Deanship of Graduate Studies

Al-Quds University

Exhaustive Extraction and Screening the Antimicrobial Activities of *Bupleurum subovatum*: A member of the Palestinian flora

Aya Ali Ahmad Alqadi

M.Sc. Thesis

Jerusalem- Palestine

1437/ 2016

Exhaustive Extraction and Screening the Antimicrobial Activities of *Bupleurum subovatum*: A member of the Palestinian flora

Prepared By:

Aya Ali Ahmad Alqadi

B.Sc. of Medical Laboratory Science (An- Najah National University) / Palestine

Supervisor: Prof. Mahmoud M. Abu-hadid

A thesis submitted in partial fulfillment of requirements for the degree Master of Medical Laboratory Sciences/ Department of Medical Laboratory Sciences/ Program of Microbiology and Immunology/ Faculty of Heath Professions/ Al-Quds University

1437/2016

Al Quds University

Deanship of Graduate studies

Master Program in Medical Laboratory Science

Thesis Approval

Exhaustive Extraction and Screening the Antimicrobial Activities of Bupleurum subovatum: A member of Palestinian flora

Prepared By: Aya Ali Ahmad Alqadi

Registration No: 21310195

Supervisor: Prof. Mahmoud M. Abu-hadid

Master thesis submitted and accepted Date: 14/5/2016

The names and signatures of the examining committee members:

1. Head of committee: Prof. Mahmoud Abu- hadid Signature .....
2. Internal Examiner: Dr. Hateim Eideh Signature .....
3. External Examiner: Dr. Nidal Jaradat Signature .....

Jerusalem- Palestine

1437/2016

Dedication

To my lovely parents

To my lovely husband

To my grandparents

To my brothers and sisters

To my friends Asma and Bayan

To all whom I love

Acknowledgements

Praise be to Allah who helped me to complete this modest effort. I would like to thank, with much appreciation and gratefulness, my supervisor Prof. Mahmoud Abu-hadid, who graciously accepted to supervise my thesis, and has spared no effort in order to present this thesis in the proper manner. He has given me a lot of knowledge and time, so many thanks to him.

Also I extend my thanks and appreciation to Dr. Sameer Al-Barghouthi, graduate program coordinator and a faculty member in the College of Health Professions at Al-Quds University, that he has made for the time and effort, and I have reported many of his observations.

I also extend my sincere thanks and appreciation to this prestigious university, Al-Quds University, the Faculty of Graduate Studies-Department of Medical Laboratory Sciences, for giving me the opportunity to pursue graduate studies.

I also thank Mr. Sameh Nusseibeh, Mr. Mohammed Kurd, Mr. Mahmoud Zhaykah, Ms. Walaa and Ms. Dina for the help that gave me in the complete this work, thanks.

Many thanks go to my family with all my love, specially my lovely husband Dr. Mohammed Alqadi, my mother, my father, my sisters, and my brothers for providing me with all kinds of support and encouragement.

Researcher

Aya Alqadi

Declaration:

I Certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or Institution.

Signed: 

Aya Ali Ahmad Alqadi

Date: 14/5/2016

Abbreviations used

Abbreviations	Meaning
B. subovatum	*Bupleurum subovatum*
TLC	Thin layer chromatography
HPTLC	High performance thin layer chromatography
ATCC	American Type Culture Collection
N. glauca	*Nicotiana glauca*
DMSO	Dimethyl sulfoxide
CFU	Colony Forming Unit
PBS	Phosphate- buffered saline
MIC	Minimum Inhibitory Concentration
MBC	Minimum bactericidal Concentration
MFC	Minimum fungicidal Concentration
C. iphionoides	*Chiliadenus iphionoides*
U. Maritima	*Urginea Maritima*

Table of Contents

Dedication .....

Acknowledgment .....

