Evaluation of Antibacterial And Synergistic/Antagonistic Effect of some Medicinal Plants Extracted By Microwave And Conventional Methods

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Declaration

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحث/ وسليم عبد الله عالي البرشي لائبل درجة الماجستير في كلية العلوم قسم التكنولوجيا الحيوية وموضوعها:

تقييم الفعالية الضد بكتيرية والتنظيمية/الاستفادة من نباتات طبية تم استخلاصها بطريقة تقليدية وللمايكروليف

Evaluation of Antibacterial and Synergistic/antagonistic Effect of Medicinal Plants Extracted By Microwave And Conventional Methods

وبعد المناقشة العلمية التي تمت اليوم الاثنين 20 ربيع أول 1438هـ الموافق 19/12/2016م الساعة الواحدة، اجتمعت لجنة الحكم على الأطروحة وال委ونة من:

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وبعد المداولة أوصت اللجنة بمنح الباحث درجة الماجستير في كلية العلوم قسم التكنولوجيا الحيوية.

الجنة إذ تمنح هذه الدرجة فإنها توصيه بتبقي على الله ونوره طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله والى التوفيق ،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

أ.د. عبادالروموف علي المناعمة
Abstract

The present study was designed for evaluation of a modern method of extraction using Microwave (MW) as an apparatus of extraction process and comparing this method with other conventional method as a soxhlet which used in this study. The bioactive compounds were extracted from the dried plants (*Ficus sycomorus* leaves, *Lawsonia inermis* leaves and *Glycerrhiza glabra* linn) were successively extracted with ethanol 80% and distilled water. Nine samples of each plant for both solvents were irradiated with MW at several power output (180w, 360w and 540w) in several interval times (1, 2 and 3 minuets). The antibacterial activities of extracts and the synergistic effect between plants and antibiotics were evaluated using disk diffusion method against clinical isolated *Staphylococcus aureus* and *Escherichia coli*. The result revealed that, the inhibition zone for more than 50% of aquatic and ethanolic samples results (extracted in two minutes and MW power 180w) had shown the optimum extract for each plant; as on a consideration sample has had shown a maximum magnitude of inhibition zone, though was the lowest time and power. The results of this study showed that ethanolic extracts used against selected microorganisms were showed antimicrobial and synergistic effect with most antibiotics better than aquatic extracts. Where in case of aquatic extracts; *G. glabra* had the best against *E. coli*. In case of ethanolic extracts, the best activity was observed with *G. glabra* against *E. coli*. Also, synergistic activity of the plant extracts, in case of aquatic extracts; *F. sycomorus* had the best synergism against *S. aureus* & *E. coli*. In case of ethanolic extracts, the best synergism was observed with *L. innermis* against *E. coli*, and with *F. sycomorus* against *S. aureus*. High levels of antagonism reaction exhibited by all aquatic plant extracts of both methods when combined with antibiotics which showed sensitivity when tested as alone against *S. aureus*. Also all ethanolic extracts of *L. innermis* and *G. glabra* of both methods exhibited antagonism reactions when combined with antibiotics which showed sensitivity when tested as alone against *S. aureus*. In addition, antagonism reaction occurred against *E. coli* for antibiotics which were resistant against *E. coli* when combined with aquatic & ethanolic extracts for both methods of *G. glabra* which showed sensitivity when tested as alone against *E. coli*. In addition, a dilution reactions against *S. aureus* occurred with some antibiotics combined with ethanolic extracts of *F. sycomorus* & *L. innermis*. 
Thereby, our results indicate the possibility of using MW apparatus as an extractor to obtain bioactive compounds from plants and thus used in the treatment of bacterial infections, and some results of this study was encouraging. However, the antagonistic reactions of some extracts with some antibiotics and their use in combination should be further studied for in vitro activities. It is clearly needs to be furthermore evaluated, to identify the effective components, the mode of action and the possible toxic effect in-vivo of these ingredients.

Key words.
Microwave, soxhlet, antibacterial, synergistic, antagonistic, aquatic extract & ethanolic extract.
المخصص

الملخص

صممت الدراسة الحالية لتقديم طريقة الاستخلاص باستخدام جهاز مايكروريف ومقارنتها بالطريقة التقليدية

باستخدام جهاز السوكليت، المواد الفعالة التي تم استخدامها من الأجزاء المحفزة من البتلات ( أوراق شجرة الجميز، أوراق شجرة الحانة، ودنجور العرق سوس) باستخدام المذيب الابتيات 80٪، والماء المغطر. حيث تم تجهيز تسع عينات لكل من بذول وتعرضها لطاقات موجية مختلفة (180 وات، 360 وات، 540 وات) من جهاز المايكروريف وذلك في فترات زمنية مختلفة (ديفقات، دقيقة، ثقل، ثلاثة دقائق). تم تغيم النشاط البكتيري والانتشار التفشي مع المضادات الحيوية للمستخلصات النباتية باستخدام طريقة الانتشار في القرص ضد كل من بكلياً المكورة العقدية، والاتجاهة الكولونيا. وقد أظهرت النتائج ولاحظت أن قطر مناطق التتبيط ضد البكتيريا المرضية الناتجة عن تأثير المستخلصات النباتية وتأثيرها البكتيري مع المضادات الحيوية لأكثر من 50٪ في مقداره دقيقين تحت تأثير موجود المايكروريف بجهد طاقة مقداره 180 وات (التي تم استخدامها في جميع البتلات التي تم اختيارها من كل بذول). على اعتبار أن البعيدة d أظهرت نتائجها بأعلى قيم جوية لمناطق الثقبية، على الرغم أنها تم استخدامها بجدير جودة وأقل مدة زمنية. في حين أظهرت تأثير هذه الدراسة أن البكتيريا المبتغاء من البتلات الطبية له تأثير ضد البكتيريا المستخدمة بالإضافة إلى تأثير تفشي مع مضادات الحيوية أفضل من المستخلص المائي. حيث أظهر المستخلص المائي لبتلة العرق سوس نتائج أنه يملأ أفضل تأثير ضد الإشريكية القولونية. في حين أظهر المستخلص الإبتياتي للعرق سوس أن يملأ أفضل تأثير ضد الإشريكية القولونية. وقد أظهرت النتائج للتأثير التفشي للمستخلصات النباتية، حيث أن المستخلص المائي للجميز أنه يملأ أفضل تأثير تفشي ضد المكورة العقدية والإشريكية القولونية. في حين أظهر المستخلص الإبتياتي للحمص أنه يملأ أفضل تأثير تفشي ضد الأشريكية القولونية. أيضاً أظهرت النتائج للتأثير الملمع لجميع البتلات المائية والإبتياتية المرضية وتاثيرها المبكر بالاضافة إلى تأثير تفشي مع مضادات حيوية أفضل من المستخلص المائي والإبتياتي. وعند دمجها مع بعض مضادات حيوية، أظهرت النتائج مصالح بالاضافة إلى تأثير ملمع. أيضاً أظهرت التأثيرات وعده تأثير وعده بين التأثير والملمع ضعف الكوة العقدية للمستخلصات البكتيرية لبتلة العرق وظهور وظهور عند دمجها مع المضادات الحيوية، وبالتالي فإن النتائج تشير إلى إمكانية استخدام جهاز مايكروريف لعملية الاستخلاص والحصول على مواد فعالة من النباتات، وهذا يعني إمكانية استخدامها في علاج الالتهابات البكتيرية. وكانت بعض نتائج هذه الدراسة مشجعة. على الرغم من ظهور بعض التفاعلات المشبعة للمستخلصات مع المضادات الحيوية، إذا فإن استخدامها في تركيبة مع مضادات حيوية ينبغي أن تتناول الدراسات الحيوية عليه. فمما واضح أنه يحتاج إلى تقييم وأيضاً للتعرف على مكونات فعالة، والله حديث التفاعل واحتمال التأثير السام في الجسم الحي من هذه المكونات.

الكلمات المفتاحية: مايكروريف، سوكليت، ضد بكتيري، تفشي، مثبط، مستخلص مائي، مستخلص إبتياتي
يقول تعالى:

(إِنَّ رَبِّي لَطِيفٌ لِّمَا يَشَآءُ إِنَّهُ وَهُوَ أَلْعَلِيمُ أَحْكَمِيمُ)

[يوسف:100]
Dedication

To my mother and my father who supported me all the way since the beginning of my life.

To my wife and my children who have been a great source of motivation and inspiration.

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List of Abbreviations

MW Microwave
MAE Microwave Assisted Extraction
EM Electromagnatic
PMAE Pressurized Microwave Assisted Extraction
FMAE Focused Microwave Assisted Extraction
SFE Supercritical Fluid Extraction
UAE Ultrasound-Assisted Extraction
ASE Accelerated Solvent Extraction
NBCs Natural Bioactive Compounds
AMR Antimicrobial Resistance
GAS Group A Streptococcus
ZIs Zone of Inhibition
DMSO Di-Methyl Sulphoxide
F Nitrofurantoin
OFX Ofloxacin
LEV Levofloxacin
AK Amikacin
CTX Cefotaxim
CI Ciprofloxacin
C Chloramphinicol
Chapter 1

Introduction
CHAPTER 1
INTRODUCTION

1.1. Overview

Research in herbal medicine has increased in developing countries as a way to rescue ancient traditions as well as an alternative solution to health problems. Therefore, with the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important (Saadoun, et al., 2014).

The emergence and spread of multidrug resistance as a phenomenon among bacterial pathogens has been a major problem confronting the field of antibacterial chemotherapy in the recent times. However, it has been found that, in addition to the production of intrinsic antimicrobial compounds, some medicinal plants also produce multidrug resistance inhibitors which enhance the activities of antibiotics against multidrug resistant bacteria pathogens. It is this finding that prompted efforts in screening of crude extracts for synergistic interaction with standard antibiotics against resistant bacteria as this would have the way for possible isolation of multidrug resistance inhibitors of plant origin (Eze, et al., 2013).

Now days, microwaves are used for extraction of bioactive compounds from plant materials because of tremendous research interest and potential. Conventional extraction techniques are time consuming and require more solvent and most of them are not suitable for thermolabile constituents (Yashwant, et al., 2013). In Microwave (MW), the process acceleration and high extraction yield may be the result of a synergistic combination of two transport phenomena: heat and mass gradients working in the same direction. On the other hand, in conventional extractions the mass transfer occurs from inside to the outside, although the heat transfer occurs from the outside to the inside of the substrate (Farid & Giancarlo 2013).

In this technique MW heat was generated using microwave energy. The parameters that influence extraction will be solvent nature, volume, extraction time, microwave power. Also, the MW technique in this study was compared with other extraction techniques in other studies (Yashwant et al., 2013 ). The antimicrobial activities of
extracts were evaluated using the disk diffusion method; the inhibitory zones were recorded in millimeters (Mohamed et al., 2015). This study had throwing light on importance of extraction step of bioactive compounds from *F. sycomorus, L. inermis* and *G. glabra* by using microwave irradiation through evaluation of antimicrobial and synergism/antagonism activity of each aquatic and ethanolic activity of the extracts.

1.2. Main objective

To evaluate antimicrobial and synergism/antagonism activity of each aquatic and ethanolic extracts which extracted from *F. sycomorus, L. inermis* and *G. glabra* by using microwave and conventional methods.

1.3. Specific objectives

- To collect of medicinal plants.
- To evaluate the efficiency of the MW method by comparing it with a conventional method through evaluation of antimicrobial and synergism/antagonism activity of each aquatic and ethanolic extracts samples.
- To evaluate parameters affecting on MW extraction process.

1.4. Signification

To my knowledge this study will be the first in Palestine, to extract bioactive compound from *F. sycomorus, L. inermis* and *G. glabra* by using microwave technique. Due to lower yield bioactive compound and long time by using conventional extraction methods, it has been observed in previous researches about several types of herbs that give high yield of bioactive compounds in short time through using microwave technique.
Chapter 2

Literature Review
2.1. Microwave technology

2.1.1. Microwave Oven

2.1.1.1. Definition of Microwave

Microwaves are a form of electromagnetic energy, like light waves or radio waves, and occupy a part of the electromagnetic spectrum (Fig. 2.1). Microwaves are used to relay long-distance telephone signals, television programs and computer information across the earth or to a satellite in space. They are used to detecting speeding cars. Yet, the microwave is perhaps most familiar as the energy source for cooking food.

All wave energy changes polarity from positive to negative with each cycle of the wave. In microwaves, these polarity changes happen millions of times every second. Food molecules - especially the molecules of water - have a positive and negative end, in the same way a bar magnet has a north and a south pole. When microwaves at the right frequency bombard food, they cause the polar molecules to rotate at the same frequency, millions of times a second. All this agitation on the molecular level creates friction, which heats up the food. Because microwaves don't interact with molecules of glass, plastic or paper, only the food is heated (Xiaofeng et al., 2002).

2.1.1.2. General mechanism of microwave oven

A microwave oven works as follows (Xiaofeng et al., 2002): (Fig. 2.2)

1. Electrical energy, in the form of low-voltage alternating current and high-voltage direct current, is transformed and converted into direct current.
2. A magnetron uses this direct current and generates microwaves with a frequency of 2450 megacycles per second or 2.45 GHz (gigahertz).

3. The microwaves are directed by an antenna at the top of the magnetron into a waveguide.

4. The waveguide channels microwaves to a fanlike device called a stirrer which disperses them inside the oven cavity.

5. The microwaves then reflect off the metal walls of the oven's interior and are absorbed by molecules in the food.

6. Because each wave has a positive and negative component, the molecules in the food are jostled back and forth at twice the rate of the microwave frequency, namely 4.9 billion times a second.

