* biofilm formation 86.7%. The Aquatic extracts of *L. siceraria* peels, *G. glabra* roots, *M. domestica* peels and *M. officinalis* inhibited *S.aureus* biofilm by 83.1%, 82.9%, 80.6% and 78.3%, respectively as shown in Table (4.15).
The ethanolic extracts of *L. siceraria* peels, *L. nobili*, *M. domestica* peels, *M. officinalis* and *G. glabra* roots inhibited *S. aureus* biofilm by 82%, 81.4%, 77.5%, 77.1.5% and 77.1%, respectively shown in Table (4.15).

**Table (4. 15)** Effects of ethanolic and aquatic extracts on biofilm formation and biofilm inhibition of *S. aureus*.

<table>
<thead>
<tr>
<th>Plant Ethanolic extracts</th>
<th>Sub-PMIC50 in mg/Ml</th>
<th>(MBIC50) in mg/mL</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glabra roots</em></td>
<td>12.5</td>
<td>6.25</td>
<td>77.1%</td>
</tr>
<tr>
<td><em>L. nobili</em></td>
<td>50</td>
<td>25</td>
<td>81.4%</td>
</tr>
<tr>
<td><em>M. domestica</em> peels</td>
<td>50</td>
<td>25</td>
<td>77.5%</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>25</td>
<td>12.5</td>
<td>77.1%</td>
</tr>
<tr>
<td><em>L. siceraria</em> peels</td>
<td>25</td>
<td>12.5</td>
<td>82%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant Aquatic extracts</th>
<th>Sub-PMIC50 in mg/Ml</th>
<th>(MBIC50) in mg/mL</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glabra roots</em></td>
<td>25</td>
<td>12.5</td>
<td>82.9%</td>
</tr>
<tr>
<td><em>L. nobili leaves</em></td>
<td>25</td>
<td>12.5</td>
<td>86.7%</td>
</tr>
<tr>
<td><em>M. domestica</em> peels</td>
<td>50</td>
<td>25</td>
<td>80.6%</td>
</tr>
<tr>
<td><em>M. officinalis</em></td>
<td>50</td>
<td>25</td>
<td>78.3%</td>
</tr>
<tr>
<td><em>L. siceraria</em> peels</td>
<td>25</td>
<td>12.5</td>
<td>83.1%</td>
</tr>
</tbody>
</table>

4.6 Evaluation the Synergistic Effect

4.6.1 The synergistic effect between plant extracts and antibiotics

We evaluated in vitro synergism between extracts of (*G. glabra* roots, *L. nobili*, *M. domestica* peels, *M. officinalis* and *L. siceraria*) and antimicrobial drugs utilized against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumonia* using disk diffusion method.

4.6.1.1 Against *Escherichia coli*

4.6.1.1.1 Ethanolic Extraction and Antibiotics

As shown in Table 4.16. Ethanol extract of *G. glabra* roots has the best synergistic effect with Neomycin (11.7mm) as shown in Figure (4.62) followed by Ceftazidime and Tetracycline with the same effect (10.3mm) on *E. coli* as shown in Figure (4.57 and 4.58). While *G. glabra* roots extract did not exhibit any synergistic effect with Rifampicin and
Amikacin disk against *E. coli*. *G. glabra* roots extract showed antagonistic effect when added on Aztreonam disk (6.3mm) against *E. coli* as shown in Table (4.16).

Ethanolic extract of *L. nobili* showed a potent synergistic effect on *E. coli* with ceftazidime disk (12.3 mm) as shown in Table (4.16). While *L. nobili* extract with Rifampicin disk showed intermediate effect on *E. coli* as shown in Figure (4.56). While *L. nobili* extract did not exhibit any synergistic effect with Cefazolin disk (7.3mm). *L. nobili* extract showed antagonistic effect with Aztreonam disk (6.3mm) against *E. coli* as shown in Table (4.16).

Ethanolic extract of *M. domestica* peels showed a potent synergistic effect on *E. coli* with Doxycycline disk (11.3mm) as shown in Figure (4.61) followed by Neomycin disk (9.3 mm). While *M. domestica* peels extract showed antagonistic effect with on Aztreonam, Cefazolin, Ceftazidime and Rifampicin disk against *E. coli* as shown in Table (4.16).

Ethanolic extract of *M. officinalis* showed a potent synergistic effect with Amikacin disk (27mm) as shown in Figure (4.65) followed by Neomycin and Tetracycline disk with same effect (12mm) as shown in Figure (4.59). *M. officinalis* extract showed antagonistic effect with Rifampicin and Ceftazidime disk (6.3mm) against *E. coli* as shown in Table (4.16).

Finally the synergistic effect of Ethanolic extract for *L. siceraria* with Aztreonam disk (14.7mm) was the highest synergistic effect on *E. coli* followed by Amikacin disk that showed strong synergistic effect with this extract (23.3mm) on *E. coli*. While *L. siceraria* peels extract did not exhibit any synergistic effect with Neomycin disk (7mm) against *E. coli*. *L. siceraria* peels extract showed antagonistic effect when added on Tetracycline disk (6.3mm) as shown in Table (4.16).
Table (4.16) Synergism Between Antibiotics and Ethanolic Extracts of Plant against E. coli

<table>
<thead>
<tr>
<th>Anti.</th>
<th>Antibiotics alone</th>
<th>G. glabra roots</th>
<th>L. nobili</th>
<th>M. domestica peels</th>
<th>M. officinalis</th>
<th>L. siceraria peels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ex7.3±0.577</td>
<td>Ex7.3±0.577</td>
<td>Ex7.3±0.577</td>
<td>Ex7.3±0.577</td>
<td>Ex7.3±0.577</td>
<td>Ex7.3±0.577</td>
</tr>
<tr>
<td>CAZ30</td>
<td>7</td>
<td>10.3±0.577</td>
<td>12.3±0.577</td>
<td>6.3±0.577</td>
<td>6.3±0.577</td>
<td>9.3±0.577</td>
</tr>
<tr>
<td>RIF</td>
<td>7</td>
<td>7.3±0.577</td>
<td>10.3±0.577</td>
<td>6.3±0.577</td>
<td>6.3±0.577</td>
<td>9.3±0.577</td>
</tr>
<tr>
<td>N30</td>
<td>7</td>
<td>11.7±0.577</td>
<td>9.3±0.577</td>
<td>9.3±0.577</td>
<td>12±0.0</td>
<td>7±0.0</td>
</tr>
<tr>
<td>TE30</td>
<td>7</td>
<td>10.3±0.577</td>
<td>7.7±0.577</td>
<td>6.3±0.577</td>
<td>12±0.0</td>
<td>6.3±0.577</td>
</tr>
<tr>
<td>DO</td>
<td>8</td>
<td>10.3±0.577</td>
<td>9.3±0.577</td>
<td>11.3±0.577</td>
<td>9.3±0.577</td>
<td>10.3±0.577</td>
</tr>
<tr>
<td>ATM</td>
<td>7</td>
<td>6.3±0.577</td>
<td>6.3±0.577</td>
<td>6.3±0.577</td>
<td>9.3±0.577</td>
<td>14.7±0.577</td>
</tr>
<tr>
<td>AK</td>
<td>18</td>
<td>18±0.0</td>
<td>18.7±0.577</td>
<td>19±0.0</td>
<td>27±0.0</td>
<td>23.3±1.52</td>
</tr>
<tr>
<td>KZ</td>
<td>0</td>
<td>6.3±0.577</td>
<td>7.3±0.577</td>
<td>0</td>
<td>8±1.0</td>
<td>6.3±0.577</td>
</tr>
</tbody>
</table>

Ceftazidime (CAZ30), Rifampicin (RIF), Neomycin (N30), Tetracycline (TE30), Doxycycline (DO), Aztreonam (ATM), Amikacin (AK), Cefazolin (KZ).