Declaration ..... **I**

Abbreviations ..... **II**

Table of contents ..... **III**

List of Tables ..... **VI**

List of Figures ..... **VII**

Abstract ..... **VIII**

Chapter 1: Introduction **1**

1.1 Background: 1

1.2 Plant classification: 2

1.3 Extraction: 2

1.4 *Bupleurum subovatum*: 4

1.5 *Arum palaestinum:* 4

1.6 *Nicotiana glauca:* 4

1.7 *Chiliadenus iphionoides:* 5

1.8 *Urginea Maritima:* 5

1.9 Screening methods of plant products to determine antimicrobial activity: 5

1.10 Thin layer chromatography 6

1.11 Problem statment: 7

1.12 Objective 7

Chapter 2: Literature review **8**

2.1 *Bupleurum subovatum* 8

2.2 *Arum palaestinum* 8

2.3 *Nicotiana glauca* 9

2.4 *Chiliadenus iphionoides* 10

2.5 *Urginea Maritima* 10

2.6 Screening methods of plant products to determine antimicrobial activity: 11

2.7 Thin layer chromatography 12

Chapter 3: Methodology **13**

3.1. Collection and identification of *Bupleurum subovatum* : 13

3.2.Extraction: 13
3.3.Antimicrobial assay: 14
3.3.1. Test microorganisms and control 14
3.3.2.Preparation of the bacterial and candidal suspensions 14
3.3.3. Screening for antibacterial and anticandidal activity of the plant extracts 15
3.3.4.Measuring the minimum inhibitory concentration (MIC) 15
3.3.5.Minimum bactericidal/fungicidal concentration (MBC/MFC) 16
3.3.6. Thin layer chromatography 16
3.3.7. Anticancer activity 16
Chapter 4: Results **17**
4.1. Classification: 17
4.2.Screening well diffusion method 18
4.3. Measuring the minimum inhibitory concentration (MIC) 18
4.3.1. MIC/ *Bupleurum subovatum* : 18
4.3.2. MIC/ *Arum palaestinum* 19
4.3.3. MIC/ *Nicotiana glauca*: 20
4.3.4. MIC/ *Chiliadenus iphionoides*: 20
4.3.5. MIC/ *Urginea Maritima* 21
4.4. Measuring the minimum bactericidal/fungicidal concentration (MBC/MFC). 22
4.4.1. MBC/MFC- *Bupleurum subovatum* : 22
4.4.2. MBC/MFC- *Arum palaestinum* 24
4.4.3. MBC/MFC- *Nicotiana glauca*: 24
4.4.4. MBC/MFC- *Chiliadenus iphionoides*: 25
4.4.5. MBC/MFC- *Urginea Maritima* 25
4.5. Thin layer chromatography 27
4.6. Anticancer activity: 27
Chapter 5: Discussion **28**
5.1 Screening by well diffusion method: 28
5.2 MIC- MBC/ MFC : 29
5.2.1. MIC-MBC/MFC- *Bupleurum subovatum* : 29
5.2.2. MIC-MBC/MFC- *Arum palaestinum* 29
5.2.3. MIC-MBC/MFC- *Nicotiana glauca*: 30
5.2.4. MIC-MBC/MFC- *Chiliadenus iphionoides*: 30
5.2.5. MIC-MBC/MFC- *Urginea Maritima* 30

5.3. Thin layer chromatography: 31
5.4. Limitation: 31
5.5.Conculsion: 31
5.6.Future study: 31
References **32**
الملخص **37**

List of tables

Table 1: The tested microorganism and their sources.

Table 2: The weights of the resulted extracts.

Table 3: The MIC for the test microorganisms for *Bupleurum subovatum*.

Table 4: The MIC for the test microorganisms for *Arum palaestinum*.

Table 5: The MIC for the test microorganisms for *Nicotiana glauca* .

Table 6: The MIC values for the test microorganisms for *Chiliadenus iphionoides*.