![Figure (2.2): Microwave oven (Yashwant et al., 2013).](image)

**2.1.1.3. Generating microwaves in magnetrons**

The most powerful microwaves produced by solid state devices, such as used in cell phones, are far too weak for cooking. Instead electron beams in vacuum tubes under the combined effect of electric and magnetic fields are made to follow curved trajectories (the detailed mechanism for which is described below). Most microwave ovens use magnetrons. First invented in 1921 and strongly improved around 1940,
magnetrons allow either continuous or pulsed microwave generation with powers up to megawatts and frequencies between 1 and 40 GHz. Efficiencies are around 80% and lifetimes about 5000 hours. A cylindrical cathode is at the axis, several millimetres from a hollow circular anode (Figure 2.3). Inside the anode there are a number of cavities designed to resonate at 2.45 GHz. A voltage of several kV is applied between the electrodes and a magnetic field is applied parallel to the axis such that electric and magnetic fields are perpendicular to each other. Electrons ejected by the cathode accelerate radially at first, but because of the magnetic field they start to follow cycloidal paths. If the magnetic field is strong enough, the electrons cannot reach the anode but form a rotating space charge. The resonant cavities of the anode interact with the electrons by either accelerating or decelerating them. Finally this leads to electron bunches which move around the cathode at microwave frequencies, which in turn leads to self-sustaining oscillations of the resonant cavities. Part of the microwave power is extracted by a coupling loop. The magnetrons in domestic microwave ovens emit microwaves at 2.45 GHz (repeatable, each time the magnetron is switched on, to ±10 MHz) with bandwidths of only a few MHz (Michael 2004).

![Schematic diagram of a magnetron](image)

**Figure (2.3):** Schematic diagram of a magnetron (Michael 2004).

### 2.1.1.4. Microwave Heating Theory

From conventional heating where the heat must diffuse in from the surface of the material. Volumetric heating means that materials can absorb microwave energy directly and internally and convert it to heat. It is this characteristic that leads to advantages using microwaves to process materials. Now, let’s represent generated
heat in a view of electromagnetic wave. The relation between the power density absorbed by a material and the electric field has been given next equation.

\[ p_{\text{dis}} = \omega \varepsilon_{\text{eff}}' |E|^2 = q_{\text{abs}} \quad (2.1) \]

From an electromagnetic (EM) point of view, \( p_{\text{dis}} \) is the power density dissipated in the materials. In the heat transfer equation, \( q_{\text{abs}} \) represents the heat generation term. It is this equation that connects the electromagnetic waves with heat transfer phenomenon. The variable \( \varepsilon'' \) plays an important role in microwave heating. For Maxwell’s equations, \( \varepsilon'' \), combined with \( \varepsilon' \) (which is the real part of the complex permittivity of the material), represents the material.\((\varepsilon=\varepsilon' - \varepsilon''j)\). The fields can be expressed as;

\[ \vec{E} = E_0 e^{-i\gamma \cdot \hat{\eta}} \] \[ \vec{H} = \frac{E_0}{\sqrt{\mu/\varepsilon}} e^{-i\gamma \cdot \hat{\eta}} \quad (2.2) \]

where \((\varepsilon=\varepsilon' - \varepsilon''j)\) and \(\mu\) is the permeability of the material. Throughout this dissertation, \(\mu=\mu_0\), the free space permeability. In the above equations, \(\gamma\), the propagation constant, is the most important parameter to describe a EM wave. The definition of \(\gamma\) is

\[ \gamma = \alpha + j\beta = \sqrt{j\mu_0 \varepsilon_0 \omega} = \omega \sqrt{\varepsilon_0 \mu_0} \sqrt{-\varepsilon_r' + j \varepsilon_r''} \]
\[ = \frac{\pi (2 \varepsilon_r')^{1/2}}{\lambda_0} \left[ \sqrt{1 + \left( \frac{\varepsilon_r''}{\varepsilon_r'} \right)^2} \right]^{1/2} - 1 + j \sqrt{\left( 1 + \left( \frac{\varepsilon_r''}{\varepsilon_r'} \right)^2 \right)}^{1/2} + 1 \quad (2.3) \]

where \(\varepsilon_r'\) and \(\varepsilon_r''\) are relative dielectric constant and relative loss factor, which are defined as

\[ \varepsilon_r' = \varepsilon'/\varepsilon_0 \quad , \quad \varepsilon_r'' = \varepsilon''/\varepsilon_0 \quad (2.4) \]

where \(\varepsilon_0\) is the permittivity of the free space and \(\lambda_0\) is the wavelength in free space defined as

\[ \lambda_0 = \frac{2\pi}{\omega \sqrt{\mu_0 \varepsilon_0}} \quad (2.5) \]

If the frequency of the microwave source is 2450 MHz, \(\lambda_0\) is equal to 12.24 cm. Now the fields are written as
The Poynting vector, which defines the power flux associated with a propagating EM wave, is given by

\[
\vec{S} = \vec{E} \times \vec{H} = \frac{(\sqrt{\sigma})^2}{\sqrt{\mu}} |E_0|^2 e^{-2\alpha z} \tag{2.7}
\]

From an energy balance, the power density dissipated in the material is

\[
p_{\text{dis}} = -\text{Re}(\nabla \cdot \vec{S}) = \frac{2\alpha \text{Re}\left(\sqrt{\sigma}\right)}{\sqrt{\mu}} |E_0|^2 e^{-2\alpha z} = \frac{2\alpha \text{Re}\left(\sqrt{\sigma}\right)}{\sqrt{\mu}} |E|^2 \tag{2.8}
\]

The power density dissipated in the lossy materials can be expressed as

\[
p_{\text{dis}} = \frac{2\alpha \text{Re}\left(\sqrt{\sigma}\right)}{\sqrt{\mu}} |E_0|^2 e^{-2\alpha z} = \omega \sigma |E_0|^2 e^{-2\alpha z} = q_{\text{abs}} \tag{2.9}
\]

The variable \(E_0\) is the amplitude of the electric wave at \(z = 0\), from where the wave originates. \(E_0\) is related to the microwave power source through the reflection coefficient \(\Gamma\) and transmission coefficient \(T\). In microwave heating, at least two media exist in the system. Microwaves travel from medium 1 into medium 2 and heat medium 2. Since the materials have different properties, only part of the energy from the power source can be transmitted into the heated material, while the remainder will be reflected. The reflection and transmission are described by \(\Gamma\) and \(T\). Normally, and \(T\) of a specified mode are functions of the media. For a uniform plane wave traveling in half infinite space, the formulas to calculate \(\Gamma\) and \(T\) can be found in any EM waves book.

From Eq. (2.9), part of the energy carried by waves changes into heat as EM waves travel in the lossy material. As a result, the amplitude of the wave and the carrying power attenuate exponentially. A useful parameter describing the attenuation effect is the penetration depth, which is defined as

\[
D_p = \frac{1}{2\alpha} \tag{2.10}
\]
The physical meaning of penetration depth is the distance that the EM wave must travel in a lossy medium to reduce its power to $e^{-1} = 0.368$ of the original value, as seen from Eq. (2.8). This value is one half of the skin depth used in electromagnetics.

The above discussion can be applied only in an infinite half space. In reality, all materials have finite dimensions. Multiple reflections will occur on the interfaces; thus, the form of the waves existing in materials is the superposition of traveling waves and standing waves. It is the interferences among the waves that make the problem more interesting. (Xiaofeng et al., 2002; Farid & Giancarlo 2013).

### 2.1.2. Microwave Extraction Theory

The principle of heating using microwave is based upon its direct impact with polar materials/solvents and is governed by two phenomenons: ionic conduction and dipole rotation, which in most cases occurs simultaneously. Ionic conduction refers to the electrophoretic migration of ions under the influence of the changing electric field. The resistance offered by the solution to the migration of ions generates friction, which eventually heats up the solution. Dipole rotation means realignment of the dipoles of the molecule with the rapidly changing electric field. Microwave energy is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles. As the field decreases, thermal disorder is restored which results in thermal energy being released (Faridah et al., 2010).

Lately, microwave system has received a lot attention in the extraction of natural substances. An original method for extracting natural products by using microwave energy has been developed. At 2450 MHz (which is the frequency used in commercial systems), the alignment of the molecules followed by their return to disorder occurs $4.9 \times 10^9$ times per second, which results in rapid heating. Unlike conventional heating which depends on conduction–convection phenomenon with eventually much of the heat energy being lost to the environment. This unique heating mechanism can significantly reduce the extraction time (usually less than 30 min) as compared to Soxhlet. During irradiation, the cells of sample matrix were thermally stressed, continuously raising the temperature of the cells and consequently, rupture of the cell walls and oil glands was made faster and the induction stage was eliminated. Evidence has been presented that during the extraction of essential oils from plant
material; microwave extraction allows the migration of the compounds out of the matrix. Indeed, microwaves interact selectively with the free water molecules present in the gland and vascular systems, this leads to localized heating, and the temperature increases rapidly near or above the boiling point of water. Thus, such systems undergo a dramatic expansion, with subsequent rupture of their walls, allowing the essential oil to flow towards the organic solvent. This process is quite different from classical solvent extraction, where the solvent diffuses into the matrix and extracts the components by solubilization. In addition, in microwave extraction a wider range of solvents could be used, as the technique should be less dependent on a high solvent affinity (Faridah et al., 2010).

2.1.3. Microwave Assisted Extraction (MAE)

MAE can be practiced in two different modes— one is closed vessel operation, that is under controlled (elevated) pressure and temperature, another is open vessel operation performed at atmospheric pressure. These technologies are named as pressurized microwave assisted extraction (PMAE) and focused microwave assisted extraction (FMAE), respectively. In closed vessel system the solvent may be heated much above their atmospheric boiling point. Both extraction speed and efficiency are enhanced in this procedure. In closed vessels the temperature may be elevated by simply applying the correct pressure. The closed vessel system is most suitable for volatile compounds. In open vessel system the maximum temperature is determined by the boiling point of the solvent used. Compared to closed vessel extractions, open cells offer increased safety in sample handling and, furthermore, they allow larger amounts to be extracted. Open cells can accommodate multiple extraction vessels at a time. Advantage of improved mass transfer due to agitation is available in both modes of MAE. Though superheating has been indicated to occur during microwave processes, MAE is not likely to suffer from thermal degradation of phytoconstituents by superheating because superheating is reported to occur in homogenous systems, and not in heterogeneous ones—in which MAE falls. The ability of microwave radiation to heat solid material effectively can be used for obtaining essential oils. This yields essential oils consisting of relatively low volatile fractions as compared to hydro distillation. MAE is highly effective for obtaining extracts under mild conditions. MAE has shorter extraction time, lesser solvent requirement, improved purity of the
extract, low cost, and better extraction yield in comparison to Soxhlet extraction. Therefore it has been considered as a potential alternative to conventional methods. Microwaves have been reported to cause little or no quality deterioration when applied to substances of plant origin such as ascorbic acid, where as moist heat application resulted in quality deterioration. MAE has been shown to be faster than the reflux method for extraction of phenolic compounds (Ankit et al., 2012).

2.1.4. Parameters In Microwave Extraction

Microwave extraction process influenced by solvent nature and volume, extraction time microwave power, matrix characteristics and temperature. Solvent choice for microwave extraction is dictated by the solubility of the target analyte, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent. Generally, by increasing the extraction time, the quantity of analyses extracted is increased, although there is the risk that degradation may occur. Microwave power and irradiation time are two such factors, which influences each other to a great extent. A combination of low or moderate power with longer exposure may be a wise approach. The plant particle size and the status in which it is presented for microwave extraction can have a profound effect on the recoveries of the compounds. The particle sizes of the extracted materials are generally in the range of 100 μm – 2 mm. Fine powders can enhance the extraction by providing larger surface area, which provides better contact between the plant matrix, and the solvent, also finer particles will allow improved or much deeper penetration of the microwave. Microwave power and temperature are very interrelated to each other and needs to be given special attention particularly when working with closed vessel system. In closed vessel systems, temperature may reach well above the boiling point of the solvent (Faridah et al., 2010).

2.1.4.1. Effect of Solvent System

The most important factor that affects MAE process is solvent selection. A proper solvent choice will provide a more efficient extraction process. Solvent selection depends on the solubility of the compounds of interest, solvent penetration and its interaction with the sample matrix and its dielectric constant, and the mass transfer kinetics of the process. The solvent should preferably have a high selectivity toward
the solutes of interest excluding undesired matrix components. Another important aspect is that the optimal extraction solvents cannot be selected directly from those used in conventional extractions: it depends on the capacity of the solvent to absorb the microwave energy and consequently heat up.

In general, the capacity of the solvent to absorb microwave energy is high when the solvent presents high dielectric constant and dielectric loss. Solvents that are transparent to microwaves do not heat when submitted to them. Hexane is an example of microwave-transparent solvent whereas ethanol is an excellent microwave-absorbing solvent. Both polar and nonpolar solvents can be used in MAE, and solvents such as ethanol, methanol, and water are sufficiently polar to be heated by microwave energy (Farid & Giancarlo 2013).

2.1.4.2. Effect of Extraction Time and Cycle
In MAE the period of heating is another important factor to be considered. Extraction times in MAE are very short compared to conventional techniques and usually vary from a few minutes to a half-hour, avoiding possible thermal degradation and oxidation, which is especially important for target compounds sensitive to overheating of the solute–solvent system. Overheating occurs because of the high dielectric properties of the solvent, especially ethanol and methanol, and further dilution with water that increases the heat capacity of the solvent combination. Higher extraction time usually tends to increase the extraction yield. However, this increase was found to be very small with longer time. Irradiation time is also influenced by the dielectric properties of the solvent. Solvents such as water, ethanol, and methanol may heat up tremendously on longer exposure, thus risking the future of thermolabile constituents (Farid & Giancarlo 2013).