Values are mean ± SD of three separate experiments

4.6.1.1.2 Aquatic Extraction and Antibiotics

As shown in Table 4.17. Aquatic extract of G. glabra roots has the best synergistic effect with Doxycycline disk with zone inhibition (12 mm) followed by Ceftazidime with intermediate zone inhibition (10.3 mm) on E. coli as shown in Figure (4.57). While G. glabra roots extract did not exhibit any synergistic effect with Rifampicin and Tetracycline disk (7 mm) against E. coli. G. glabra roots extract showed antagonistic effect when added on Aztreonam and Neomycin disk (6.3 mm) against E. coli as shown in Table (4.17).

Aquatic extract of L. nobili showed intermediate synergistic effect on E. coli with Tetracycline disk (10.3 mm) as shown in Figure (4.58). While the same of extract showed equal synergistic effect with Doxycycline and Amikacin disk (11 mm, 20 mm, Respectively) as shown in Figure (4.63 and 4.64). L. nobili extract exhibited antagonistic effect with Aztreonam, Ceftazidime and Cefazolin disk (6.3 mm) disk against E. coli as shown in Table (4.17).
Aquatic extract of *M. domestica* peels showed a good synergism effect on *E. coli* with Tetracycline and Doxycycline disk (10 mm, 10.3mm, Respectively) as shown in Figure (4.61 and 4.63). While *M. domestica* peels extract did not exhibit any synergistic effect when added on Rifampicin disk (7.3mm) against *E. coli*. *M. domestica* peels extract showed antagonistic effect with Aztreonam, Neomycin, Ceftazidime disk with zone inhibition (6.3mm) on *E. coli* as shown in Table (4.17).

Aquatic extract of *M. officinalis* had a synergistic effect with Amikacin disk (21.3mm) followed by Tetracycline, Aztreonam and Cefazolin that exhibit the same effect with this extract (10mm, 10.3mm, 10.3mm, Respectively) as shown in Figure (4.59, 4.60 and 4.60). While *M. officinalis* extract exhibited antagonistic effect when added on Rifampicin and Ceftazidime and disk (6.3mm) against *E. coli* as shown in Table (4.17).

Finally the synergistic effect of Aquatic extract for *L. siceraria* peels with Ceftazidime disk (12mm) was the highest synergistic effect on *E. coli* Figure (4.57). Aquatic extract of *L. siceraria* peels exhibit the same synergistic effect with both of Amikacin and Doxycycline (20mm,10.3mm, Respectively) against *E. coli*. While *L. siceraria* peels extract did not exhibit any synergistic effect with Cefazolin disk (7mm) against *E. coli*. *L. siceraria* peels extract showed antagonistic effect with Tetracycline and Aztreonam disk (6.3mm) on *E. coli*. 
Table (4. 17) Synergism Between Antibiotics and Aquatic Extracts of Plant against *E. coli*

<table>
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<tr>
<th>Anti.</th>
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*Ceftazidime (CAZ30), Rifampicin (RIF), Neomycin (N30), Tetracycline (TE30), Doxycycline (DO), Aztreonam (ATM), Amikacin (AK), Cefazolin (KZ).*

Values are mean ± SD of three separate experiments.

**Figure (4.56)** The effect of *L. nobili* extract and *L. siceraria* peels with *Rifampicin* against *E. coli*

**Figure (4.57)** The effect of *G. glabra* roots extract and *L. siceraria* peels with *Ceftazidime* against *E. coli*
**Figure (4.58)** The effect of *L. nobili* extract and *G. glabra* roots with *Tetracycline* against *E. coli*

**Figure (4.59)** The effect of *M. officinalis* with *Neomycin* and *Tetracycline* against *E. coli*

**Figure (4.60)** The effect of *M. officinalis* with *Aztreonam* and *Cefazolin* against *E. coli*

**Figure (4.61)** The effect of *L. nobili* extract and *M. domestica* peels with *Tetracycline* against *E. coli*

**Figure (4.62)** The effect of *G. glabra* roots extract and *L. siceraria* peels with *Neomycin* against *E. coli*

**Figure (4.63)** The effect of *L. nobili* extract and *M. domestica* peels with *Doxycycline* against *E. coli*

**Figure (4.64)** The effect of *L. nobili* extract and *M. domestica* peels with *Amikacin* against *E. coli*

**Figure (4.65)** The effect of *M. officinalis* extract with *Amikacin* against *E. coli*
4.6.1.2 Against *Staphylococcus aureus*

4.6.1.2.1 Ethanolic Extraction and Antibiotics

As shown in Table 4.18. Ethanolic extract of *G. glabra* roots showed a potent synergistic effect on *S. aureus* with Cefazolin, Aztreonam and ceftazidime disk (14.3 mm) as shown in Figure (4.66, 4.66 and 4.69) followed by Doxycycline with zone inhibition (29 mm) as shown in Figure (4.72A). *G. glabra* roots extract gave the same synergistic effect when added to Tetracycline and Rifampicin disk (26 mm, 12 mm, Respectively) as shown in Figure (4.68 and 4.70). While *G. glabra* roots extract did not exhibit any synergistic effect with Amikacin disk against *S. aureus*. *G. glabra* roots extract showed antagonistic effect effect when added on Neomycin disk (14.3 mm) and Ciprofloxacin disk (25 mm) against *S. aureus* as shown in Table (4.18).

Ethanolic extract of *The L. nobili* showed a potent synergistic effect on *S. aureus* with most antibiotics. The strongest when added on Tetracycline disk (32 mm) followed by Neomycin (26 mm) followed by Doxycycline disk (29 mm). While *L. nobili* extract exhibited antagonistic effect when added on Ciprofloxacin disk (29.3 mm) and Cefazolin disk (10 mm) against *S. aureus* as shown in Table (4.18).

Ethanolic extract of *M. domestica* peels showed a potent synergistic effect on *S. aureus* with most antibiotics. The strongest with Tetracycline disk (32 mm) followed by Aztreonam disk (13 mm). While *M. domestica* peels extract with Doxycycline and Ceftazidime disk showed strong effect with zone inhibition (30.3 mm, 12 mm, Respectively) on *S. aureus*. While *M. domestica* peels extract did not exhibit any synergistic effect when added on Ciprofloxacin disk (30 mm) and Amikacin disk (19 mm) against *S. aureus* as shown in Table (4.18).