Table 7: The MIC values for the test microorganisms for *Urginea Maritima*

Table 8: The MBC/MFC values for test microorganisms for *Bupleurum subovatum.*

Table 9: The MBC/MFC values for the test microorganisms for *Arum palaestinum*

Table 10: The MBC/MFC values for the test microorganisms for *Nicotiana glauca*

Table 11: The MBC/MFC values for the test microorganisms for *Chiliadenus iphionoides*

Table 12: The MBC/MFC values for the test microorganisms for *Urginea Maritima*

Table 13: Comparison between plants for MIC values for aqueous extracts

Table 14: Comparison between plants for MBC/MFC values for aqueous extracts

List of Figures

Figure 1 *Bupleurum-subovatum* plant

Figure 2 *Arum palaestinum* plant

Figure 3 *Nicotiana glauca* plant

Figure 4 *Chiliadenus iphionoides* plant

Figure 5 *Urginea Maritima* plant

Figure 6 Closer look for the *Bupleurum-subovatum* leaves

Figure 7 Closer look for the *Bupleurum-subovatum* fruits

Figure 8 *Bupleurum-subovatum* whole plant (Our sample)

Figure 9 MFC for *Bupleurum subovatum* for *Candida albican*

Figure 10 MBC for *Bupleurum subovatum* for *Escherichia coli*

Figure 11 MBC for *Bupleurum subovatum* for *Pseudomonas aerginosa*

Figure 12 MBC for *Bupleurum subovatum* for *Staphylococcus aureus*

Abstract

Natural product extraction from plants to isolate new compounds that will be used in therapy is under many current investigations. *Bupleurum subovatum* plant with unknown biological activity was selected for our study. We extracted the plant by exhaustive extraction method that we developed and modified in our laboratory. This extraction method is able to extract almost all active compounds from this plant. We have tested the biological activity of the extracts by well diffusion method, minimum inhibitory concentration and minimum bactericidal/fungicidal concentration for gram positive bacteria *Staphylococcus aureus* (ATCC 25923), gram negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and yeast *Candida albican* (ATCC 10231). We determined the antibacterial and antifungal activity of crude extracts first aqueous, second aqueous and organic extracts of *Bupleurum subovatum*. The percent of total extract from initial weight (25 g) was (9.34 g) 37.36 %. The percent of organic extract (0.14 g) to 25 g was 0.56% while the percent of aqueous extracts (9.2 g) to 25 g was 36.8%. The percent of each extract to total extracts (9.34 g) was 1.5 % for organic extract, while first aqueous extract was 67.6%, and 30.9% for second aqueous extract. The most sensitive organisms was *Pseudomonas aeruginosa* at concentration 0.16 mg/ml then *Candida albican* at concentration 0.8 mg/ml and *Staphylococcus aureus, Escherichia coli* at concentration 4 mg/ml based on MIC assessment. Our plant has bactericidal activity against *Pseudomonas aeruginosa* for first and second aqueous extracts at 20 mg/ml but no activity for organic extract and fungicidal activity against *Candida albican* for all (first, second aqueous and organic) extracts at 20 mg/ml, as no growth occurred in these sub-cultured pure tubes. While it has bacteriostatic activities against *Staphylococcus aureus* at 20 mg/ml for all extracts, *Escherichia coli* at 20 mg/ml for first aqueous and organic extracts. And it has many growths occur for *Escherichia coli* at 20 mg/ml for second aqueous extract. Our plant was also compared to four other plants extracted similarly. We proved that this extraction method is effective for isolating most of the plant metabolites as well as preserving their biological activity.