2.1.4.3. Effect of Microwave Power and Extraction Temperature
Microwave power and temperature are interrelated because high microwave power can bring up the temperature of the system and result in the increase of the extraction yield until it becomes insignificant or declines. It is known that the temperature is controlled by incident microwave power that controls the amount of energy provided to the matrix, which is converted to heat energy in the dielectric material. At high temperatures the solvent power increases because of a drop in viscosity and surface tension, facilitating the solvent to solubilize solutes, and improving
matrix wetting and penetration. In addition, when MAE is performed in closed vessels, the temperature may reach far above the boiling point of the solvent, leading to better extraction efficiency by the desorption of solutes from active sites in the matrix. Microwave power is directly related to the quantity of sample and the extraction acts as a driving force for MAE to destroy the plant matrix so that the solute can diffuse out and dissolve in the solvent. Therefore, increasing the power will generally improve the extraction yield and result in shorter extraction time. On the other hand, high microwave power can cause poor extraction yield because of the degradation of thermally sensitive compounds. Also, rapid rupture of the cell wall takes place at a higher temperature when using higher power, and as a result impurities can also be leached out into the solvent together with the desired solute. Therefore, it is important to properly select the MAE power to minimize the time needed to reach the set temperature and avoid a “bumping” phenomenon in temperature during the extraction. Moreover, the overexposure to microwave radiation, even at low temperature or low operating power, was found to decrease the extraction yield because of the loss of chemical structure of the active compounds (Farid & Giancarlo 2013).

2.2. Conventional extraction methods

2.2.1. Maceration, infusion, percolation and decoction

Maceration is a technique use in wine making and has been adopted and widely used in medicinal plants research. Maceration involved soaking plant materials (coarse or powdered) in a stoppered container with a solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation. The processed intended to soften and break the plant’s cell wall to release the soluble phytochemicals. After 3 days, the mixture is pressed or strained by filtration. In this conventional method, heat is transferred through convection and conduction and the choice of solvents will determine the type of compound extracted from the samples. Infusion and decoction uses the same principle as maceration; both are soaked in cold or boiled water. However, the maceration period for infusion is shorter and the sample is boiled in specified volume of water (eg. 1:4 or 1:16) for a defined time for decoction. Decoction is only suitable for extracting heat-stable compounds, hard plants materials (e.g. roots and barks) and usually resulted in more oil-soluble
compounds compared to maceration and infusion. Unique equipment called percolator is used in percolation, another method that shares similar fundamental principle. Dried powdered samples are packed in the percolator, added with boiling water and macerated for 2 hours. The percolation process is usually done at moderate rate (e.g. 6 drops /min) until the extraction is completed before evaporation to get a concentrated extracts.

This technique is the easiest and simple method. However, organic waste come into an issue as large volume of solvents is used and proper management of the waste is needed. Alteration in temperature and choice of solvents enhance the extraction process, reduce the volume needed for extraction and can be introduced in the maceration technique, when such alteration is not objectionable. Boiling Centella asiatica at 90°C showed to increase phenolics content and antioxidant activities, but jeopardized the pH of the extracts with increase extraction time. In this method, solvents used in the soaking process play a critical role (Azwanida 2015).

2.2.2. Soxhlet extraction or hot continuous extraction:

In this method, finely ground sample is placed in a porous bag or “thimble” made from a strong filter paper or cellulose, which is place, is in thimble chamber of the Soxhlet apparatus. Extraction solvents is heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued.

This method requires a smaller quantity of solvent compared to maceration. However, the Soxhlet extraction comes with disadvantage such as exposure to hazardous and flammable liquid organic solvents, with potential toxic emissions during extraction. Solvents used in the extraction system need to be of high-purity that might add to cost. This procedure is considered not environmental friendly and may contribute to pollution problem compared to advance extraction method such as supercritical fluid extraction (SFE). The ideal sample for Soxhlet extraction is also limited to a dry and finely divided solid and many factors such as temperature, solvent-sample ratio and agitation speed need to be considered for this method (Azwanida 2015).
2.3. Other extraction methods

2.3.1. Ultrasound-assisted extraction (UAE) or sonication extraction

UAE involves the use of ultrasound ranging from 20 kHz to 2000 kHz. The mechanic effect of acoustic cavitation from the ultrasound increases the surface contact between solvents and samples and permeability of cell walls. Physical and chemical properties of the materials subjected to ultrasound are altered and disrupt the plant cell wall; facilitating release of compounds and enhancing mass transport of the solvents into the plant cells. The procedure is simple and relatively low cost technology that can be used in both small and larger scale of phytochemical extraction. The benefits of UAE is mainly due reduction in extraction time and solvent consumption. However, use of ultrasound energy more than 20 kHz may have an effect on the active phytochemicals through the formation of free radicals (Azwanida 2015).

2.3.2. Accelerated solvent extraction (ASE)

ASE is an efficient form of liquid solvent extraction compared to maceration and Soxhlet extraction as the method use minimal amount of solvent. Sample is packed with inert material such as sand in the stainless steel extraction cell to prevent sample from aggregating and block the system tubing. Packed ASE cell includes layers of sand-sample mixture in between cellulose filter paper and sand layers. This automated extraction technology is able to control temperature and pressure for each individual samples and requires less than an hour for extraction. Similar to other solvent technique, ASE also critically depend on the solvent types. Cyclohexaneacetone solution at the ratio of 6:4 v/v with 5 minute heating (50°C) showed to yield highest bixin from Bixa orellana with 68.16% purity. High recoveries (~94%) of flavonoids from Rheum palmatun were observed using 80% aqueous methanol by ASE, suggesting the suitability of this method for quality control evaluation (Azwanida 2015).

2.3.3. Supercritical fluid extraction (SFE)

Supercritical fluid (SF) or also called as dense-gas is a substance that shares the physical properties of both gas and liquid at its critical point. Factors such as temperature and pressure are the determinants that push a substance into its critical region. SF behaves more like a gas but have the solvating characteristic of a liquid.
An example of SF is CO2 that become SF at above 31.1°C and 7380 kPa. Interest in Supercritical- CO2 (SC-CO2) extraction due to excellent solvent for nonpolar analytes and CO2 is readily available at low cost and has low toxicity. Even though SC-CO2 has poor solubility for polar compounds, modification such as adding small amount of ethanol and methanol enable it to extracts polar compounds. SC-CO2 also produces analytes at concentrate form as CO2 vaporizes at ambient temperature. SC-solvents strength can be easily altered by changing the temperature, pressure or by adding modifiers that lead to reduce extraction time. Optimization of SC-CO2 on Wadelia calendulacea achieved its optimum yield at 25 MPa, 25 °C temperature, 10% modifier concentration and 90 minute extraction time. A major drawback of this method is the initial cost of the equipment is very high ( Azwanida 2015 ).

2.3.4. Pressurised solvent extraction
Pressurised solvent extraction (PSE) is a SLE technique which has been developed as an alternative to current extraction methods such as Soxhlet, maceration, percolation or reflux, offering advantages with respect to extraction time, solvent consumption, extraction yields and reproducibility. PSE uses organic solvents at elevated pressure and temperature in order to increase the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics and elevated pressure keeps the solvent in the liquid state, thus enabling safe and rapid extractions. Furthermore, high pressure forces the solvent into the matrix pores and hence should facilitate extraction of analytes. High temperatures decrease the viscosity of the liquid solvent, allowing a better penetration of the matrix and weakened solute–matrix interactions. In addition, elevated temperatures enhance diffusivity of the solvent resulting in increased extraction speed. An evaluation of ASE has been made for the extraction of various metabolites covering a large range of structures and polarities (curcuminoids, saponins, flavonolignans, terpenes) present in different vegetal matrixes such as leaves, roots, fruits, herbs and rhizomes. Performances were compared to corresponding European Pharmacopoeia methods. Yields were found to be equivalent or even higher with ASE, with a reduction in the extraction time (especially when consecutive extractions with solvents of increasing polarity were made) and solvent consumption (by a factor from two to five), and with good reproducibility probably
occasioned by the minimal sample handling required during the extraction procedure (Be’atrice & Philippe 2002).

2.3.5. Enzyme-assisted extraction

As described before, one of the main sources for the extraction of antioxidants are plant tissues. Plant cell walls contain polysaccharides such as cellulose, hemicellulose, and pectins which act as barriers to the release of intracellular substances. Some enzymes such as cellulase, β-glucosidase, xylanase, β-gluconase, and pectinase help to degrade cell wall structure and depolymerize plant cell wall polysaccharides, facilitating the release of linked compounds. Hence, these enzymes have been proposed as tools to optimize the extraction of compounds from plant matrix. Most of the Natural Bioactive Compounds NBCs, such as flavonoids, are present in different forms, interacting with the cell wall components (cellulose, hemicellulose, and pectin). For the release of these compounds, these interactions need to be broken by cell wall-hydrolyzing enzymes. β-Glycosidase breaks the β-1,4 glucosidic linkages in glucosides (flavonoids in conjunction with glucose). On the other hand, phenolic compounds are often linked to cell wall polysaccharides and cell wall degradation is a key step in releasing phenols from the cell wall. In this sense, the use of xylanases, β-gluconases, and cellulases has been shown to be effective, since they can hydrolyze the ester-linked phenolic acids. Nevertheless, such release depends on compositional and structural characteristics of compounds (Joana et al., 2013).

2.4. Medicinal plants

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin and toothpaste.

Medicinal plants have many characteristics when used as a treatment, as follow:
• Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.
• Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.
• Preventive medicine- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment.

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies (Bassam 2012).

2.5. Plants used in this study

2.5.1. *F. sycomorus*

Table (2.1): Classification of *F. sycomorus*.

| Kingdom: | Plantae |
| Division: | Magnoliophyta |
| Class: | Magnoliopsida |
| Order: | Urticales |
| Family: | Moraceae |
| Genus: | Ficus |
| Species: | *sycomorus* |

Leaves of *F. sycomorus*

The Sycamore Fig belongs to family Moraceae (Table 2.1) is one of the old and historic plant species in the Palestine coastal valley and the study area as well. It is known and called in Palestine as “Balami or Jummaze”. It is a tree attaining up to a height of 20 meters and sometimes reaching 6 meters in, growth with widely spreading branches and a massive crown. Sheep and cattle eat its young foliage. Fruits of this plant are mixed ration for cows in order to increase milk flow. The fresh fruits of this plant are taken orally by human beings in Palestine. The trees have some medicinal values as the sap extracted from the trunk can cure some skin diseases. The
antibacterial activity of *F. sycomorus* could be related to the presence of bioactive compounds, such as tannins, saponins, flavonoids, steroids, anthraquinone glycosides and reducing sugars (Mohammed et al., 2015).

*F. sycomorus* have been suspected to possess anti-diarrhoeal activities and sedative and anticonvulsant (are a diverse group of pharmaceuticals used in the treatment of epileptic seizures) properties of this plant have also been reported. Reported different solvent extracts of some plants to have different pharmacological properties. Reported organic stem extracts of *F. sycomorus* with higher antifungal activity than aqueous extracts (Mohamed et al., 2013). The fruit extracts of *F. sycomorus* L exhibited antitumor activity in the potato disc bioassay. it had significant antibacterial activity, but no antifungal activity (Bello et al., 2013).

### 2.5.2. *L. inermis*

**Table (2.2): Classification of *L. inermis***

| Kingdom:     | Plantae       |
|--------------|---------------|
| Division:    | Angiosperms   |
| Class:       | Eudicots      |
| Order:       | Myrtales      |
| Family:      | Lythraceae    |
| Genus:       | Lawsonia      |
| Species:     | *L. inermis*  |

*L. inermis* belongs to family *Lythraceae* (Table 2.2) is a much-branched glabrous shrub or small tree 2-6 m in height, which may be spiny. Bark greyish-brown, unarmed when young, older plants with spine-tipped branchlets. Young branches quadrangular, green but turn red with age (Orwa et al., 2009). *L. inermis* Linn. most commonly known as ‘Henna”, leaves have been used traditionally in northern Nigeria as a remedy against diarrhea, dysentery and other related diseases, which are caused by *Corynebacterium* spp., *S. aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Shigella dysentriae*. In addition the leaves are frequently used as a herbal remedy for an array of human disorders including wounds, ulcers, strangery, cough, bronchitis, lumbago, hemi crania, leucoderma, scabies, boil, hepatopathy, spleenopathy, ophthalmic conditions, falling of hairs and jaundice. The main
constituents in the plant are carbohydrates, glycosides, tannins, phenolic compounds and gums and mucilage. Plant henna extract is used as hair growth stimulators for the treatment of dandruff and as hair colorant or dye. Mechanism of action appears to be done by acceleration of blood circulation, activation of dermal papilla and increase nutrition to the hair follicles. Henna is a perennial shrub native to Northern Africa and cultivated in the Tropical regions of America, Egypt, India, Palestine and other parts of Middle East (Jain et al., 2010).

2.5.3. *G. glabra*

Table (2.3): Classification of *G. glabra*

| Kingdom: | Plantae |
| Division: | Angiospermae |
| Class: | Dicotyledoneae |
| Order: | Rosales |
| Family: | Leguminosae |
| Genus: | Glycyrrhiza |
| Species: | *glabra* Linn |

*Dried sticks of G. glabra root*

*G. glabra* Linn is one of the most extensively used medicinal herb. It is also used as a flavoring herb. The word Glycyrrhiza is derived from the Greek term glykos (meaning sweet) and rhiza (meaning root). *G. glabra* Linn, commonly known as ‘liquorice’ and ‘sweet wood’ belongs to Leguminosae family (Table 2.3).