Ethanolic extract of *M. officinalis* a potent synergistic effect on *S. aureus* with most antibiotics. The strongest with Tetracycline disk (30.7 mm) followed by Ceftazidime, Rifampicin and Doxycycline disk (11.3 mm, 10.3 mm, 29 mm, Respectively) followed by Neomycin and Amikacin disk (21.3 mm). While *M. officinalis* extract showed antagonistic effect when added on Ciprofloxacin disk (29 mm) against *S. aureus* as shown in Table (4.18).

Finally the synergistic effect of *L. siceraria* peels extract with Ceftazidime (15.3 mm) was the highest synergistic effect on this bacteria as shown in Figure (4.69) followed by Tetracycline
disk that showed a potent inhibition zone (27.7 mm) as shown in Figure (4.68). While *L. siceraria* peels extract did not exhibit any synergistic effect when with Cefazolin disk (6 mm) and Ciprofloxacin disk (30 mm) against *S. aureus* as shown in Table (4.18).

**Table (4.18) Synergism Between Antibiotics and Ethanolic Extracts of Plant against *S. aureus.***

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**Ceftazidine (CAZ30), Rifampicin (RIF), Neomycin (N30), Tetracycline (TE30), Doxycycline (DO), Ciprofloxacin (CIP), Aztreonam (ATM), Amikacin (AK), Cefazolin (KZ).**

Values are mean ± SD of three separate experiments

**4.6.1.2 Aquatic Extraction and Antibiotics**

As shown in Table 4.19. Aquatic extract of *G. glabra* roots exhibit the strongest synergistic effect on *S. aureus* with Tetracycline disk (30.7 mm) as shown in Figure (4.68). Amikacin and Rifampicin disk showed low effect with this extract (20.7 mm, 8 mm, Respectively) as shown in Table (4.19). While *G. glabra* roots extract did not exhibit any synergistic effect with Cefazolin disk (7 mm), Aztreonam (7 mm) and Ceftazidine disk (7 mm) against *S. aureus*. While *G. glabra* roots extract showed antagonistic effect effect with Neomycin disk (18.3 mm), Doxycycline disk (24 mm) and Ciprofloxacin disk (29 mm) against *S. aureus* as shown in Table (4.19).
Aquatic extract of *L. nobili* exhibit the strongest synergistic effect on *S. aureus* with Tetracycline disk (33.7 mm) followed by Neomycin and Doxycycline disk (25 mm, 30.7mm, Respectively) that showed the same synergistic effect on *S. aureus*. While *L. nobili* extract showed antagonistic effect with Ceftazidime disk (10mm) and Cefazolin disk (10 mm) against *S. aureus*. The *L. nobili* extract did not exhibit any synergistic effect when added on Ciprofloxacin disk (30 mm) against *S. aureus* as shown in Table (4.19).

Aquatic extract of *M. domestica* peels showed a potent synergistic effect on *S. aureus* with most antibiotics. The strongest with Doxycycline disk (48.7 mm) as shown in Figure (4.73A) followed by Tetracycline disk (30.7mm). *M. domestica* peels extract gave the same synergistic effect when added to Neomycin and Amikacin disk (24.7mm, 25mm, Respectively) against *S. aureus* as shown in Table (4.19) and Figure (4.73C).

Aquatic extract of *M. officinalis* showed the best synergistic effect on *S. aureus* with Tetracycline disk (29 mm) followed by Ceftazidime disk (13.3mm) as shown in Table (4.19) followed by Doxycycline disk (29mm) as shown in Table (4.19) and Figure (4.72B). *M. officinalis* extract exhibited antagonistic effect with Cefazolin disk, Neomycin and Aztreonam disk against *S. aureus* as shown in Table (4.19).

Aquatic extract for *L. siceraria* peels showed the strongest synergistic effect with Tetracycline (28mm) as shown in Figure (4.68) followed by Rifampicin disk that showed a potent synergistic effect with with extract (12mm) on *S. aureus* as shown in Figure (4.70) *L. siceraria* peels extract gave the same synergistic effect with Aztreonam and Doxycycline disk (8mm, 27.3mm, Respectively) on *S. aureus* as shown in Figure (4.67 and 4.72A) followed by Neomycin, Ciprofloxacin and Amikacin disk (20mm, 31mm, 20mm, Respectively ).While *L. siceraria* peels extract did not exhibit any synergistic effect when added on Cefazolin disk (6.3mm) against *S. aureus* as shown in Table (4.19).
Table (4.19) Synergism Between Antibiotics and Aquatic Extracts of Plant against \textit{S. aureus}.

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\textit{Ceftazidime (CAZ30), Rifampicin (RIF), Neomycin (N30), Tetracycline (TE30), Doxycycline (DO), Ciprofloxacin (CIP), Aztreonam (ATM), Amikacin (AK), Cefazolin (KZ).}

Values are mean ± SD of three separate experiments.

\textbf{Figure (4.66)} The effect of \textit{G. glabra} roots extract with Aztreonam and Cefazolin against \textit{S. aureus}.

\textbf{Figure (4.67)} The effect of \textit{L. siceraria} peels extract with Aztreonam against \textit{S. aureus}. 
**Figure (4.68)** The effect of *L. siceraria* peels and *G. glabra* roots extract with *Tetracycline* against *S. aureus*

**Figure (4.69)** The effect of *L. siceraria* peels and *G. glabra* roots extract with *Ceftazidime* against *S. aureus*

**Figure (4.70)** The effect of *L. siceraria* peels and *G. glabra* roots extract with *Rifampicin* against *S. aureus*

**Figure (4.71)** The effect of *L. nobili* extract and *M. domestica* peels with *Rifampicin* against *S. aureus*

**Figure (4.72)** (A) The effect of *L. siceraria* peels and *G. glabra* roots extract with *Doxycycline*, (B) *M. officinalis* extract with *Doxycycline*, (C) *M. domestica* peels extract with *Doxycycline* against *S. aureus*.

**Figure (4.73)** (A) The effect of *L. nobili* extract with *Doxycycline*, (B) *M. officinalis* extract with *Amikacin*, (C) *M. domestica* peels and *L. nobili* extract with *Amikacin* against *S. aureus.*
4.6.1.3 Against *Pseudomonas aeruginosa*

### 4.6.1.3.1 Ethanolic Extraction and Antibiotics

As shown in Table 4.20. Ethanolic extract of *G. glabra* roots extract the same synergistic effect with both of Rifampicin, Cefazolin disk (10.3mm) as shown in Figure (4.75, 4.76) and ceftazidime on *P. aeruginosa*. While *G. glabra* roots extract did not exhibit a synergistic effect with Doxycycline disk (8mm). *G. glabra* roots extract exhibited antagonistic effect with Neomycin, Aztreonam and Amikacin disk against *P. aeruginosa* as shown in Table (4.20).

Ethanolic extract of *L. nobili* extract showed a potent synergistic effect on *P. aeruginosa* with ceftazidime disk (13.3mm) as shown in Figure (4.74) followed by Doxycycline disk (12mm). While *L. nobili* extract did not exhibit a synergistic effect with tetracycline and Amikacin disk. *L. nobili* extract showed antagonistic effect with Aztreonam disk against *P. aeruginosa* as shown in Table (4.20).