Chapter 1:

Introduction

1.1 Background

Plants in many places in the world are used for treatment by themselves or in semi synthetic drugs production and they are considered as an important materials for therapy (Jaradat and Abu-hadid 2014). Many studies indicate that in some plants there are many compounds such as essential oils, peptides and others that have therapeutic activity against human microbes, including bacteria, fungi, protozoa and virus (El Astal, Ashour et al. 2005). Bacterial resistance to antibiotics is driving the world to search for new antibacterial agents (Rahman, Abd-Ellatif et al. 2013). Many plants found to have antimicrobial activities by investigation (Rahman, Abd-Ellatif et al. 2013). Because of failure to treat infectious diseases by available antimicrobials, many researchers have focused on the investigation of natural products as source of new bioactive molecules (Silver and Bostian 1993). Numerous plants have anti-inflammatory properties and anti-tumor agents (Kaileh, Berghe et al. 2007). In addition, there are plants which have demonstrated wound healing properties (Martins, Marques et al. 2009). Moreover, many plants are used to treat sexually transmitted diseases (Tshikalange, Meyer et al. 2005).

The use of plant compounds to treat infections is an old habit in large area of the world, particularly in developing countries, where there is dependence on natural treatment for many diseases (Abu-Shanab, Adwan et al. 2007). Over 75% of people according to the World Health Organization (WHO) still depend on plant based traditional medicines for primary health care in underdeveloped or developing countries (Sarker, Latif et al. 2005). Palestine Mountains are rich in plant species. Where about 2600 species are found on this area (Ali-Shtayeh, Yaniv et al. 2000).

Natural drugs from the plants are popular because they are cheap, safe, have few side effects and have better patient tolerance (Tabassum and Hamdani 2014). Natural products are not drugs; they are produced in the nature and through biological assays, which become candidates for drug discovery. More than 60% of the drugs that are in use for disease treatment world wild are derived from natural sources (Molinari 2009).

1.2 Classification

Early classification started by the Greek philosopher Theophrastus who was the farther of botany, and he classified all plants into annuals, biennials, and perennials according to life spans, and into herbs, shrubs, and trees according to their growth habit (Grene and Depew 2004). The modern taxonomy for plant classification is based on Linnaeus who was the father of modern taxonomy, and he revolutionized the field of plant and animal classifications, so the plants were classified in the plant kingdom (Calisher 2007).

In our laboratory there are four plants that we used for comparison with our plant *Bupleurum subovatum*, these plants are *Arum palaestinum* (fruits and seeds), *Nicotiana glauca* (all parts of plants), *Chiliadenus iphionoides* (all parts of plants) and *Urginea Maritima* (flowers and stalks).

1.3 Extraction

So, in order to determine whether plants have a biological activity we will extract as much as possible the chemicals that are present in the plants. Extraction, involves the separation of therapeutically active portions of plants from the inactive components by using selective solvents in standard extraction procedures (Sukhdev, Suman et al. 2008). A wide range of technologies is available for the extraction of active components from plants. There are many factors affecting the choice of extraction depending on the nature, stability and cost of crude drug, selection of the solvent depends on the solubility of the required components, concentration of the product, and recovery of solvent (Singh 2008).

The general principles in maceration, percolation and infusion referred to as leaching (Singh 2008). Maceration is suitable for both initial and bulk extraction of plant material (Regasini, Vellosa et al. 2008). The advantage of maceration is you can extracting the crude drug many times (Singh 2008) and the major disadvantage of the maceration technique is the loss of tissue structure and the structural relationship of cells to each other (David 1973). Another disadvantage for this technique is time consuming; taking from a few hours up to several weeks with occasional shaking, and it consumes lots of solvent (Regasini, Vellosa et al. 2008). Percolation is adequate for both initial and large-scale extraction (Sun, Lou et al. 2008) and the main disadvantages are plants powders and materials that swell widely which can block the

percolator (Singh 2008). However, the process can be time consuming and required large volumes of solvents (Seidel 2012). In infusion the main disadvantage it cannot be stored longer than 12 hours because it's susceptible to bacterial and fungal growth (Singh 2008). Decoction (decoction: hot aqueous extraction) is water based extraction and the liquid plant preparation is made by boiling the plant with water, so it is suitable for extracting water soluble and heat stable chemicals and it is unsuitable for water insoluble and heat labile substances (Rane 2008).