In traditional medicine, liquorice has been recommended as a prophylactic agent for gastric and duodenal ulcers. It is employed in dyspepsia as an anti-inflammatory agent during allergenic reactions. It is used as a contraceptive, laxative, anti-asthmatic, emmenagogue, galactagogue, antiviral agent in folk therapy. Glycyrrhiza roots are useful for treating cough because of its demulcent and expectorant property. It is also effective against anemia, gout, sore throat, tonsillitis, flatulence, sexual debility, hyperdypsia, fever, skin diseases, swellings. Liquorice is effectively used in acidity, leucorrhoea, bleeding, jaundice, hiccough, hoarseness, bronchitis, vitiated conditions of Vata dosha, gastralgia, diarrhea, fever with delirium and anuria. It is a vital
ingredient in medicinal oils used for the treatment of rheumatism, hemorrhagic diseases, epilepsy and paralysis.

A large number of components have been isolated from the liquorice roots. 40-50 percent of total dry material weight of *G. glabra* is accounted by water-soluble, biologically active complex. Starches (30%), pectins, polysaccharides, simple sugars, gums, mucilage (Rhizome), amino acids, triterpene saponin, flavonoids, mineral salts, bitters, essential oil, fat, asparagines, female hormone estrogen, tannins, glycosides, protein, resins, sterols, volatile oils and various other substances are components of this complex. The primary active ingredient, Glycyrrhizin (glycyrrhizic acid; glycyrrhizinate) constitutes 10–25% of liquorice root extract. It is a saponin compound (60 times sweeter than cane sugar) comprised of a triterpenoid aglycone, glycyrrhetic acid (glycyrrhetinic acid; enoxolone) conjugated to a disaccharide of glucuronic acid. The yellow color of liquorice is because of the flavonoid content of the plant. Flavonoids include liquiritin, a chalcone (isoliqurtitin) and other compounds. Reported antibacterial activity because of the presence of secondary metabolites such as; saponins, alkaloids, flavonoids in hydro-methanolic root extract of *G. glabra*, the extract exhibits potent antibacterial activity. In vitro studies have proved that aqueous and ethanolic extracts of liquorice show inhibitory activity on cultures of *S. aureus* and *S. pyogenes*.

Reported Pharmacological activities such as anti-tussive, expectorant activity, antioxidant activity, skin lightening, anti-inflammatory activity, Anti-malarial activity, Anti hyperglycemic activity, Immunostimulatory effects, Memory enhancing activity, Hepatoprotective activity, anticoagulant and hair growth stimulatory activity (Lakshmi & Geetha 2011; Asha et al., 2012).

2.6. Microorganisms and bacterial resistance

Microorganisms have existed on the earth for more than 3.8 billion years and exhibit the greatest genetic and metabolic diversity. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems. It is believed that they compose about 50% of the living biomass. In order to survive, they have evolved mechanisms that enable them to respond to selective pressure exerted by various environments and competitive challenges. The
disease-causing microorganisms have particularly been vulnerable to man’s selfishness for survival who has sought to deprive them of their habitat using antimicrobial agents. These microorganisms have responded by developing resistance mechanisms to fight off this offensive. Currently antimicrobial resistance among bacteria, viruses, parasites, and other disease-causing organisms is a serious threat to infectious disease management globally. Antibiotics were discovered in the middle of the nineteenth century and brought down the threat of infectious diseases which had devastated the human race (Denis, 2009).

Antimicrobial resistance (AMR) within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. Increasingly, governments around the world are beginning to pay attention to a problem so serious that it threatens the achievements of modern medicine (Keiji, 2014).

The resistance problem can be seen simplistically as an equation with two main components: the antibiotic or antimicrobial drug, which inhibits susceptible organisms and selects the resistant ones; and the genetic resistance determinant in microorganisms selected by the antimicrobial drug. Drug resistance emerges only when the two components come together in an environment or host, which can lead to a clinical problem. Selected resistance genes and their hosts spread and propagate under continued antimicrobial selection to amplify and extend the problem to other hosts and other geographic locations. There are more than 15 classes of antibiotics whose targets are involved in essential physiological or metabolic functions of the bacterial cell. None has escaped a resistance mechanism. Millions of kilograms of antimicrobials are used each year in the prophylaxis and treatment of people, animals and agriculture globally driving the resistance problem by killing susceptible strains and selecting those that are resistant (Stuart & Bonnie 2004).

In general, the reasons for increasing resistance levels include the following:
- Suboptimal use of antimicrobials for prophylaxis and treatment of infection,
- Noncompliance with infection-control practices,
- Prolonged hospitalization, increased number and duration of intensive care unit stays,
- Multiple comorbidities in hospitalized patients,
- Increased use of invasive devices and catheters,
- Ineffective infection-control practices, transfer of colonized patients from hospital to hospital,
- Grouping of colonized patients in long-term-care facilities,
- Antibiotic use in agriculture and household chores, and
- Increasing national and international travel.

The level of antibiotic resistance is dependent on the following:
- The population of organisms that spontaneously acquire resistance mechanisms as a result of selective pressure either from antibiotic use or otherwise,
- The rate of introduction from the community of those resistant organisms into health care settings, and
- The proportion that is spread from person to person.

All of these factors must be addressed in order to control the spread of antimicrobial-resistant organisms within health care settings. Community acquired antimicrobial resistance is increasing in large part because of the widespread suboptimal use of antibiotics in the outpatient settings and the use of antibiotics in animal husbandry and agriculture ( Denis, 2009 ).

### 2.6.1. E. coli

**Table (2.4): Classification of E. coli**

| Domain       | Bacteria                                      |
|--------------|-----------------------------------------------|
| Phylum       | Proteobacteria                                |
| Class        | Gammaproteobacteria                           |
| Order        | Enterobacteriales                             |
| Family       | Enterobacteriaceae                            |
| Genus        | *Escherichia*                                 |
| Species      | *coli*                                        |

*E. coli* is the most commonly encountered member of the family *Enterobacteriaceae* in the normal colonic flora and the most common cause of opportunistic infections ( Sherris 1984 ).

*Escherichia coli* is a gram negative, non-spore forming rod. It may or may not be mobile. (Some rods are flagellated and some are not.) The organism is a facultative anaerobe and ferments simple sugars such as glucose to form lactic, acetic, and formic.
acids. Growth Conditions The optimal conditions for growth are a temperature of 98.6°F, with a range of 45 to 114°F (Samuel et al., 2006).

*Escherichia coli* is the predominant facultative organism in the human gastrointestinal tract. Pathogenic forms of *E. coli* can cause a variety of diarrhoeal diseases in hosts due to the presence of specific colonisation factors, virulence factors and pathogenicity associated genes which are generally not present in other *E. coli*. Of the strains that cause diarrhoeal diseases, six pathotypes are now recognized (O’Sullivan et al. 2007).

The biology of the different *E. coli* pathovars is complex. what makes each pathovar distinct is the subset of genes involved in the subversion of host responses and hijacking of host cell machinery. In many pathovars, the same host machinery or process is targeted but the mechanism and outcome is different (Matthew & Brett 2010).

Within the community, *E. coli* strains are commonly susceptible to all agents active against the Enterobacteriaceae. However, because of the frequent occurrence of R plasmids, strains acquired in hospitals may be resistant to any combination of potentially effective antimicrobics and therapy must therefore be guided by susceptibility testing (Sherris 1984).

### 2.6.2. *S. aureus*

**Table (2.5): Classification of *S. aureus***

| Domain   | Bacteria             |
|----------|----------------------|
| Phylum   | Firmicutes           |
| Class    | Bacilli              |
| Order    | Bacillales           |
| Family   | Staphylococcaceae    |
| Genus    | *Staphylococcus*     |
| Species  | *Aureus*             |

*S. aureus* belongs to the family *Staphylococcaceae* and is part of the genus *Staphylococcus*, which contains more than 30 species such as *S. epidermidis*, *S.
saprophyticus and S. haemolyticus. Among the staphylococcal species, S. aureus is by far the most virulent and pathogenic for humans. S. aureus is a 1 μm, Gram-positive cell that in the laboratory may be observed as single cells, in pairs or as grape-like irregular clusters. It is characterized as coagulase- and catalase positive, non-motile, non-spore-forming and as facultative anaerobic. It grows in yellow colonies on nutrient rich media and is referred to as the yellow staphylococci (Lisa 2013).

S. aureus is a major cause of nosocomial infections worldwide, especially methicillin-resistant S. aureus. Patients subjected to broad-spectrum antibiotics and immunosuppressive therapies have higher risk of infection by this microorganism. S. aureus infection are often extremely difficult to treat due to the large population heterogeneity, phenotypic switching, intra-strain diversity, hypermutability and most importantly the small colony variants. It is very important to emphasise that host immune responses against persistent infections by S. aureus is insufficient resulting normally into chronic infections, which in turn can lead to life threatening situations (Ana et al., 2013).

S. aureus is considered to be a major pathogen that colonises and infects both hospitalised patients with decreased immunity, and healthy immuno-competent people in the community. This bacterium is found naturally on the skin and in the nasopharynx of the human body. It can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening (Harris et al., 2002).

To all the virulence factors described earlier it is important to mention that a key factor for the success of S. aureus as a pathogen is its remarkable capacity to acquire antibiotic resistance. Therefore, from a clinical point of view, the major problem that physicians have to face when treating S. aureus infections is antibiotic resistance, due to the likelihood of therapeutic failure and consequently poor prognostic (Ana et al., 2013).
2.7. Previous Studies
2.7.1. *F. Sycomorus*

- In 2015, Basel et al used soxhlet apparatus to extract bioactive compounds and evaluate antibacterial activity of methanol and acetone in leaf (LE) and stem-bark (SBE) of *F. sycomorus* crude extracts against sensitive and resistant species of *S. aureus* and *Acinetobacter baumannii* pathogens. Antimicrobial activity expressed by disc-diffusion method (zone of inhibitions – ZIs). Zone of inhibition (ZIs) was varied according to the tested bacteria pathogens and plant fraction extract. This value ranged between 15–23.5 mm and 16–27 mm for methanol and acetone extracts, respectively. It was noticed that sensitive *A. baumannii* isolate was the pathogen most inhibited by the both extracts. It was recorded to be 26, 27 mm for acetone LE and SBE; while it was recorded to be 23, 23.5 mm for methanol LE and SBE; respectively.

- In 2010, Adeshina et al evaluate antibacterial activities of ethanolic extracts of *F. sycomorus* Linn. and *Ficus platyphylla* Del. Powdered leaves and stem barks were extracted with 70% aqueous ethanol at room temperature. The zones of inhibition showed by *F. sycomorus* ranged between 11.5 - 21.5 mm.

- In 2015, Mosad et al studied an extraction process which was carried out via soaking it in 85% MeOH in room temperature with shaking day by day followed by (two weeks). The antimicrobial screening was carried out via disc diffusion method toward four strains of the clinical antibiotic resistant pathogens including *E. coli*, *S. aureus*, *Candida albicans* and *Aspergillus niger*. The results revealed that the methanolic extract of *F. sycomorus* showed very strong activity against *S. aureus* (27 mm) and moderate activity against *E. coli* and *C. albicans* of inhibition zones 14 and 16 mm respectively. On the other hand, the ethyl acetate fraction showed strong activity against *S. aureus* (18 mm)

- In 2015, Zumbes et al used soxhlet apparatus to extract bioactive compounds from leaf and stem bark of *F.sycomorus*. In-vitro agar diffusion of the leaf and stem bark extracts of the plant using methanol, water and hexane were investigated on Salmonella Typhi and Salmonella Paratyphi A respectively. The findings demonstrated that the stem bark extract was sensitive to all the
test organisms and thus showed that the extract contained potential antimicrobial agents. However, the leaf extracts of *Ficus sycomorus* was observed to be less potent against S. Typhi and S. Paratyphi A respectively. The extract with the greatest antibacterial activity was methanol stem bark extract (13mm inhibition zone) at 100mg/ml for S. Typhi and S. Paratyphi A respectively.

- In 2015, **Mohammed et al** was extract *F. sycomorus* in ethanol and methanol by a soxhlet extractor. Aqueous extraction was done by boiled on slow heat for 2 hours. The study was to assess the antibacterial effect of *F. sycomorus* extracts and their synergistic antibiotics against *E. coli, S. aureus* and *Pseudomonas aeruginosa*. The results showed that the methanol and ethanol extracts of *F. sycomorus* bark showed the highest effect towards *S. aureus* (with a 15 mm zone of inhibition), While only methanol extract of *F. sycomorus* (bark) was showed effect against *E. coli* with a zone of inhibition = 9 mm. The largest zone of inhibition against *P. aeruginosa* was observed with the ethanol and methanol extracts of *F. sycomorus* bark with a zone of inhibition (10 mm).

2.7.2. *L. inermis*

- In 2013, **Kannahi & Vinotha** evaluated antimicrobial effect of henna extract which extracted in soxhlet apparatus using methanol, ethanol and aqueous extracts. Hence, the isolated bacterial isolates were confirmed as *S.aureus, S.mutans, P.aeruginosa*. Antibacterial activity was determined by agar disk diffusion method at four different concentrations 100, 75, 50 and 25 mg/ml.

The results showed that the activity of methanol extracts of *L. inermis* leaves against *S. aureus* showed minimum activity (2.3±2.01mm) at 25% concentration and maximum activity (9.3±8.9mm) at 100% level. The activity of methanol extracts of *L. inermis* against *S. mutans* showed minimum activity (2.3±1.51mm) at 25% followed by 50% (3.6±3.23mm), 75% (5±5.12mm) and 100% 17±16.04mm). The activity of methanol extracts of *L. inermis* against *Pseudomonas aeruginosa*, showed maximum activity was obtained at 75% (4.6±3.16mm) followed by 100% (3.3±2.16mm), 50% (2.8±2.4mm) and 25% (2.6±2.1mm). The ethanol extracts of *L. inermis leaves* against *S. aureus*
showed minimum activity (3.1±3.2 mm) at 25% concentration and maximum activity (8.1±6.2 mm) at 100% level. The ethanol extracts of L. inermis leaves S. mutans showed minimum activity (2.0±4.3 mm) at 25% concentration maximum activity (9.1±5.1 mm) at 100%. The ethanol extracts of L. inermis leaves against pseudomonas aeruginosa showed minimum activity (9.1±8.6 mm) an 25% concentration and maximum activity (7.6±6.4 mm) at 100%. There was no activity showed by aqueous extracts of L. inermis against S. aureus, S. mutans, and Psseudomonas aeruginosa.