Ethanolic extract of *M. domestica* peels has the strongest synergistic effect on *P. aeruginosa* with Doxycycline disk (17.3mm), followed by ceftazidime disk (10.3mm) that gave a potent synergistic effect on *P. aeruginosa* as shown in Figure (4.74). While *M. domestica* peels extract did not exhibit any synergistic effect with tetracycline disk (7mm) on *P. aeruginosa*. *M. domestica* peels extract showed antagonistic effect with Aztreonam and Neomycin disk on *P. aeruginosa* as shown in Table (4.20).

Ethanolic extract of *M. officinalis* showed synergistic effect with different antibiotics. The highest synergistic effect to this extract was with Doxycycline disk (14mm) as shown in Figure (4.83) followed by Rifampicin disk that gave intermediate effect with zone inhibition (11.3mm) as shown in Figure (4.82). *M. officinalis* extract exhibited antagonistic effect with Aztreonam and Amikacin disk on *P. aeruginosa* as shown in Table (4.20).

Finally the synergistic effect of Ethanolic extract for *L. siceraria* peels with Doxycycline disk (14mm) exhibits the highest synergistic effect on this bacteria as shown in Figure (4.80) followed by Ciprofloxacin disk (34.3mm) as shown in Figure (4.79) followed by Rifampicin disk (10.3mm) as shown in Figure (4.75). While *Lagenaria siceraria peels* extract did not exhibit any synergistic effect when added on tetracycline disk (7mm) on *P. aeruginosa*.
Lagenaria siceraria peels extract showed antagonistic effect with Aztreonam disk against P. aeruginosa as shown in Table (4.20).

Table (4. 20) Synergism Between Antibiotics and Ethanolic Extracts of Plant against P. aeruginosa.

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Ceftazidime (CAZ30), Rifampicin (RIF), Neomycin (N30), Tetracycline (TE30), Doxycycline (DO), Ciprofloxacin (CIP), Aztreonam (ATM), Amikacin (AK), Cefazolin (KZ).

Values are mean ± SD of three separate experiments

4.6.1.3.2 Aquatic Extraction and Antibiotics

Aquatic Extract of G. glabra roots showed a potent synergistic effect on P. aeruginosa with Ciprofloxacin disk (35mm) as shown in Figure (4.79) followed by Rifampicin disk (11.3mm) as shown in Figure (4.75). While G. glabra roots extract did not exhibit any synergistic effect with Amikacin (17mm) and tetracycline disk (7mm). G. glabra roots extract showed antagonistic effect with Doxycycline disk on P. aeruginosa as shown in Table (4.21).

Aquatic Extract of L. nobili showed a potent synergistic effect synergistic effect on P. aeruginosa with Doxycycline disk (17.3mm) followed by ceftazidime disk (11.3mm) as shown in Figure (4.74) followed by Ciprofloxacin disk (34.3mm) as shown in Figure (4.78).
While *L. nobili* extract did not exhibit any synergistic effect with tetracycline and Rifampicin disk (7mm), *L. nobili* leaf extract showed antagonistic effect when added on Aztreonam disk against *P. aeruginosa* as shown in Table (4.21).

Aquatic Extract of *M. domestica* peels exhibited the strongest synergistic effect on *P. aeruginosa* with Doxycycline disk (19.7mm) followed by ceftazidime and Ciprofloxacin disk (11.3mm, 35mm, Respectively) as shown in Figure (4.74 and 4.78). While *M. domestica* peels did not exhibit any synergistic effect with tetracycline disk (7mm) against *P. aeruginosa*. *M. domestica* peels extract showed antagonistic effect with Cefazolin disk against *P. aeruginosa* as shown in Table (4.21).

Aquatic Extract of *M. officinalis* showed a synergistic effect with different antibiotics. The highest synergistic effect to this extract was with Doxycycline disk (16.3mm) as shown in Figure (4.83). *M. officinalis* extract showed intermediate effect with Rifampicin and ceftazidime disk (11.3mm) as shown in Figure (4.82). While *M. officinalis* extract did not exhibit any synergistic effect with Ciprofloxacin disk (31mm) and Cefazolin disk (8mm) on *P. aeruginosa*. *M. officinalis* extract showed different levels of antagonistic effect when added on Aztreonam, tetracycline, Neomycin and Amikacin disk against *P. aeruginosa* as shown in Table (4.21).

Finally the synergistic effect of Aquatic Extract for *L. siceraria* peels with tetracycline disk (12mm) was the highest synergistic effect on *P. aeruginosa* as shown in Figure (4.77), followed by Rifampicin disk (11.3mm) as shown in Figure (4.75). This extract showed intermediate synergistic effect with cefazolin and ceftazidime against *P. aeruginosa* as shown in Figure (4.76 and 4.81). While *L. siceraria* peels extract did not exhibit any synergistic effect when added on Neomycin disk (11mm) against *P. aeruginosa*. *L. siceraria* peels extract showed different levels of antagonistic effect when added on Aztreonam, Doxycycline, Ciprofloxacin and Amikacin disk against *P. aeruginosa* as shown in Table (4.21).
Table (4. 21) Synergism Between Antibiotics and Aquatic Extracts of Plant against *P. aeruginosa*.

<table>
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<th>M. officinalis</th>
<th>L. siceraria peels</th>
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<td>6±0.0</td>
<td>8±0.0</td>
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</tr>
</tbody>
</table>

Ceftazidime (CAZ30), Rifampicin (RIF), Neomycin (N30), Tetracycline (TE30), Doxycycline (DO), Ciprofloxacin (CIP), Aztreonam (ATM), Amikacin (AK), Cefazolin (KZ).

Values are mean ± SD of three separate experiments.

**Figure (4.74)** The effect of *L. nobili* extract and *M. domestica* peels with Cefazidime extract against *P. aeruginosa*

**Figure (4.75)** The effect of *G. glabra* roots extract and *L. siceraria* peels with Rifampicin against *P. aeruginosa*
Figure (4.76) The effect of *G. glabra* roots extract and *L. siceraria* peels with *Cefazolin* against *P. aeruginosa*

Figure (4.77) The effect of *G. glabra* roots extract and *L. siceraria* peels with *Tetracycline* against *P. aeruginosa*

Figure (4.78) The effect of *L. nobili* extract and *M. domestica* peels with *Ciprofloxacin* against *P. aeruginosa*

Figure (4.79) The effect of *G. glabra* roots extract and *L. siceraria* peels with *Ciprofloxacin* against *P. aeruginosa*

Figure (4.80) The effect of *L. siceraria* peels with *Doxycycline* against *P. aeruginosa*

Figure (4.81) The effect of *L. siceraria* peels with *Cefazidime* against *P. aeruginosa*

Figure (4.82) The effect of and *M. officinalis* extract with *Rifampicin* against *P. aeruginosa*

Figure (4.83) The effect of and *M. officinalis* extract with *Doxycycline* against *P. aeruginosa*
4.6.1.4 Against *klebsiella pneumonia*

4.6.1.4.1 Ethanolic Extraction and Antibiotics

As shown in Table 4.22. Ethanolic Extract of *G. glabra* roots showed a potent synergistic effect on *K. pneumonia* with ceftazidime disk (13.3mm) as shown in Figure (4.84). This extract showed the same synergistic effect with Cefazolin and Rifampicin disk (11.3mm) as shown in Figure (4.85 and 4.94). *G. glabra roots* extract showed antagonistic effect when with Tetracycline, Doxycycline, Amikacin and Neomycin disk against *K. pneumonia* as shown in Table (4.22).