Ultrasound assisted solvent extraction (sonication) the procedure increases the permeability of cell walls and produces bores by using ultrasound with frequencies ranging from 20 kHz to 2000 kHz (Handa 2008). It's limited for large scale extraction due to the high costs; it is used mostly for extraction of a small amount of material (Conceiçao, Ferreres et al. 2006). The main disadvantage of this procedure is the deleterious effect of ultrasound energy on the active components of plants through formation of free radicals and so undesirable changes in the drug molecules (Handa 2008).

The main advantages of soxhlet (soxhlet: hot continuous extraction) that it can extract large amounts of drug with a much smaller quantity of solvent, so it is a great economy in time, financial inputs and energy (Handa 2008). While the disadvantage is the possibility of thermal decomposition of the aim compounds as the extraction generally occurs at the boiling point of the solvent for a long time (Rane 2008). It is widely used for both initial and bulk extraction (Sun, Lou et al. 2008). Distillation is the most popular method for extracting essential oils throughout the world, distillation shows evaporating the oils from the plant membranes in the existence of humidity under the effect of high temperature and then cooling the vapor mixture to separate the oil from the water (Tandon 2008).

Pressurized solvent extraction also called accelerated solvent extraction, this method maintain the solvent in a liquid state by using high pressure at high temperatures (Seidel 2012). The advantages for pressurized solvent extraction are low solvent requirements, environment friendly (Benthin, Danz et al. 1999) and improve metabolite solubilization by high temperatures and pressures. Thus enhancing extraction yield and speed (Waksmundzka-Hajnos, Petruczynik et al. 2004). This method is best suited for the reproducible and rapid initial extraction of a high number of samples (Seidel 2012).

Because all these noted extraction methods were not expected to be suitable for our plant. We used exhaustive extraction; the more exhaustive the plant extraction, the better the yield. We expect that this improved method will give the best results for our plant, so we chose it.

1.4 *Bupleurum subovatum*

Bupleurum subovatum is an annual herbaceous plants, length 10 to 100 cm, usually branched in the upper half, have 3 to 25 umbels, umbellules usually 15 to 25 flowered, fruit crowded to a semi-globose cluster and yellow petals. Cotyledons on this plant rarely observed, from 20 to 30 mm, linear. The plant first leaves soon withering, with petiole up to 5 mm, from 5 to 30 mm long and from 2 to 7 mm across, gradually changing from narrowly lanceolate to elliptical, perfoliate, upper most leaves ovate to suborbicular, perfoliate, yellow, veining parallel to divergent, median vein thicker. *B. subovatum* plant flowering time is from April to May, and fruiting time is from May to July. It grows and spreads in fields, vine yards, open forests, usually on limestone. Globally this plant distributed in north Africa and South West Asia (Snogerup and Snogerup 2001).

1.5 *Arum palaestinum*

There is 26 species of the Arum genus, *Arum palaestinum* one of them, which are flowering plants belonging to Araceae, and native to Europe, Western Asia and Northern Africa, with the highest species diversity in the Mediterranean region. *Arum palaestinum* aerial parts are considered edible after being soaked in salty water or dried, also used as animal fodder (El-Desouky, Kim et al. 2007). It is an atypical "cryptic" species, producing a smell of rotten fruit (Gibernau, Macquart et al. 2004).

1.6 *Nicotiana glauca*

Nicotiana glauca belonging to family Solanaceae, also called tree tobacco or wild tobacco. It is closely related to commercial tobacco, *Nicotiana tabacum* L., and is widespread throughout South Africa (Bromilow 2003). The smoking of *N. glauca* has been reported and the plant has also been used medicinally (Steenkamp, Van Heerden et al. 2002).