- In 2013, Wasim et al evaluated antmicrobial effect of L. inermis L. Leaves which was defatted with petroleum ether and the residue was extracted in 50% methanol with the help of separating funnel. The test organisms included the gram positive bacteria; Bacillus cereus, S. aureus and Gram than negative bacteria; Klebsiella pneumoniae, E. coli and Pseudomonas pseudoalcaligenes. Antibacterial activity was determined by agar disk diffusion method at four different concentrations 100, 75, 50 and 25 mg/ml. The results showed that the extracts has a concentration dependent antibacterial activity with more sensitivity for Gram negative bacteria than Gram positive bacteria used in the study. The extracts of L. inermis showed considerable antibacterial activity at all the four concentrations 100, 75, 50 and 25mg/ml.

- In 2007, Abdulmoneim evaluated antimicrobial effect of henna extract which extracted in soxhelt apparatus using methanol, chloroform and aqueous extracts. Hence, the isolated bacterial isolates were confirmed as S.aureus, bacillus subtilitis, P. aeruginosa and E. coli. Antibacterial activity was determined by agar disk diffusion method. The results showed that three types of henna extract has a substantial inhibitory effects against the four tested bacterial strain. Still the water extract was superior in suppressing the bacterial growth, followed by methanol and chloroform extracts. The maximum inhibition zone was observed against S. aureus (19mm) followed by P. aeruginosa (18mm) and (16mm) for both bacillus and E. coli. the weakest activity was observed in chloroform extracts with a maximum zone of
inhibition (15mm) observed against \textit{P. aeruginosa} and minimum zone of inhibition (13mm) in \textit{S. aureus}.

- In 2010, Arun \textit{et al} was used methanol for an ultrasonic bath extraction process. The bacteria used for antibacterial study were \textit{S. aureus, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa & Proteus mirablis}. The plate hole diffusion assay was used to determine the growth inhibition of bacteria by plant extracts. Various concentrations of methanolic extract were used (1000μg/ml, 500μg/ml, 250μg/ml, and 62.5μg/ml) to test the antibacterial activity. From the results of antibacterial screening, 7.2% of methanolic extract were active in the lowest tested concentration of 62.5μg/ml, 5% active in a concentration of 250μg/ml, 75.7% active in a concentration of 500μg/ml, and 92.8% active in a concentration of 1000μg/ml. Ampicillin (10μg) was used as standard drug.

- In 2013, Nagarajan \textit{et al} used cold maceration method for henna leaf. Ethanol, chloroform, hexane and methane were used as extraction solvents. The test microorganisms namely, \textit{Bacillus subtites, Staphylococcus aurous, and Escherichia coli}. The plant extracts showed antibiotic activity against the test organisms used. The zone of inhibition was increased with increase in the concentration of extracts. At 4000 μg/μL, among all the plant extracts of \textit{La inermis}, ethanol extract showed the maximum antibacterial activity against all the test organisms. This indicates that most of the active compounds in the plant might be soluble in particular solvent then other solvents. \textit{Bacillus subtilis} and \textit{s. aurus} were highly susceptible in all the four extracts of the plant.

- In 2011, Al-Mehna \& Kadhum used henna with 70% ethanol and mixed by hot plate magnetic steror for 24 hours. The antibacterial effects of aqueous and alcoholic extracts of \textit{L. inermis} leaves against \textit{S. pyogenes} were investigated in vitro by using agar well diffusion method. Results showed that both extracts demonstrated antimicrobial activity against the tested organism but the efficiency of the extracts was significantly affected by the solvent used in the extraction as well as the concentration of extract. Alcoholic extracts had the
highest antibacterial activity it exhibited an inhibition zone 18.2-28.2 mm in comparison with 26.2 mm for gentamicin.

2.7.3. *G. glabra* roots

- In 2013, Aparajita et al used soxhlet extractor to obtain the plant extract (root of *G. glabra*) and then evaluated the antibacterial potency against some gram-positive and gram-negative bacterial strains. Two solvents (methanol and acetone) were used to extract the phytochemicals from the test material. Four different concentrations (100%, 75%, 50% and 25%) of methanolic and acetic extract were used to investigate the inhibiting properties against *Salmonella typhi*, *E. coli*, *Vibrio cholerae*, *S. aureus*, *Bacillus cereus* and *Bacillus subtilis* strains. The results showed that the maximum effective inhibition in methanolic extract at 100% was found against *B. subtilis* (18.6 mm) followed by *E. coli* (18.3 mm), *S. aureus*, *B. cereus* (17.6 mm) and *S. typhi* (16.3 mm), whereby the minimum inhibition zone was recorded against *V. cholerae*. Acetonic extract did not show the same trend of antimicrobial activity of bacterial strains as were found in methanolic extract. Maximum effective inhibition in 100% acetonic drug extract was recorded against *B. cereus* (16.3 mm) followed by *S. typhi* (16.0 mm), in *E. coli* (15.3 mm), in *V. cholerae* and *S. aureus* (15.0 mm), whereas the minimum inhibition zone was (14.3) recorded against *B. subtilis*. In general, the effective pattern of inhibition zone with dilution was recorded in all dilutions of both extracts.

- In 2009, Patil et al evaluate antimicrobial effect of liquorice which was extracted with ethanol (95%) in a Soxhlet extractor. The tested microorganisms used for the antimicrobial activity screening were four bacterial spp. (two Gram positive and two Gram negative) - *Bacillus subtilis*, *S. aureus*, *Pseudomonas aeruginosa*, *E. coli* and one fungal spp. - Candida albicans. The agar diffusion method11 was used to evaluate the antimicrobial activity. A stronger and broader spectrum of antimicrobial activity was observed in aqueous and ethanolic extracts of *G. glabra*, as compared to vehicle, dimethyl formamide (DMF). Also, the diethyl ether fraction of ethanolic extract of *G. glabra* showed significant antifungal and antibacterial
activity. Increased inhibition was found at higher levels of extract concentration.

- In 2010, Manoj et al made studies about liquorice which was extracted with chloroform, acetone and ether by soxhalation method. The tested microorganisms used for the antimicrobial activity screening were four bacterial spp. *S. aureus, Bascilus subtilis, E. coli* and *Psudomonas aerugenosa*. The agar diffusion method was used to screen the antibacterial activity of all extracts. The results showed that the extracts of the roots of *G. glabra* has shown magnificent antibacterial effect. Although ethereal extract has shown good effect on *E. Coli* strain. The acetone extract has shown excellent effect than Streptomycin. Overall the acetone extract of the roots has shown significant antibacterial effect on studied organisms.
Chapter 3

Materials & Methods
CHAPTER 3

Materials and Methods

3.1. Materials

3.1.1. Plant Sample Collection

The plant materials used in this study consisted of *F. sycomorus*, *L. inermis* and *G. glabra* which are collected from different area in Gaza strip (Table 3.1).

Table (3.1): Plant materials used in this study

| Plant/Part used     | Place                      | Time of collection      |
|---------------------|----------------------------|-------------------------|
| *F. sycomorus*/leaves | Jabalia-North Of Gaza | March & April 2015        |
| *L. inermis*/leaves  | Market                     | April 2015               |
| *G. glabra*/Roots    | Market                     | April 2015               |

3.1.2. Chemicals and Culture Media

Two types of media were used for carrying out this study, Nutrient broth, Nutrient agar and Muller Hinton agar. Distilled water and ethanol was used for extraction process. Cefotaxime, Ofloxacin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, ceftriaxon, chloramphinicol and Amikacin used as reference antibiotics (Table 3.2). Dimethyl sulfoxide (DMSO) and ethanol 80%. These media, the solvent and antibiotics discs were purchased from Zant company in Gaza.

Table (3.2): list of antibiotic potency

| Antibiotics         | symbol | Antibiotics potency | Manufactured by      |
|---------------------|--------|---------------------|----------------------|
| Cefotaxime          | CTX    | 30 μg               | Bioanalyse, Turkey   |
| Ofloxacin           | OF     | 5 μg                | Himedia, Indian      |
| Ceftriaxon          | CTR    | 30 μg               | Himedia, Indian      |
| Amikacin            | AK     | 30 μg               | Bioanalyse, Turkey   |
| Chloramphenicol     | C      | 30 μg               | Bioanalyse, Turkey   |
| Ciprofloxacin       | CI     | 5 μg                | Bioanalyse, Turkey   |
| Levofloxacin        | LEV    | 5 μg                | Bioanalyse, Turkey   |
| Nitrofurantoin      | F      | 300 μg              | Bioanalyse, Turkey   |
3.1.3 Bacteria
Clinical isolated species of *S. aureus*, *S* and *E. coli* were obtained from microbiology department at laboratory of Balsam hospital, and were maintained on Nutrient slants agar at 4 °C for further experiments.

3.1.4 Equipments

**Microwave apparatus**: commercial microwave oven (Panasonic) with ten power levels (80 to 800 W) was used.

**Various glassware**

**Filter 0.2 micron**

3.2. Methods

3.2.1. Preparation of plant extract

3.2.1.1. Soxhlet extraction

**Aqueous extraction**

Air dried powder (20g) was added to 150 ml of distilled water as a solvent for 8 hours, using soxhlet equipment. Then the extract was filtered and allowed to evaporate in oven (45 °C) through 48hr (Mohamed et al., 2016).

**Ethanol extraction**

According to (Mohamed et al., 2016), air dried powder (20g) was added to 150 ml of 150 ml of 80 % ethanol as a solvent for 8 hours, using soxhlet equipment. Then the extract was filtered and allowed to evaporate in oven (45 °C) through 48hr.

3.2.1.2. Microwave extraction

**Aqueous extraction**

According to (Mahaveer et al., 2009), 4 g of the powder was mixed with 100 mL distilled water. Then the mixture was irradiated with microwave at several power output (180, 360 and 540) in several interval times (1, 2 and 3) minuets to obtain nine samples.
Table (3.3): list of aquatic samples extracted in microwave

| Sample symbol | Power output | Time(minutes) |
|---------------|--------------|---------------|
| a             | 180          | 1             |
| b             | 360          |               |
| c             | 540          |               |
| d             | 180          | 2             |
| e             | 360          |               |
| f             | 540          |               |
| g             | 180          | 3             |
| h             | 360          |               |
| i             | 540          |               |

Ethanol extraction

According to (Mahaveer et al., 2009) 4 g of the powder was mixed with 100 mL (80%) ethanol. Then the mixture was irradiated with microwave at several power (180, 360 and 540) in several interval times (1, 2 and 3 minuets) to obtain nine samples.

Table (3.4): list of Ethanolic samples extracted in microwave

| Sample symbol | power | Time(minutes) |
|---------------|-------|---------------|
| a             | 180   | 1             |
| b             | 360   |               |
| c             | 540   |               |
| d             | 180   | 2             |
| e             | 360   |               |
| f             | 540   |               |
| g             | 180   | 3             |
| h             | 360   |               |
| i             | 540   |               |

3.2.2. Preparation of plant extracts standard concentrations

Aqueous extract was dissolved in sterile distilled water, while alcoholic extracts were dissolved in Di-Methyl Sulphoxide (DMSO). Thus 200 mg/ml of stock was obtained as a standard concentration of aqueous and alcoholic extracts. Aqueous extracts were sterilized using 0.22 μm membrane filters and alcoholic extracts were pasteurization for 15 minutes at temperature 62 °C (Muhammad & Muhammad 2005).
3.2.3. Preparation of inoculums

According to (Mohammed et al., 2015) stock cultures were maintained at 4°C on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring 0.5ml of culture to 5 ml of nutrient broth and incubated at 37°C for 24 hours. The optical density of each active culture was adjusted to 0.1 at 625 nm using fresh broth to give a standard inoculums of 10⁶ colony forming units (cfu) per ml (Alzoreky & Nakahara 2003).

3.2.4. Antibiotics activity assay

The antibiotic sensitivity of the isolates was determined using the disc diffusion method. Standardized inoculum (100μl) of the overnight grown nutrient broth cultures were spread on Mueller-Hinton agar plates using sterile swabs. The plates were dried at room temperature for 10 min, before placing the antibiotic discs at equidistance. The plates were incubated for 24 h at 37°C and the diameter of zone of inhibition was measured (Mabrouk, 2012; Donald, 2006). Organisms were classified as sensitive, intermediate or resistant, based on the NCCLS standards. A total of 8 antibiotics were used in this study as shown in Table 3.1.