Ethanolic Extract of The *L. nobili* has the highest synergistic effect on *K. pneumonia* when added on Doxycycline disk (37.3mm) as shown in Figure (4.92). While *L. nobili* extract did not exhibit any synergistic effect when added on Rifampicin, Tetracycline and Ceftazidime disk on *K. pneumonia*. This extract showed antagonistic effect when added on Neomycin and Ciprofloxacin disk against *K. pneumonia* as shown in Table (4.22).

Ethanolic Extract of *M. domestica* peels extract has the best synergistic effect on *K. pneumonia* when added on Amikacin disk (23.3mm) as shown in Table (4.22). *M. domestica* peels showed low synergistic effect with Neomycin as shown in Figure (4.86) and Ciprofloxacin disk on *K. pneumonia* as shown in Figure (4.97). This extract did not exhibit a synergistic effect when added on Aztreonam disk (10.3mm). *M. domestica* peels extract showed antagonistic effect when added on Cefazolin disk (6mm) on *K. pneumonia* as shown in Table (4.22).

Ethanolic Extract of *M. officinalis* had a synergistic effect with different antibiotics. The highest synergistic effect to this extract with Doxycycline disk (48.7mm) as shown in Figure (4.93). *M. officinalis* showed a potent synergistic effect with Aztreonam and Amikacin disk (12mm, 24.3mm, Respectively) as shown in Figure (4.87 and 4.90). *M. officinalis* extract showed antagonistic effect with Neomycin and Rifampicin disk against *K. pneumonia* as shown in Table (4.22).
Ethanolic Extract of *L. siceraria* peels with Neomycin disk (25mm) was the highest synergistic effect on this bacteria as shown in Figure (4.87 and 4.88). It had the same synergistic effect with both of Rifampicin and Tetracycline disk Figure (4.91)(11.3mm, 20.3mm, Respectively) against *K. pneumonia*. While *L. siceraria* peels extract showed antagonistic effect when added on Aztreonam and Ciprofloxacin disk against *K. pneumonia* as shown in Table (4.22).

**Table (4.22) Synergism Between Antibiotics and Ethanolic Extracts of Plant against *K. pneumonia*.

<table>
<thead>
<tr>
<th>Anti.</th>
<th><strong>Antibiotic alone</strong></th>
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Ceftazidime (CAZ30), Rifampicin (RIF), Neomycin (N30), Tetracycline (TE30), Doxycycline (DO), Ciprofloxacin (CIP), Aztreonam (ATM), Amikacin (AK), Cefazolin (KZ).

Values are mean ± SD of three separate experiments.

**4.6.1.4.2 Aquatic Extraction and Antibiotics**

Aquatic extract of *G. glabra* roots has the best synergistic effect on *K. pneumonia* when added on ceftazidime disk (12mm) as shown in Figure (4.84) followed by Rifampicin disk (12mm). While *G. glabra* roots extract did not show a synergistic effect when added on Amikacin disk (19mm). *G. glabra* roots extract showed antagonistic effect when added on Doxycycline and Neomycin disk against *K. pneumonia* as shown in Table (4.23).
Aquatic extract of *L. nobili* showed low synergistic effect on *K. pneumonia* with some antibiotics as shown in Table (4.23). This extract did not exhibit a synergistic effect when added on Aztreonam and Rifampicin disk (10.3mm). *L. nobili* extract showed antagonistic effect when added on Doxycycline disk on *K. pneumonia* as shown in Table (4.23).

Aquatic extract of *M. domestica* peels showed a potent synergistic effect on *K. pneumonia* with Ciprofloxacin and Tetracycline disk (20.3mm,28.3mm, Respectively) as shown in Figure (4.97 and 4.89). While *M. domestica* peels extract did not exhibit any synergistic effect with Aztreonam, Neomycin and Rifampicin disk (10.3mm,14mm and10.3mm, Respectively) against *K. pneumonia*. This extract showed antagonistic effect when added on Cefazolin disk against *K. pneumonia* as shown in Table (4.23).

Aquatic extract of *M. officinalis* showed the highest synergistic effect to this extract was with Aztreonam disk (12.3mm) as shown in Figure (4.87) and Rifampicin disk (12mm) as shown in Figure (4.95). This extract showed low synergistic effect with other antibiotics exclude Tetracycline disk (16mm) which did not show a synergistic effect with this extract. *M. officinalis* extract showed antagonistic effect with Ciprofloxacin (23mm) disk against *K. pneumonia* as shown in Table (4.23).

Aquatic extract of *L. siceraria* peels with Ciprofloxacin disk (30.3mm) was the highest synergistic effect on this bacteria as shown in Figure (4.96) followed by ceftazidime, Doxycycline as shown in Figure (4.84, 4.98), Aztreonam and Amikacin disk (12mm, 12.3mm, 37.3mm, 24.3mm Respectively) which showed a potent synergistic effect on against *K. pneumonia* as shown in Table (4.23).
Table (4. 23) Synergism Between Antibiotics and Aquatic Extracts of Plant against *K. pneumonia*.

<table>
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<tr>
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<th><strong>M. domestica peels</strong></th>
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_Ceftazidime (CAZ30), Rifampicin (RIF), Neomycin (N30), Tetracycline (TE30), Doxycycline (DO), Ciprofloxacin (CIP), Aztreonam (ATM), Amikacin (AK), Cefazolin (KZ)._  
Values are mean ± SD of three separate experiments.

**Figure (4.84)** The effect of *G. glabra* roots extract and *L. siceraria* peels with _Ceftazidime_ against *K. pneumonia*

**Figure (4.85)** The effect of *G. glabra* roots extract and *L. siceraria* peels with _Cefazolin_ against *K. pneumonia*
Figure (4.86) The effect of *L. nobili* extract and *M. domestica* peels with *Neomycin* against *K. pneumonia*

Figure (4.87) The effect of *G. glabra* roots extract and *M. officinalis* with *Aztreonam* against *K. pneumonia*

Figure (4.88) The effect of *G. glabra* roots extract and *L. siceraria* peels with *Neomycin* against *K. pneumonia*

Figure (4.89) The effect of *L. nobili* extract and *M. domestica* peels with *Tetracycline* against *K. pneumonia*

Figure (4.90) The effect *M. officinalis* with *Amikacin* against *K. pneumonia*

Figure (4.91) The effect of *G. glabra* roots extract and *L. siceraria* peels with *Tetracycline* against *K. pneumonia*

Figure (4.92) The effect of *L. nobili* extract and *M. domestica* peels with *Doxycycline* against *K. pneumonia*

Figure (4.93) The effect *M. officinalis* with *Doxycycline* against *K. pneumonia*
Figure (4.94) The effect of *G. glabra* roots extract and *L. siceraria* peels with *Rifampicin* against *K. pneumonia*

Figure (4.95) The effect *M. officinalis* with *Rifampicin* against *K. pneumonia*

Figure (4.96) The effect of *G. glabra* roots extract and *L. siceraria* peels with *Ciprofloxacin* against *K. pneumonia*

Figure (4.97) The effect of *L. nobili* extract and *M. domestica* peels with *Ciprofloxacin* against *K. pneumonia*
Chapter 5 Discussion
Chapter 5 Discussion

It has been found that many compounds found in plants, herbs have antimicrobial activities and are an important source of treatment for pathogenic microbes (Raji & Raveendran, 2013). Bacterial infectious diseases are one of the leading causes of increased morbidity and mortality worldwide. Therefore, increased attention has been paid to the development of antimicrobial agents to treat bacterial infections. The main objective of the study was to determine the ability of plant extract to inhibit the growth of bacteria and biofilms and to determine the ability of plant extracts to enhance the activity of antibiotics. Antimicrobial activity was recorded when the zone of inhibition is greater than 6 mm.