1.7 *Chiliadenus iphionoides*

Chiliadenus iphionoides (syn. *Varthemia iphionoides*) a member of the Asteraceae family, is endemic to the Mediterranean region. It grows throughout most regions of Palestine, as well as in Jordan, Lebanon, Syria and the Sinai peninsula (Tamir, Satovic et al. 2011). *C.iphionoides* has small leaves covered with hairs and glands containing the essential oil, which gives the plant its unique aroma (Feinbrun Dothan 1978).

1.8 *Urginea Maritima*

Urginea Maritima is a species of flowering plant in the family Asparagaceae (Chase, Reveal et al. 2009). It have several common names, including sea squill, sea onion, and maritime squill. It is native to western Asia, southern Europe and northern Africa (Pascual-Villalobos 2002).

1.9 Screening methods of plant products to determine antimicrobial activity

There are three screening methods currently available for the detection of antimicrobial activity of natural products, including diffusion, bioautographic and dilution methods. The diffusion and bioautographic (agar diffusion and chromatogram layer) methods are known as qualitative techniques which gives only an idea of the presence or absence of the antimicrobial activity. On the other hand, dilution methods are considered quantitative assays which determine the minimal inhibitory concentration (MIC) (Valgas, Souza et al. 2007). The test systems should be rapid, simple, reproducible and inexpensive (Hostettmann, Wolfender et al. 1997).

Agar diffusion test has two different types, filter paper disc impregnated with compound test and wells in dishes. “The diffusion process may be defined as the process by which molecules intermingle as a result of their kinetic energy of random motion from high concentrations areas to lower ones. The diffusion process depends on numerous factors including number, size and shape of particles” (Valgas, Souza et al. 2007). Simplicity of the well and disc diffusion methods and little time consuming are reasons for stimulating using it. In general, both diffusion methods are similar to each other in terms of cost, although discs method may be a little more expensive because is using filter paper Whatman, which is composed of cellulose [β-(1-4) linked glucose monomers] (Valgas, Souza et al. 2007). If natural products were cationic,

they would be expected to adsorb to the surface of the disc and not diffuse into the medium, because of the many free hydroxyl groups present on each glucose residues making the surface of the disc hydrophilic. Polar compounds would not be influenced by the hydroxyls on the surface of the paper and would diffuse easily (Burgess, Jordan et al. 1999). Although there are many advantages of the well diffusion method, one must be aware of its limitations. Diffusion methods are not the best choice for testing non-polar or other samples as aqueous dispersions containing high molecular weight solutes, which are difficult to diffuse in the media. Alternative methods are suggested in those cases, as direct bioautography or semi-quantitative dilution methods (Begue and Kline 1972).

The bioautography method may represent a useful tool for purification of antibacterial substances if tests are performed through the use of chromatograms; TLC (Thin Layer Chromatography) (Hamburger and Cordell 1987). Micro-dilution method can be used as semi-quantitative or quantitative assay, depending on the aim of the test (for screening or determination of minimum inhibitory concentration MIC). MIC values were defined as the lowest concentration of each natural product, which completely inhibit microbial growth (Souza, Monache et al. 2005).

The minimum bactericidal concentration (MBC) of antibiotic is determine for an infecting organism and is now more frequently requested of clinical microbiology laboratories because immunocompromised and other patient's infections may require treatment with bactericidal rather than bacteriostatic antibiotic levels (Taylor, Schoenknecht et al. 1983).

1.10 Thin layer chromatography(TLC)

We did thin layer chromatography to separate and identify components in a compound mixture, TLC is routinely used by researcher in the field of phyto-chemicals. It is a semi quantitative method of analysis and its sophisticated version or quantitative method is high performance thin layer chromatography (HPTLC) (Sukhdev, Suman et al. 2008). TLC analyses with various solvent system for each solvent type extract revealed the presence of spots (Jagessar, Mars et al. 2008).

1.11 Problem Statement

Does *Bupleurum subovatum* have any antimicrobial activity?