3.2.5. Plant extracts activity assay

3.2.5.1. Paper Disk Diffusion Assay

A modification of previously described procedures (Manoj, 2009) was followed to evaluate of antibacterial activity of plant extracts. Standardized inoculums of each bacterium, i.e., 10⁶ CFU ( Colony Forming Units)/ml to 0.1 at 625 nm was introduced onto the surface of sterile Nutrient agar plates and a sterile cotton swab was used for even distribution of inoculums. After a few minutes, sterile filter paper discs of 5 mm diameter were placed on the surface of inoculated and labeled nutrient agar plates and impregnated with 20 μL of known concentration of extracts (200 mg /ml) for aquatic and ethanolic extracts. Sterile paper discs containing Dimethyl sulfoxide alone was served as negative control. The plates were placed at 4°C for 2 h. and then subsequently incubated at 37° C for 24 Hrs. After incubation, the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. For each test solution, three replicates were maintained.
3.2.5.2. Synergism/Antagonism between plant extract and antibiotics

A modification of previously described procedures (Mohammed et al., 2015) was followed to evaluate of the synergistic effect. The bacterial cultures were grown in sterile nutrient broth medium at 37º C. After 4 h of growth, standardized inoculums of each bacterium, i.e., 10^6 CFU /ml to 0.1 at 625 nm was introduced onto the surface of sterile Nutrient agar plates and a sterile cotton swab was used for even distribution of inoculums. After a few minutes, the antibiotic filter paper disk of 5 mm in diameter were placed on the surface of inoculated and labeled Nutrient agar plates and impregnated with 20 μL of known concentration of extracts (200 mg /ml for aquatic and ethanolic extracts. The plates were incubated at 37º C for 24 h. The diameters of cleared zones were measured and compared with that of the antibiotic alone. For each test solution, three replicates were maintained.

3.3. Statistical Analysis:

All data were expressed as the mean ± standard deviation (SD) by measuring three independent replicates. One-way analysis of variance (SAS, 1990; ANOVA procedure).
Chapter 4
Results
CHAPTER 4

Results

4.1. Evaluation of antibiotics activity

4.1.1 Against E. coli

By disc plate method (section 3.2.4) the effectiveness of a range of antibiotics was determined against E. coli (Table 4.1). It was resistance to all antibiotics Cefotaxime, Ofloxacin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, ceftriaxon, chloramphenicol and Amikacin.

4.1.2 Against S. aureus

As shown in (Table 4.1and Figure 4.1); Ofloxacin, Levofloxacin and Nitrofurantoin had the highest inhibition zone (25 mm) followed by Cefotaxime (22 mm). While there was no effect of Ciprofloxacin, Ceftriaxon, Chloramphenicol & Amikacin against S. aureus.

| Microorganism | Antibiotics | E. coli | S. aureus |
|---------------|-------------|---------|-----------|
|               | Inhibition zone (mm) |          |           |
| Ofloxacin     | -ve         | 25±1.0* | -ve       |
| Ceftriaxone   | -ve         | -ve     | -ve       |
| Ciprofloxacin | -ve         | 22±1.58*| -ve       |
| Cefotaxime    | -ve         | -ve     | -ve       |
| Amikacin      | -ve         | -ve     | -ve       |
| Chloramphenicol| -ve      | -ve     | -ve       |
| Levofloxacin  | -ve         | 25±0.7* | -ve       |
| Nitrofurantoin| -ve         | 25 ±2.0*| -ve       |

mm= millimeter, *Mean ± Standard Deviation, n=3, -ve= Negative effect
Figure (4.1): Inhibition zone (mm) of some antibiotics against S. aureus

F:Nitrofurantoin ; OFX:Ofloxacin ; LEV: Levofoxacin ; AK: Amikacin ; CTX: Cefotaxim ; CI:Ciprofloxacin ; C:Chloramphinicol

4.2. Evaluation of antibacterial activity of plant extracts:

The results of antibacterial activity of aquatic and ethanolic extracts of all the three plants when tested individually for their antibacterial activity against the four isolated bacterial species, which are known to cause infection in humans shown below.

4.2.1. Evaluation of antibacterial activity of F. sycomorus extracts:

Antibacterial activity of F. sycomorus extracts against S. aureus and E.coli

There was no significant antibacterial activity of aquatic and ethanolic F. sycomorus extracts that extracted by MW and soxhlet methods against clinical isolated S. aureus, and E. coli.

4.2.2. Evaluation of antibacterial activity of L. inermis extracts:

There was no significant antibacterial activity of aquatic and ethanolic L. inermis extracts that extracted by MW and soxhlet methods against clinical isolated bacteria.

4.2.3. Evaluation of antibacterial activity of G. glabra extracts:

4.2.3.1. Antibacterial activity of G. glabra extracts against S. aureus:
The disc diameters of zone of inhibition of *G. glabra* extracts against *S. aureus* and are shown in table 4.2.

There was no significant antibacterial activity of aquatic and ethanolic *G. glabra* extracts that extracted by MW and soxhlet methods against clinical isolated *S. aureus*.

### 4.2.3.2. Antibacterial activity of *G. glabra* extracts against *E. coli*:

The disc diameters of zone of inhibition of *G. glabra* extracts against *E. coli* are shown in table 4.2.

- **Soxhlet extracts:**
  - **The aquatic extracts:**
    A significant antibacterial activity of aquatic *G. glabra* extract were found against pathogenic *E. coli* (inhibitory zone 15mm).
  - **The ethanolic extracts:**
    A significant antibacterial activity of ethanolic *G. glabra* extract were found against pathogenic *E. coli* (inhibitory zone 17mm).

- **Microwave extracts:**
  - **The aquatic extracts:**
    Samples extracted in tow and three minutes c, d, e, f, g, h & I of aquatic *G. glabra* extracts were showed antibacterial activity against pathogenic *E. coli* (inhibitory zone 15mm).
  - **The ethanolic extracts:**
    Samples extracted in tow and three minutes d, e, f, g, h & I of aquatic *G. glabra* extracts were showed antibacterial activity against pathogenic *E. coli* (inhibitory zone 17mm). samples a,b & c had (inhibitory zone 15mm).
### Table (4.2.): Antibacterial Effect of *G. glabra* Extracts Against *S. aureus* and *E. coli* in mm

| Plant extract | Extraction method | Solvent | Sample symbol | Power (W) | Time (min) | Inhibition zone in (mm) | *S. aureus* | *E. coli* |
|---------------|-------------------|---------|---------------|-----------|------------|-------------------------|-------------|----------|
| *G. glabra*   | Soxhlet D.W.      | C       | -             | 8 hr.     | -ve        | 15±0.7*                 |             |          |
|               | Ethanol           | C       | -             | 8 hr.     | -ve        | 17±1.0*                 |             |          |
|               |                   | a       | 180           | 1         | -ve        | -ve                     |             | -ve      |
|               |                   | b       | 360           | 1         | -ve        | -ve                     |             | -ve      |
|               |                   | c       | 540           | 2         | -ve        | -ve 15±0.7*             |             |          |
|               |                   | d       | 180           | 2         | -ve        | -ve 15±0.7*             |             |          |
|               |                   | e       | 360           | 2         | -ve        | -ve 15±0.7*             |             |          |
|               |                   | f       | 540           | 2         | -ve        | -ve 15±1.0*             |             |          |
|               |                   | g       | 180           | 3         | -ve        | -ve 15±0.0*             |             |          |
|               |                   | h       | 360           | 3         | -ve        | -ve 15±0.0*             |             |          |
|               |                   | i       | 540           | 3         | -ve        | -ve 15±1.5*             |             |          |
|               | Microwave D.W.    | C       | -             | 8 hr.     | -ve        | 15±0.7*                 |             |          |
|               | Ethanol           | a       | 180           | 1         | -ve        | 15±1.0*                 |             |          |
|               |                   | b       | 360           | 1         | -ve        | 15±1.0*                 |             |          |
|               |                   | c       | 540           | 1         | -ve        | 15±0.7*                 |             |          |
|               |                   | d       | 180           | 2         | -ve        | 17±0.7*                 |             |          |
|               |                   | e       | 360           | 2         | -ve        | 17±0.7*                 |             |          |
|               |                   | f       | 540           | 2         | -ve        | 17±2.0*                 |             |          |
|               |                   | g       | 180           | 3         | -ve        | 17±1.0*                 |             |          |
|               |                   | h       | 360           | 3         | -ve        | 17±1.0*                 |             |          |
|               |                   | i       | 540           | 3         | -ve        | 17±0.0*                 |             |          |

mm= millimeter, *Mean ± Standard Deviation, n=3, -ve= Negative effect*
4.3. Evaluation the Synergistic/Antagonistic Effect

4.3.1. The Synergistic/Antagonistic Effect between *F. sycomorus* extracts and Antibiotics

We evaluated in vitro synergism between extracts of *F. sycomorus* and antimicrobial drugs disks against *S. aureus* and *E. coli* using disk diffusion method as mentioned in (section 3.2.5.2).

4.3.1.1. The Synergistic/Antagonistic Effect between *F. sycomorus* extracts and Antibiotics against *S. aureus*:

The disc diameters of zone of inhibition of synergistic effect between *F. sycomorus* extracts and antibiotics against *S. aureus* are shown in Table 4.3, Figures 4.5-4.9.

- **Soxhlet extracts and antibiotics:**
  
  **The aquatic extracts:**

  As shown in Table 4.3, *F. sycomorus* extract has a synergistic effect on *S. aureus* when added on Ceftriaxon disk (inhibition zone 12mm). With the rest antibiotics there was either no effect or there was antagonism.

  **The ethanolic extracts:**

  A dilution effect of ethanolic *F. sycomorus* extract were found against clinical isolated *S. aureus* when added on Cefotaxim disk (inhibition zone 20mm), followed by Nitrofurantoin (17mm). With the rest antibiotics there was either no effect or there was antagonism.

- **Microwave extracts:**

  **The aquatic extracts:**

  As shown in Table 4.3, *F. sycomorus* extracted samples d, e, f, g, h, and I has a synergistic effect on *S. aureus* when added on Ceftriaxon disk (15mm). With the rest antibiotics there was either no effect or there was antagonism.

  **The ethanolic extracts:**
The extracted samples d, e, f, g, h, and I showed the best synergistic effect on *S. aureus* when added on Amikacin disks (inhibition zone 21mm). Samples b, c, d, e, f, g, h, and I has a dilution effect with Cefotaxim and Ofloxacin (inhibition zone 21mm). Also, sample-c showed dilution effect with Nitrofurantoin (15mm) and (17mm) with sample-g.
Figure (4.4): Inhibition zone (mm) of aquatic *F. Sycomorus* extracts in combination with Cefotaxim against *S. aureus*.

Figure (4.5): Inhibition zone (mm) of aquatic *F. sycomorus* extracts in combination with Ceftriaxon against *S. aureus*.

Figure (4.6): Inhibition zone (mm) of ethanolic *F. sycomorus* extracts in combination with Nitrofurantoin against *S. aureus*.

Figure (4.7): Inhibition zone (mm) of ethanolic *F. sycomorus* extracts in combination with Amikacin against *S. aureus*.

Figure (4.8): Inhibition zone (mm) of ethanolic *F. sycomorus* extracts in combination with Ofloxacin against *S. aureus*. 
Table (4.3.): Synergistic/Antagonistic Effect of *F. sycomorus* Extracts and Antibiotics Against *S. aureus* in mm

| P.E Extraction method | Solvent | S.S | Power (W) | Time (min) | Synergism with | CTR | CTX | OFX | LEV | AK | F |
|-----------------------|---------|-----|-----------|------------|---------------|-----|-----|-----|-----|----|---|
| **Soxhlet**           | D.W.    | A   | -         | 8 hr.      |               | -ve | -ve | -ve | -ve | -ve | -ve |
| Ethanol               |         | A   | -         | 8 hr.      |               | -ve | 20±0.7* | -ve | -ve | -ve | 17±2.0* |
| **F. sycomorus**      |         |     |           |            |               |     |     |     |     |     |    |
| D.W.                  |         |     |           |            |               |     |     |     |     |     |    |
| Soxhlet               |         |     |           |            |               |     |     |     |     |     |    |
| a                     | 180     |     |           |            |               | -ve | -ve | -ve | -ve | -ve | -ve |
| b                     | 360     |     |           |            |               | -ve | -ve | -ve | -ve | -ve | -ve |
| c                     | 540     |     |           |            |               | -ve | -ve | -ve | -ve | -ve | -ve |
| d                     | 180     |     |           |            |               | 15±1.0* | -ve | -ve | -ve | -ve | -ve |
| e                     | 360     |     |           |            |               | 15±1.0* | -ve | -ve | -ve | -ve | -ve |
| f                     | 540     |     |           |            |               | 15±1.0* | -ve | -ve | -ve | -ve | -ve |
| g                     | 180     |     |           |            |               | 15±0.0* | -ve | -ve | -ve | -ve | -ve |
| h                     | 360     |     |           |            |               | 15±0.0* | -ve | -ve | -ve | -ve | -ve |
| i                     | 540     |     |           |            |               | 15±2.0* | -ve | -ve | -ve | -ve | -ve |
| Ethanol               |         |     |           |            |               |     |     |     |     |     |    |
| Microwave             |         |     |           |            |               |     |     |     |     |     |    |
| a                     | 180     |     |           |            |               | -ve | -ve | -ve | -ve | -ve | -ve |
| b                     | 360     |     |           |            |               | -ve | -ve | 7±1.0* | -ve | -ve | -ve |
| c                     | 540     |     |           |            |               | -ve | 17±0.7* | 21±1.5* | -ve | -ve | 15±1.0* |
| d                     | 180     |     |           |            |               | -ve | 21±2.0* | 21±0.0* | -ve | 21±0.7* | -ve |
| e                     | 360     |     |           |            |               | -ve | 21±0.0* | 21±1.0* | -ve | 21±0.7* | -ve |
| f                     | 540     |     |           |            |               | -ve | 21±0.0* | 21±2.0* | -ve | 21±1.0* | -ve |
| g                     | 180     |     |           |            |               | -ve | 21±1.0* | 21±0.7* | -ve | 21±1.5* | 17±1.0* |
| h                     | 360     |     |           |            |               | -ve | 21±1.5* | 21±0.7* | -ve | 21±1.5* | -ve |
| i                     | 540     |     |           |            |               | -ve | 21±1.0* | 21±0.0* | -ve | 21±0.0* | -ve |

mm= millimeter, *Mean ± Standard Deviation, n=3, -ve= Negative effect, D.W.: Distilled water. P.E.: Plant Extract, S.S.: Sample Symbol, F:Nitrofurantoin ; OFX:Ofloxacin ; LEV: Levofloxacin ; AK: Amikacin ; CTX: Cefotaxim.