5.1 Antibacterial Activity of the Plant Extracts

The results showed the effective ability of plant extracts tested as antibacterial agents against *E.coli, S.aureus, P.aurgenosa* and *k. pneumonia*. This confirms the role of active constituents of plant extracts as agents to stop bacterial growth. Generally medicinal plants appear to be more inhibitor against Gram-positive than Gram-negative bacteria (Rakholiya & Chanda, 2012), which could be due to the difference in the structure of the bacterial cell wall (Elbashiti, Elmanama, & Masad, 2011).

5.1.1 Against *Escherichia coli*

Results of our experiments, well diffusion method was used to evaluate the activity of plant extracts was showed higher activity than disc diffusion method against *E. coli* because the paper disk saturated with plant extract retains the active component and is not allowed to spread into the Muller Hinton Agar, because some compounds does not diffuse in the agar but *M. officinalis* extracts was showed low inhibition zone by disc diffusion method in comparison with well diffusion method. The extract had no inhibitory effect when used because the plant extract spread in the bottom of the dish away from the growth of bacteria on the surface.

Previous Studies reported antibacterial activity of *G. glabra* roots against *E. coli* by well diffusion method. The results indicated that extract different solvents has shown good effect on *E. coli*. Ether with zone inhibition (16mm), Acetone (15mm), Chloroform (11mm) (Nitalikar et al., 2010). Similar to the results obtained by (Sedighinia et al., 2012), which showed that the ethanolic extract of *G. glabra* roots had higher inhibitory effect against *E.coli* with MIC50s values at 50mg/ml. The results of the study presented by (Gnanamoorthy,
Sridharan, Dhayananth, & G, 2017) using disc diffusion method, indicated that G. glabra roots ethanolic extract has a good inhibitory effect against E. coli. These results are similar to the results of our study, however our study showed slightly higher antibacterial activity of G. glabra roots against E. coli.

In previous studies, the results indicated that ethanolic and aqueous G. glabra roots had no inhibitory effect against E. coli (Irani, Sarmadi, Bernard, Ebrahimi Pour, & Shaker Bazarnov, 2010), while our results showed that roots extracts can inhibit the growth of E. coli.

Research suggests that G. glabra roots has antibacterial effects due to to the presence of glabrene, licoisoflavone B, solicoflavonol, gancaonin (Sultana, Haque, Hamid, Urmi, & Roy, 2010).

Previous Studies reported antibacterial activity of L. nobilis against E. coli by dsic diffusion method. The results indicated that aqueous L. nobilis extract exhibited good inhibitory effect agiants E. coli (Marwaha, Khan, & Abhyankar, 2015). These results are close to the results of our study, however our study showed good antibacterial activity of G. glabra roots against E. coli.

Research suggests that L. nobilis has antibacterial effects due to carvacrol, 1,8-cineole, fenchone, and trans-anethole (Ghadiri et al., 2014).

There no previous studies about antibacterial effect of M. domestica peels, M. officinalis and L. siceraria peels against E. coli.

5.1.2 Againts Staphylococcus aureus

Results of our experiments, well diffusion method was used to evaluate the activity of plant extracts was showed higher activity than disc diffusion method against S. aureus.

Previous Studies reported antibacterial activity of G. glabra roots against S. aureus by well diffusion method. The results indicated that different solvents extract has shown good effect on S. aureus. Ether with zone inhibition (22mm), Acetone (32mm), Chloroform (18mm) (Nitalikar et al., 2010).

Similar to the results obtained by (Sedighinia et al., 2012) which showed that the extract of G. glabra roots had higher inhibitory effect against S. aureus with MIC50s values at 50mg/ml.

The results of the study presented by (Gnanamoorthy et al., 2017) using disc diffusion method indicated that G. glabra roots ethanolic extract has a good inhibitory effect against
S. aureus. These results are similar to the results of our study, however our study showed slightly higher antibacterial activity of G. glabra roots against S. aureus.
Previous Studies reported antibacterial activity of methanolic Laurus nobilis extract against S. aureus by well diffusion method (Shan, Cai, Brooks, & Corke, 2007). The results indicated that extract has shown inhibitory effect on S. aureus but less than result of our study.
There no previous studies about antibacterial effect of M. domestica peels, M. officinalis and L. siceraria peels against S.aureus.

5.1.3 Against of Pseudomonas aeruginosa

Results of our experiments, disc diffusion method was used to evaluate the activity of plant extracts was showed low activity against P. aeruginosa. The results indicated that aqueous g. glabra roots was showed the highest activity against P. aeruginosa by well diffusion method compared to disc diffusion method.
In previous studies, the results indicated that ethanolic and aqueous G. glabra roots had no inhibitory effect against P. aeruginosa (Irani et al., 2010) while our results showed that roots extracts can inhibit the growth of P. aeruginosa.
The results of the study presented by (Nitalikar et al., 2010) using well diffusion method indicated that extract different with solvents has shown good effect on P. aeruginosa. Ether with zone inhibition (14mm), Acetone (22mm), Chloroform (14mm). These results are close to the results of our study, however our study showed good antibacterial activity of G. glabra roots against P. aeruginosa.
There no previous studies about antibacterial effect of M. domestica peels, M. officinalis and L. siceraria peels against P. aeruginosa.

5.1.4 Against klebsiella pneumonia

Results of our experiments, well diffusion method was used to evaluate the activity of plant extracts was showed higher activity than disc diffusion method against K. pneumonia.
M. officinalis extracts was showed low inhibition zone by disc diffusion method in comparison with well diffusion method. The extract had no inhibitory effect when used because the plant extract spread in the bottom of the dish away from the growth of bacteria on the surface.
In previous studies, the results indicated that ethanolic and aqueous G. glabra roots extract had no inhibitory effect against K. pneumonia (Irani et al., 2010).
Also the results of the study presented by (Karahan, Avsar, Ozyigit, & Berber, 2016) indicated that methanolic *G. glabra* roots extract had no inhibitory effect against *K. pneumonia* while our results showed that roots extracts can inhibit the growth of *K. pneumonia*.

The results of the study presented by (Al-Mariri & Safi, 2014) using disc diffusion method indicated that ethanolic *L. nobilis* has good antibacterial effect with zone inhibition (15mm) against *K. pneumonia*. The results of our study showed antibacterial effect with zone inhibition (10mm) against *K. pneumonia*.

There no previous studies about antibacterial effect of *M. domestica* peels, *M. officinalis* and *L. siceraria* peels against *K. pneumonia*.

5.2 Minimum inhibitory concentration (MIC) of plant extracts

Microdilution method was used to determine the lowest plant extracts concentration that inhibiting the growth of the bacteria and found effective in the evaluation of MIC. The MIC value of *M. domestica* peels was found as the lowest (1.56 mg/ml) against *E. coli* and the aquatic extracts of *M. domestica* peels gave the best antibacterial activity against *E. coli*.

The extract of *G. glabra* roots, *M. officinalis* and *L. siceraria* peels was to be significantly active exhibiting the medium potency with all solvents used (25 mg/ml) against *S. aureus*, and this confirms of the need for a high concentration of plants until affect of the bacteria. Also ethanolic extracts of *Laurus nobilis* was to be significantly active exhibiting the medium potency with MIC (25 mg/ml).

The extract of *M. domestica* peels and *L. siceraria* peels was to be active exhibiting the medium effect with all solvents used (25 mg/ml) against *K. pneumonia*. Also aquatic extracts of *G. glabra* roots and *L. nobilis* showed significantly active with MIC (25mg/ml), this confirms of the need for a high concentration of plants until affect of the bacteria.

The MIC values obtained showed that ethanol extract of *G. glabra* roots has the most potent effect against *P. aeruginosa* with MIC (12.5-6.25mg/ml).

5.3 Minimum bactericidal concentration (MBC)

The minimum bactericidal activity is determined by the semi-inhibitory concentrations of the extracts used after reading their MIC. The MBC of the plants extracts was observed with the ethanolic and aquatic extracts, which came out to be in a range between 100 to 200 μg/mL for the concentration of 200 μg/mL.
The MBC of *G. glabra* roots extract against *E. coli* was observed at 200 μg/mL that indicates of the efficacy of the plant extract, The MBC of others extracts was observed more than 200 μg/mL. The MBC of *G. glabra* roots ethanolic extract against *S. aureus* was observed at 200 μg/mL. The MBC of *M. officinalis* ethanolic extract against *P. aeruginosa* was observed at 200 μg/mL. The MBC of *M. officinalis* ethanolic and Aquatic extract against *K. pneumonia* was observed at 200 μg/mL and the MBC of *L. siceraria* peels ethanolic extract against *K. pneumonia* was observed at 200 μg/mL, the MBC of *L. siceraria* peels Aquatic extract against *K. pneumonia* was observed at 100 μg/mL.

### 5.4 Effect extract of Plants on Biofilm formation of *P. aeruginosa* and *S. aureus*

Some of *P. aeruginosa* and *S. aureus* isolates have antibiotic resistance mechanisms, such as having the ability to form biofilm and have a barrier to the permeability of antibiotic molecules. The concentration of antibiotic doses to prevent the growth of bacterial species producing the biofilm is much higher than that needed to prevent the species less able to form the biofilm. Biofilm formation by *P. aeruginosa* was more sensitive against plant extracts than biofilm formation by *S. aureus* because *P. aeruginosa* is negative gram and has a thick membrane because the membrane allows the permeability of the plant extract into the bacterial cell.

The results of this study indicated the effectiveness of plant extracts in inhibiting the formation of biofilm by *P. aeruginosa* and *S. aureus*.

The results of the study presented by (Chakotiya et al., 2016) found that the *G. glabra* roots extract has a significant inhibitory effect on Biofilm formation against *P. aeruginosa*. The effectiveness of the extract is due to its active compounds such as glycyrrhic acid (Chakotiya et al., 2016). There no previous studies about antibiofilm effect of *M. domestica peels*, *M. officinalis* and *L. siceraria* peels against *P. aeruginosa* and *S. aureus*.
5.5 Synergistic activity of plant extracts and antibiotics

In our study, plant extracts gave a different synergistic ability to inhibit the growth of microorganisms depending on extraction method. Microbial capacity was determined to be an effective and potent enhancer to enhance the activity of certain antibiotics (Chanda and Rakhholiya, 2011).

5.5.1 Against Escherichia coli

The results of the study showed that ethanolic extracts have a synergistic effect with antibiotics better than aquatic extracts against E. coli.

The protein synthesis inhibitors such as (Amikacin) was showed the highest synergistic effect with ethanol plant extracts. The better synergistic effect was found with *M. officinalis* against *E. coli*.

*Aztreonam* was showed antagonistic effect effect with most ethanolic extract except *L. siceraria* peels which exhibits strong synergistic effect with *Aztreonam*.

*Neomycin* was showed strong synergistic effect with ethanolic extracts of *M. officinalis* and *G. glabra* roots.

*Dexocycline* was showed strong synergistic activity with the ethanolic extracts of all plant extracts against *E. coli*.

Cell wall synthesis inhibitors such as *Ceftazidine* which showed strong synergistic activity with ethanolic extract of *L. nobili* and intermediate synergistic effect with ethanolic extract of *G. glabra* roots.

For the aqueous extract, The protein synthesis inhibitors such as *Dexocycline* showed a significant synergistic effect with the aqueous extracts of all plant extracts against *E. coli* except *M. officinalis* extract which showed antagonistic effect. Protein synthesis inhibitors (such as *Amikacin* and *Tetracycline*) were showed intermediate synergistic effect with most plant extract using and water as a solvent. *Rifampicin* showed the best synergistic effect with *L. nobili* and *L. siceraria* peels, respectively.

Cell wall synthesis inhibitors such as (*Ceftazidine* and *Cefazolin*). *G. glabra* roots showed intermediate synergistic effect with *Ceftazidine*. *M. officinalis* were showed the best synergistic effect with *Cefazolin* against *E. coli*. 
5.5.2 Against Staphylococcus aureus

The results of the study showed that ethanolic extracts have a synergistic effect with antibiotics better than Aquatic extracts against S. aureus.

The protein synthesis inhibitors such as (Aztreonam) was showed the strongest synergistic effect with ethanolic plant extracts. The better synergistic effect was found with G. glabra roots. Tetracycline was showed the strongest synergistic effect with ethanolic plant extracts. The better synergistic effect was found with M. domestica peels and L. nobili. Dexocycline showed strong synergistic effect with all ethanolic plant extracts against S. aureus.

Cell wall synthesis inhibitors such as (Cefazolin, Ceftazidime) showed synergistic activity with ethanolic plant extracts except L. nobili which showed antagonistic effect against S. aureus. Ciprofloxacin which antagonistic effect against S. aureus with all plant extracts.

For the aqueous extract, The protein synthesis inhibitors such as (dexocycline) was showed the strongest synergistic effect with aqueous plant extracts. The better synergistic effect was found with M. domestica peels. Neomycin showed a potent synergistic effect with M. domestica peels and L. nobili. Tetracycline showed a potent synergistic effect with aqueous plant extracts against S. aureus.

Cell wall synthesis inhibitors such as (Cefazolin, Ceftazidime) showed strong or no synergistic activity against S. aureus. Ciprofloxacin which showed the strongest synergistic effect with M. domestica peels against S. aureus.

5.5.3 Against Pseudomonas aeruginosa

The results of the study showed that ethanolic extracts have a synergistic effect with antibiotics better than Aquatic extracts against P. aeruginosa.