4.3.1.2. The Synergistic/Antagonistic Effect between *F. sycomorus* extracts and Antibiotics against *E. coli*:

The disc diameters of zone of inhibition of *F. sycomorus* extracts against *E. coli* are shown in Table 4.4. and Figure 4.11.

- Soxhlet extracts:

  The aquatic extracts:
Aquatic *F. sycomorus* extract showed a synergistic effect with the only antibiotic Ceftriaxon (inhibition zone 16mm) against pathogenic *E. coli*.

**The ethanolic extracts:**

Ethanolic *F. sycomorus* extract showed no synergism with all antibiotics against pathogenic *E. coli*.

- **Microwave extracts:**

  **The aquatic extracts:**

  Aquatic *F. sycomorus* extracts samples- d, e, f, g, h, & I were showed a synergistic effect with the only antibiotic Ceftriaxon (inhibition zone 17mm) against pathogenic *E. coli*.

  **The ethanolic extracts:**

  Ethanolic *F. sycomorus* extracts of all samples showed no synergism with all antibiotics against pathogenic *E. coli*.

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**Figure (4.9):** Inhibition zone (mm) of aquatic *F. sycomorus* extracts in combination with Ceftriaxon against *E. coli*. 

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Table (4.4.): Synergistic/Antagonistic Effect of *F. sycomorus* Extracts and Antibiotics Against *E. coli* in mm

| P.E  | Extraction method | Solvent | S.S | Power (W) | Time (min) | *E. coli* | Synergism with |
|------|-------------------|---------|-----|-----------|------------|-----------|--------------|
|      |                   |         |     |           |            | CTR       | CTX          | OFX          | LEV         | AK         | F         |
|      |                   |         |     |           |            |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
| F. sycomorus | Soxhlet | D.W. | A   | -         | 8 hr.      | 16±0.0*   | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | Ethanol | A      | -   | 8 hr.     | 1          |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | a          | 180    |     |           |             |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | b          | 360    |     |           |             |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | c          | 540    |     |           |             |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | d          | 180    |     |           | 1          |           | 17±1.5*      | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | e          | 360    |     |           | 2          |           | 17±0.7*      | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | f          | 540    |     |           |            |           | 17±0.7*      | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | g          | 180    |     |           | 3          |           | 17±0.0*      | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | h          | 360    |     |           |            |           | 17±2.0*      | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | i          | 540    |     |           |            |           | 17±1.5*      | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | Microwave | D.W. | A   | -         | 8 hr.      | 16±0.0*   | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | Ethanol | A      | -   | 8 hr.     | 1          |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | a          | 180    |     |           |             |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | b          | 360    |     |           |             |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | c          | 540    |     |           |             |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | d          | 180    |     |           | 1          |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | e          | 360    |     |           | 2          |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | f          | 540    |     |           |            |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | g          | 180    |     |           | 3          |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | h          | 360    |     |           |            |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |
|      | i          | 540    |     |           |            |           | -ve          | -ve          | -ve          | -ve         | -ve        | -ve       |

mm= millimeter, *Mean ± Standard Deviation, n=3, -ve= Negative effect, D.W.: Distilled water. P.E.: Plant Extract, S.S.: Sample Symbol; F:Nitrofurantoin; OFX:Ofloxacin; LEV: Levofloxacin; AK: Amikacin; CTX: Cefotaxim.

### 4.3.2. The Synergistic/Antagonistic Effect between *L. inermis* extracts and Antibiotics

We evaluated in vitro synergism/antagonism between extracts of *L. inermis* and antimicrobial drugs disks against *S. aureus* and *E. coli* using disk diffusion method as mentioned in (section 3.2.5.2).

#### 4.3.2.1. The Synergistic/Antagonistic Effect between *L. inermis* extracts and Antibiotics against *S. aureus*:

- **Table (4.4.): Synergistic/Antagonistic Effect of *F. sycomorus* Extracts and Antibiotics Against *E. coli* in mm**

  - Table content as shown in the original text.

  - Table notes and abbreviations as described in the original text.

  - Table description as shown in the original text.

  - Table format as shown in the original text.
The disc diameters of zone of inhibition of synergistic/antagonistic effect between *L. inermis* extracts and antibiotics against *S. aureus* are shown in Table 4.5. Figure 4.12

- **Soxhlet extracts and antibiotics:**

  **The aquatic extracts:**

  Aquatic *L. inermis* extract showed no synergism with all antibiotics against pathogenic *S. aureus*.

  **The ethanolic extracts:**

  The only antibiotic Nitrofurantoin disk showed a dilution effect with ethanolic *L. inermis* extract against pathogenic *S. aureus* (inhibition zone 17mm).

- **Microwave extracts and antibiotics:**

  **The aquatic extracts:**

  Aquatic *L. inermis* extract showed no synergism with all antibiotics against pathogenic *S. aureus*.

  **The ethanolic extracts:**

  The only samples- c & g of ethanolic *L. inermis* extracts were showed a dilution effect with the only antibiotic Nitrofurantoin disk against pathogenic *S. aureus*, sample-g has inhibition zone (17mm) followed by sample-b (15mm).
**Table (4.5.):** Synergistic/Antagonistic Effect of *L. inermis* Extracts and Antibiotics Against *S. aureus* in mm

| P.E | Extraction method | Solvent | S.S | Power (W) | Time (min) | Synergism with | CTR | CTX | OFX | LEV | AK | F |
|-----|-------------------|---------|-----|-----------|------------|----------------|-----|-----|-----|-----|----|---|
|     | Soxhlet           | D.W.    | B   | -         | 8 hr.      | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   | Ethanol | B   | -         | 8 hr.      | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | a   | 180       | 1          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | b   | 360       | 1          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | c   | 540       | 1          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | d   | 180       | 2          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | e   | 360       | 2          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | f   | 540       | 2          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | g   | 180       | 3          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | h   | 360       | 3          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | i   | 540       | 3          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     | Microwave         | D.W.    | a   | 180       | 1          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | b   | 360       | 1          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | c   | 540       | 1          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | d   | 180       | 2          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | e   | 360       | 2          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | f   | 540       | 2          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   | Ethanol | g   | 180       | 3          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | h   | 360       | 3          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |
|     |                   |         | i   | 540       | 3          | -ve -ve -ve -ve -ve -ve |     |     |     |     |    |   |

*Figure (4.10):* Inhibition zone (mm) of ethanolic *L. inermis* extracts in combination with Nitrofuantoin against *S. aureus*.
Continued Table (4.5.):

mm= millimeter, *Mean ± Standard Deviation, n=3, -ve= Negative effect, D.W.: Distilled water.  P.E.: Plant Extract, S.S.: Sample Symbol, F:Nitrofurantoin ; OFX:Ofloxacin ; LEV: Levofloxacin ; AK: Amikacin ; CTX: Cefotaxim.

4.3.2.2. The Synergistic/Antagonistic Effect between L. inermis extracts and Antibiotics against E. coli:

The disc diameters of zone of inhibition of synergistic/Antagonistic effect between L. inermis extracts and antibiotics against E. coli are shown in Table 4.6, Figures 4.14 - 4.17.

- Soxhlet extracts and antibiotics:

  **The aquatic extracts:**

  Aquatic L. inermis extract showed no synergism with all antibiotics against pathogenic E. coli.

  **The ethanolic extracts:**

  The antibiotic Amikacin disk showed a highest synergistic effect with ethanolic L. inermis extract against pathogenic E. coli (inhibition zone 20mm). And (14mm) with Levofloxacin, also (13mm) with Ofloxacin, followed by the lowest synergism (11mm) with Cefotaxim.

- Microwave extracts and antibiotics:

  **The aquatic extracts:**

  Aquatic L. inermis extract showed no synergism with all antibiotics against pathogenic E. coli.

  **The ethanolic extracts:**

  The antibiotic Amikacin disk was showed a highest synergistic effect with ethanolic L. inermis extracts samples- c, d, e, f, g, h, &I against pathogenic E.
coli (inhibition zone 20mm). With Levofloxacin samples-e, f, g, h, &I showed an inhibition zone (16mm), but samples- c &d had (inhibition zone 14mm). Also, with Ofloxacin samples- d, e, f, g, h, &I showed (inhibition zone 13mm), except sample-c has (inhibition zone 11mm). Followed by the lowest synergism (9mm) for sample-c with Cefotaxim and (11mm) for samples- d, e, f, g, h, &I . With the rest antibiotics there was either no effect or there was antagonism.

**Figure (4.11):** Inhibition zone (mm) of ethanolic *L. inermis* extracts in combination with Ofloxacin against *E. coli*.

**Figure (4.12):** Inhibition zone (mm) of ethanolic *L. inermis* extracts in combination with Levofloxacin against *E. coli*.

**Figure (4.13):** Inhibition zone (mm) of ethanolic *L. inermis* extracts in combination with Cefotaxim against *E. coli*.

**Figure (4.14):** Inhibition zone (mm) of ethanolic *L. inermis* extracts in combination with Amikacin against *E. coli*. 
Table (4.6.): Synergistic/Antagonistic Effect of *L. inermis* Extracts and Antibiotics Against *E. coli* in mm

| Synergism with | CTR | CTX | OFX | LEV | AK | F |
|----------------|-----|-----|-----|-----|----|---|
| Soxhlet D.W. B - 8 hr. | -ve | -ve | -ve | -ve | -ve | -ve |
| Ethanol B - 8 hr. | -ve | 11±1.0* | 13±0.7* | 14±0.7* | 20±1.5* | -ve |
| D.W. | | | | | | |
| A 180 | 1 | -ve | -ve | -ve | -ve | -ve |
| b 360 | -ve | -ve | -ve | -ve | -ve | -ve |
| c 540 | -ve | -ve | -ve | -ve | -ve | -ve |
| d 180 | 2 | -ve | -ve | -ve | -ve | -ve |
| e 360 | -ve | -ve | -ve | -ve | -ve | -ve |
| f 540 | -ve | -ve | -ve | -ve | -ve | -ve |
| g 180 | 3 | -ve | -ve | -ve | -ve | -ve |
| h 360 | -ve | -ve | -ve | -ve | -ve | -ve |
| i 540 | -ve | -ve | -ve | -ve | -ve | -ve |
| Microwave D.W. | | | | | | |
| a 180 | 1 | -ve | -ve | -ve | -ve | -ve |
| b 360 | -ve | -ve | -ve | -ve | -ve | -ve |
| c 540 | -ve | 9±0.7* | 11±2.0* | 14±1.5* | 20±0.0* | -ve |
| d 180 | 2 | -ve | 11±0.7* | 13±1.5* | 14±1.5* | 20±1.0* | -ve |
| e 360 | -ve | 11±1.0* | 13±0.0* | 16±1.5* | 20±1.5* | -ve |
| f 540 | -ve | 11±0.0* | 13±0.7* | 16±0.7* | 20±0.7* | -ve |
| g 180 | 3 | -ve | 11±0.0* | 13±0.7* | 16±0.7* | 20±0.7* | -ve |
| h 360 | -ve | 11±1.5* | 13±1.0* | 16±1.0* | 20±0.7* | -ve |
| i 540 | -ve | 11±2.0* | 13±1.0* | 16±1.0* | 20±1.5* | -ve |

mm = millimeter, *Mean ± Standard Deviation, n=3, -ve = Negative effect, D.W.: Distilled water. P.E.: Plant Extract, S.S.: Sample Symbol, F: Nitrofurantoin; OFX: Ofloxacin; LEV: Levofloxacin; AK: Amikacin; CTX: Cefotaxim.

4.3.3. The Synergistic/Antagonistic Effect between *G. glabra* extracts and Antibiotics

We evaluated in vitro synergism/Antagonism between extracts of *G. glabra* and antimicrobial drugs disks against *S. aureus* and *E. coli* using disk diffusion method as mentioned in (Section 3.2.5.2). There were no synergism effect for all soxhlet and microwave samples (aquatic & ethanolic) with all antibiotics against *S. aureus*. An antagonistic effect were shown against *E. coli* because the aquatic and ethanolic extract showed antimicrobial activity as alone against it.
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Discussion

5.1. Evaluation of antibacterial activity of plant extracts

In our experiments, MW and soxhlet methods was used to extract the bioactive compounds of plants. Both of these methods have comparable effect in the inhibition zone values results, and sometimes MW samples was showed the best.

It was also noticed that the ethanolic extract has greater effect in the inhibition than aqueous extract, which may be due to the fact that alcohol is the best solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts, this explanation is in agreement with the study of (Mohammed et al., 2015).

The difference results in antibacterial activity of a plant extract might be attributable to the parameters (solvent nature, irradiation time, microwave power and temperature) were systematically studied for set up of the optimal extraction conditions as in agreement with the study of (Faridah et al., 2010).

Where the samples with no inhibition zone is probably because the paper disc retains the active component and does not allow it to diffuse into the nutrient agar, because some compounds does not diffuse in the agar especially non polar compounds, this interpretation is in agreement with the study of (Olila et al., 2001).

5.1.1. Antibacterial activity of F. sycomorus

The absence of effectiveness to F. sycomorus on S. aureus and E. coli, even it has a very strong synregestic effect, which may probably due to reuse of Ficus by human that may lead to increase bacterial resistant to it even it has an effective antibacterial ingradients. Similar negative results of aquatic extracts given by (Mohamed et al., 2016; Mohammed et al., 2015). Other studies (Basel et al., 2015; Mohammed et al., 2015; Mosad et al., 2015; Mohamed et al., 2016) showed a positive result against E. coli and S. aureus, where they used aceton or methanol as a solvent; which may lead to increasing of solubility of different bioactive compounds and thus increasing the activity of the extract.
5.1.2. Antibacterial activity of *L. inermis*

The Lawsonia ethanolic extracts did not show any activity against *S. aureus* and *E. coli*. Similar results have been given by (Al-Mehna & Kadhum 2013). Other studies (Abdelraouf et al., 2011; EL-Zawahry et al., 2013) showed a positive results against *S. aureus* and *E. coli*, where they used aceton or methanol as a solvent through extraction process.

5.1.3. Antibacterial activity of *G. glabra*

Both of the aqueous and the ethanolic extracts of *G. glabra* for both methods used in this study had a strong antibacterial activity against *E. coli*. These results are in agreement with (Aparajita et al., 2013; Patil et al., 2009; Manoj et al., 2010; Yusra et al., 2013) results., but *S. aureus* was resistance in our experements compared with the other studies (Chitra et al., 2012; Mahboubeh et al., 2010) which have shown antibacterial activity of ethanolic *G. glabra* extract; it may due to using another method (cold percolation extraction process) without using heat which helping in degradation of labile bioactive compounds. Other studies (Prabhat et al., 2010) used aceton and methanol as a solvent in extraction process and had shown antibacterial activity against *S. aureus*. Also, other research (Rasha et al., 2012) worked in (Separation, fractionation and purification of the active antimicrobial components from *G. glabra*) and had shown antibacterial activity against *S. aureus*. In addition, a study by (Mona et al., 2015) used solvent solution (20 %methanol: 80 % distilled water) in extraction process and her results had shown antibacterial activity against *S. aureus* and *E. coli*.

5.2. Evaluation of the synergistic/Antagonistic effect

In our research, the plant extracts had different synergistic ability to inhibit the growth of microorganism depending on the method of extraction. Plants antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug. It has been known that one of the effective approaches to overcome bacterial resistance is restoration of antibiotic activity through the synergistic action of antibacterial materials from natural and synthesized agents. Drug synergism between known antibiotics and bioactive plant extracts is a
novel concept and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome). Despite the abundant literature about the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been used for clinical use as antibiotics, this interpretation is in agreement with (Mohamed et al. 2013). Antagonism occurs when the effect of extract and drug together is less than the effect of either alone and indifference when no effect is exhibited (Sumitra & Kalpna 2011).

5.2.1. The synergistic/Antagonistic effect between *F. sycomorus* extracts and antibiotics

Against pathogenic *S. aureus*, the only antibiotic Ceftriaxon (cell wall synthesis inhibitor) had a maximum synergistic effect with MW aquatic extracts samples (inhibitory zone 15mm). This value is shown better than soxhlet aquatic sample which has a synergistic effect (inhibitory zone 12mm). The other antibiotics had antagonistic effect because they shown an inhibition zone when used as alone. In addition, MW ethanolic extracts samples showed a maximum synergistic effect with Amikacin (protein synthesis inhibitor) with (inhibitory zone 21mm) against *S. aureus*. This value shown better than the soxhlet ethanolic extracts samples which showed no synergistic effect, but only a dilution effect with Cefotaxim and Nitrofurantoin with (inhibitory zone 20mm and 17mm respectively). As for Nitrofurantoin showed a dilution effect with sample-c (MW ethanolic extract; extracted in one minute and power 180) as (inhibitory zone 15mm) and both of sample-g (extracted in three minutes and power 180) and soxhlet ethanolic extract as (inhibitory zone 17mm), while both dilution effect were against *S. aureus*. As for Ofloxacin and Cefotaxim showed a dilution effect (inhibitory zone 21mm), but an antagonistic effect shown with Levofloxacin. Similar synergism results were given by (Mohammed et al., 2015) against pathogenic *S. aureus*. On the other hand, similar antagonism results were given by (Eze et al., 2013) for Picralima nitida extracts which exhibited high levels of antagonism with Ciprofloxacin and Norfloxacin against almost all the tested bacteria. Also, a study by (Saaadoun et al., 2014) shown antagonistic effect of Propois juliflora.

In the case of against *E. coli*, the only antibiotic Ceftriaxon (cell wall synthesis inhibitor) has a maximum synergistic effect with MW aquatic extracts samples
(inhibitory zone 17mm) against *E. coli*, this value is shown better than soxhlet aquatic sample that has a synergistic effect (inhibitory zone 16mm). Similar synergism results with aquatic extract results were given by (Mohammed et al., 2015) against pathogenic *E. coli*. The other antibiotics showed antagonistic effect when combined with aquatic or ethanolic extracts for both MW and soxhlet method against *E. coli*, this is in agreement with a study by (Eze et al., 2013).

In addition, we noticed that each of Ceftriaxon, Amikacin and *F. sycomorus* extract as alone was not give any activity against *S. aureus* or *E. coli*, but as in synergism were shown high activity

In general, as shown, the values of inhibitory zone results of MW aquatic and especially ethanolic extracts samples were better than the samples extracted by conventional method (soxhlet method in this study and some of previous studies that mentioned above)

5.2.2. The Synergistic/Antagonistic Effect between *L. inermis* extracts and Antibiotics

The antibiotic Nitrofurantoin (nucleic acid inhibitor) has a dilution effect with MW ethanolic extract sample-c (extracted in one minute and power 180) as (inhibitory zone 15mm) and both of sample-g (extracted in three minutes and power 180) and soxhlet ethanolic extract showed as (inhibitory zone 17mm) against pathogenic *S. aureus*. The results were obtained by (Ghaleb et al., 2009) who showed a asynergistic effect with ethanolic extract and protein synthesis inhibitor drugs. The antagonistic effect shown with the other antibiotics which were had high activity when used as alone.

The antibiotic Amikacin has a maximum synergistic effect with both MW and soxhlet ethanolic extracts against *E. coli* as (inhibitory zone 20mm).

As for Ofloxacin showed a synergistic effect with both MW and soxhlet ethanolic extracts as (inhibitory zone 13mm) against *E. coli*. While Levofoxacin showed (inhibitory zone 16mm) against *E. coli*. The lowest synergisem shown with Cefotaxim as (inhibitory zone 11mm). Similar results obtained by (Iqbal & Farrukh 2007) had
shown a synergistic effect with some nucleic acid inhibitor drugs against pathogenic *E. coli*.

### 5.2.3. The Synergistic/Anatgonistic Effect between *G. glabra* extracts and Antibiotics

Our results in this study showed no significant synergistic effect for all antibiotics with MW and soxhlet for aquatic and ethanolic extracts samples against *S. aureus*. But shown an antagonistic effect against *E. coli* because the aquatic and ethanolic extract showed antimicrobial activity as alone against it. In the other hand, other study by (Dawoud et al. 2013) who used triple extract mixtures (*G. glabra* was once of the mixture) with resulted in a synergistic effect upon antibiotics against *S. aureus* strains. Another study (Mona et al., 2015) used *G. glabra* methanolic extract showed a synergistic effect when mixed with other plant extracts against several types of bacteria.

### 5.3. Evaluation of parameters affecting MW extraction process

In this study, the effects of several influential extraction parameters (solvent nature, irradiation time, microwave power and temperature) were systematically studied for set up of the optimal extraction conditions (Farid & Giancarlo 2013; Faridah et al., 2010). The aim of optimization of these parameters is to obtain the maximum bioactive compounds (terpenes, phenols and flavonoids) which acting as antibacterial agents (Michael 2015).

#### 5.3.1. Effect of Solvent System

As mentioned in section 2.1.4.1, and through evaluation of antimicrobial activity of plant extracts and in synergisim, our results which obtained from ethanolic extracts (ethanol as a solvent) had shown better results than that obtained from aquatic extracts (water as a solvent). In addition most of aquatic extracts results had shown no inhibition zone. The same solvent system of samples used in extraction process in both methods MW and soxhlet in this study had shown mostly the same inhibition zone results, and some tests of MW samples had shown better than soxhlet samples. So, because of high capacity with high dielectric constant, ethanol is the best solvent in our experiments of extraction process. This evaluation is in agreement with (Noelia
et al, 2014; Farid & Giancarlo 2013; Faridah et al., 2010; Jila et al., 2011; Mahaveer et al., 2009; Ajanta et al., 2010).

5.3.2. Effect of Irradiation Time

As mentioned in section 2.1.4.2, and through evaluation of antimicrobial activity of plant extracts and in synergism, our results showed that, the inhibition zone is increased by increasing the time of extraction till reaching in a steady state values of inhibition zone. This may due to increasing of the time of extraction which lead to increasing of the yield of bioactive compounds till to reach a saturation although of increasing a time. The samples of our plants in this study which extracted in one minutes were in mostly had shown no inhibition zone except samples extracted by using a power 540w which had shown a weak inhibition zone. But in general the samples extracted in tow and three minutes had shown a maximum value and the same results of inhibition zone. So we can considered that the best with optimum time of extraction is a sample-d which extracted in tow minutes and had shown a maximum inhibition zone with a lowest power 180w.

Our results showed that, the MW method gave better results or at least same outputs as soxhlet method. We recommended MW method since it provides bioactive compounds in a very short time, mostly few minutes and a very low power. Other conventional methods may attributed to a degradation of bioactive compounds in long time of extraction process of conventional methods. This evaluation is in agreement with (Noelia et al, 2014; Farid & Giancarlo 2013; Faridah et al., 2010; Jila et al., 2011; Mahaveer et al., 2009; Ajanta et al., 2010).

5.3.3. Effect of Microwave Power and Temperature

As mentioned in section 2.1.4.3, and through evaluation of antimicrobial activity of plant extracts and synergism effect, our results showed that, the inhibition zone is increased by increasing the MW power of extraction process till reaching in a steady state results of inhibition zone. This may due to increasing of the MW power of extraction which lead to increasing of the yield of bioactive compounds till reaching a saturation although of increasing MW power. The samples of our plants in this study which extracted in one minutes were in mostly showed no inhibition zone, although of
increasing of MW power except some samples extracted by using a power 540w which showed a weak of inhibition zone. But in mostly, they didn't show any effect by increasing MW power for samples extracted in tow and three minutes which showed a maximum and the same results of inhibition zone. So we can considered that the best with optimum MW power of extraction is a sample-d which extracted at lowest power 180w in tow minutes and showed a maximum inhibition zone.

According to the temperature, the results of ehanolic samples were better than aquatic samples. Due to high dielectric properties of ethanol, it lead to increasing of temperature of the medium solvent-solute, and thus increasing of the extraction of the bioactive compound, then had shown more of antimicrobial activity of inhibition zone. This evaluation is in agreement with studies of (Noelia et al, 2014; Farid & Giancarlo 2013; Faridah et al., 2010; Jila et al., 2011; Mahaveer et al., 2009; Ajanta et al., 2010).
Chapter 6

Conclusion & Recommendations
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Conclusion

In our work, we successfully examined microwave assisted extraction process to obtain bioactive compounds from plants which required less time, less efforts, small quantity of solvents, higher extraction rate, better product with minimum loss and less risk of decomposition and oxidation of Phytoconstituents from plant. Through optimizing three parameters (solvent nature, irradiation time, microwave power) and then evaluation of antimicrobial activity of plants extracts and in combination with antibiotics. In general, as shown the positive inhibitory zone results of MW aquatic and ethanolic extracts samples were the best comparing with samples extracted by conventional method (soxhlet method in this study and some of previous studies), especially more than 50% of aquatic and ethanolic sample-d results (extracted in tow minutes and MW power 180w) had shown a maximum magnitude of inhibition zone, though it consumed less time and power. The results of this study showed that ethanolic extracts used against selected microorganisms were showed antimicrobial and synergistic effect with most antibiotics better than aquatic extracts. Where in case of aquatic extracts; G. glabra had the best against E. coli. In case of ethanolic extracts, the best activity was observed with G. glabra against E. coli. Also, synergistic activity of the plant extracts, in case of aquatic extracts; F. sycomorus had the best synergism against S. aureus & E. coli. In case of ethanolic extracts, the best synergism was observed with L. innermis against E. coli, and with F. sycomorus against S. aureus. Antagonism exhibited by all aquatic plant extracts of both methods when combined with antibiotics which showed sensitivity when tested as alone against S. aureus. Also all ethanolic extracts of L. innermis and G. glabra of both methods exhibited antagonism reactions when combined with antibiotics which showed sensitivity when tested as alone against S. aureus. In addition, antagonism reaction occurred against E. coli for antibiotics which were resistant against E. coli when combined with aquatic & ethanolic extracts for both methods of G. glabra which showed sensitivity when tested as alone against E. coli. In addition, a dilution reactions against S. aureus occurred with some antibiotics combined with ethanolic extracts of F. sycomorus & L. innermis.
Recommendations

- The present work provide baseline information for the possible use of MW apparatus for extraction processes of plant materials.

- Furthermore, a study is needed to improve MW method by adding other parameters, materials, solvents and/or other apparatus may help to increase the concentration and selectivity of bioactive compound extraction.

- In addition, further analysis towards comparing the extraction could be done quantitatively.

- The findings of the current research may encourage development of pilot industrial microwave extractors for flavors, fragrances and medicinal aroma.

- Our results support the use of some plants in traditional medicine that can be used as antimicrobial agents in the search for new drugs and the possibility of concurrent use of these antimicrobial drugs and plant extracts in combination for treating infectious diseases.

- However, the antagonistic reactions of some extracts with some antibiotics, this recommended to the development of much needed drug enhancing preparations. Their use in combination with conventional antibiotics should be further studied for in vitro activities. It is clearly needs to be furthermore evaluated, to identify the effective components, the mode of action and the possible toxic effect in-vivo of these ingredients.
The Reference List
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