The protein synthesis inhibitors such as (dexocycline) was showed the strongest synergistic effect with the ethanolic extracts of all plant extracts except for ethanolic extract of G. glabra roots showed no significant synergistic with dexocycline followed by Rifampicin gave clear synergistic with the ethanolic extracts of some plant extracts but ethanolic extract of L. nobili showed no significant synergistic with Rifampicin against P. aeruginosa.

Tetracycline and Amikacin were observed that their synergistic effect was weak with ethanolic extracts. Neomycin and Aztreonam showed antagonistic effect with all ethanolic plant.

Cell wall synthesis inhibitors such as Ceftazidime and Cefazolin were showed synergistic with the ethanolic extracts of all plant extracts. Ciprofloxacin was showed synergistic effect with M. domestica peels, G. glabra roots and L. siceraria peels against P.aeruginosa.
For the aqueous extract The protein synthesis inhibitors such as (dectocycline) was showed the strongest synergistic effect with aqueous plant extracts. The better synergistic effect was found with M. domestica peels, L. nobili and M. officinalis. Neomycin showed no significant synergistic with aqueous extract against P. aeruginosa.

Cell wall synthesis inhibitors such as Cefazolin showed weak activity against P. aeruginosa. Ciprofloxacin which showed significant synergistic activity with M. domestica peels, L. nobili and G. glabra roots. Ceftazidime was showed strong synergistic effect with all aqueous plant extracts except G. glabra roots which showed no synergistic activity with Ceftazidime against P. aeruginosa.

5.5.4 Against klebsiella pneumonia

The results of the study showed that aquatic extracts have a synergistic effect with antibiotics better than ethanolic extracts against K. pneumonia.

Protein synthesis inhibitors (such as dexocycline) was showed the strongest synergistic effect with the ethanolic extracts of M. officinalis. Ethanolic extracts of L. siceraria peels was showed strong synergistic effect with Neomycin. While tetracycline was given a potent synergistic effect with ethanolic extracts of M. officinalis and L. siceraria peels. Rifampicin gave a clear synergistic effect with L. siceraria peels and G. glabra roots. Aztreonam was showed the best synergistic effect with ethanolic extract of M. officinalis While it had no synergistic with other extracts. Amikacin was showed synergistic effect with ethanol plant extracts. The better synergistic effect was found with M. officinalis and M. domestica peels, Respectively against K. pneumonia.

Cell wall synthesis inhibitors such as Ciprofloxacin was showed synergistic effect with some ethanolic plant extracts. The better synergistic effect was found with M. officinalis, G. glabra roots and M. domestica peels, Respectively. Ceftazidime was showed synergistic effect with some ethanolic plant extracts. The better synergistic effect was found with G. glabra roots against K. pneumonia.

For the aqueous extract Protein synthesis inhibitors such as Amikacin was showed the best synergistic effect with L. siceraria peels while G. glabra roots did not give a synergistic effect with it. Tetracycline was showed the best synergistic effect with M. domestica peels while M. officinalis did not give a synergistic effect with it. Rifampicin exhibited a potent synergistic effect with G. glabra roots and M. officinalis. Aztreonam showed a potent synergistic effect with L. siceraria peels, M. officinalis and with G. glabra roots.
Cell wall synthesis inhibitors such as *Ciprofloxacin* was showed synergistic effect with some aquatic plant extracts. The better synergistic effect was found with *L. siceraria* peels while *M. officinalis* showed antagonistic effect. *Ceftazidime* showed synergistic effect ranging between a potent and intermediate with all aquatic plant extracts against *K. pneumonia*. 
Chapter 6 Conclusions and Recommendations
6.1 Conclusion

Based on the antibacterial tests that are used in our study. It was found that E. coli is more (susceptible to the employed plant extracts) than S. aureus, K. pneumonia, and P. aeruginosa.

All plant extracts were evaluated for their MIC against E. coli, S. aureus, P. aeruginosa and K. pneumonia.

The MIC value of aquatic extract of M. domestica peels against E. coli was the best value of plant extracts on E. coli followed by ethanolic extract of L. nobilis which gave 3.125mg/ml of MIC value. We conclude that these plant extracts have a potent inhibitory effect against E. coli.

The ethanolic extract of G. glabra roots against P. aeruginosa was from 12.5-6.25 mg/ml. We conclude that this plant extract has a potent inhibitory effect against P. aeruginosa. And the suggesting that very small amount of these extracts are required to inhibit the growth of the bacteria.

The ethanolic and aquatic extract of G. glabra roots, L. siceraria peels and M. officinalis against S. aureus gave intermediate MIC value.

The aquatic extract of G. glabra roots and L. nobilis gave intermediate MIC value against K. pneumonia. Ethanolic extract of M. officinalis and M. domestica peels against K. pneumonia gave intermediate MIC value. Also the ethanolic and aquatic extract of L. siceraria peels gave intermediate MIC value against K. pneumonia. We conclude that these plant extracts have moderate inhibitory effect against S. aureus and K. pneumonia.

The results obtained in this study showed that five of the extracts inhibited > 50% of P. aeruginosa and S. aureus biofilm formation. We conclude that all of the plant extracts mentioned in this study were an important source of anti-biofilm agents to develop new methods of treatment of infection caused by P. aeruginosa and S. aureus biofilm.
The strongest effect against *E. coli* was observed when ethanolic extract of *M. officinalis* were mixed with Amikacin and when ethanolic extract of *L. siceraria* peels were mixed with Aztreonam. Doxycycline and Tetracyclin showed a potent effect with most plant extracts on *S. aureus*. Ceftazidime, Aztreonam and Cefazolin exhibited a potent effect with ethanolic extract of *G. glabra* roots against *S. aureus*.

The highest effect against *P. aeruginosa* was observed when Doxycycline was combined with most plant extracts such as *M. officinalis, M. domestica* peels and *L. nobilis*.

The strongest effect against *K. pneumonia* was observed when Doxycycline was combined with ethanolic extract of *M. officinalis* and when aquatic extract of *L. siceraria* peels were mixed with Ciprofloxacin. We conclude that these plant extracts can be used to enhance inhibitory activity of antibiotics against bacteria.

### 6.2 Recommendation

**Based on the results of this study, the following recommendations were proposed:**

1. Research the efficacy of other parts of the plant to study antimicrobial capacity.
2. Toxicity of active plants should be studied to determine safety indicators.
3. Further studies to determine the effect of plant extracts against the biofilms to check their efficacies in vivo.
4. Conduct further studies aimed at finding anti-biofilm activities of different plant extracts.
5. Be careful when using some antibiotics with medicinal plants because the synergy between them may cause to reduce the effectiveness of the other on bacterial activity.
6. Synergies between plant extracts should be made to determine their effectiveness against bacterial activity.
7. Studying the effectiveness of these plant extracts on fungus species and the possibility of synergistic activity of these plants with antibiotics.
Reference
Reference


Baron. S (1996). Medical Microbiology, 4th edition. The University of Texas Medical Branch at Galveston, Texas


Karahan, F., Avsar, C., Ozyigit, I. I., & Berber, I. (2016). Antimicrobial and antioxidant...


Yanrong Lv. (2016). *Triterpenes and Phenolic Compounds in Apple Fruit (Malus domestica Borkh.).*