1.12 Objectives

Bupleurum subovatum is one of the plants that grow widely in the spring in the mountains of Palestine but it is medical importance is still unknown. The aim of our study is to evaluate antibacterial and antifungal activities of crude aqueous and organic extracts of this plant and compare them with another four plants: *Arum palaestinum* (fruits and seeds), *Nicotiana glauca* (entire plant), *Chiliadenus iphionoides* (entire plant) and *Urginea Maritima* (flowers and stalks) against the gram positive *Staphylococcus aureus* (ATCC 25923), the gram negative *Pseudomonas aeruginosa* (ATCC 27853) and the gram negative *Eschrichia coli* (ATCC 25922) and fungi represented in *Candida albicans* (ATCC 10231).

Chapter 2

Literature review

2.1 *Bupleurum subovatum*

Bupleurum (B) subgenus have four types: *B. rotundifolium, B. croceum, B.lancifolium* and *B. subovatum*. *B. subovatum* subgenus lacking bracts and has very broad, semi-perfoliate upper leaves. The perennials of this subgenus may be woody at the base, but the flowering shoots are herbaceous throughout. The *Bupleurum* annuals are often short-lived, germinating in spring and setting seed in the following summer (Snogerup and Snogerup 2001). There is no previous study of the antimicrobial activity for *B. subovatum*.

Fig. 1: *Bupleurum subovatum* plant (Our sample) (Abu-hadid 2014).

2.2 *Arum palaestinum*

Arum palaestinum, it is a plant used by grandparent to treat many diseases including atherosclerosis, stomach acidity, cancer, food toxicity and diabetes (Ali-Shtayeh, Yaghmour et al. 1998). The plant found to have many biological activities which were proved by investigations such as inhibitory effect on smooth muscle contraction in rates (Afifi, Khalil et al. 1999), anticancer activity against lymphoblastic leukemia (El-Desouky, Kim et al. 2007), antioxidant activity, have very low or no antibacterial activity against gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and gram negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and have not any activity against *Candida albicans* strains. The method of extraction was done by grounding fifty grams of dried plant using a Mulenix for a minute, and then the powder resulted was extracted by continuous stirring with 200 ml 70% ethanol at 24°C for 72 h. Then the extracts were filtered through Whatman's No. 4 filter paper, and the residue was washed with 50 ml ethanol. The combined ethanol extracts were dried using rotary evaporator followed by freeze drying and stored at -20°C for future use (Husein, Ali-Shtayeh et al. 2014).

Fig. 2: *Arum palaestinum* plant (Stökl, Strutz et al. 2010)

2.3 *Nicotiana glauca*

Nicotiana glauca warmed leaves are used for head to relieve headache and on the throat to relieve pain (Van Wyk and Gericke 2000). Animal deaths have also been reported (Watt and Breyer Brandwijk 1962). It has been used as an insecticide (Ginsbuk 1935), but its use has been stopped due to the development of more specific and less toxic insecticides. In humans, accidental ingestion of *N. glauca* does occur (Mizrachi, Levy et al. 2000) and usually results in death. *N. glauca* have antibacterial activity, the *N. glauca* methanol extract with different concentrations showed inhibitory activity against different strains of *Staphylococcus aureus* and have not activity against *Echericha coli*. The method of extraction was done by using clean fresh aerial parts, air dried and coarsely powdered using a mortar, powdered plant material was extracted with hexane and methanol; to obtain fractions with different polarities. Extraction was done along a period of two weeks under occasional shaking. The extract was collected and concentrated to dryness using rotary evaporator at 40°C. The dried extract were weighed and then stored in air tight container and kept at 4°C until it was used for further analysis (Rahman, Abd-Ellatif et al. 2013).

Fig. 3: *Nicotiana glauca* plant (Giordana)

2.4 *Chiliadenus iphionoides*

Chiliadenus iphionoides is used to treat fever, influenza, stomach pain, nervousness, and depression (Tamir, Satovic et al. 2011). Preliminary studies showed its antioxidant (Al-Mustafa and Al-Thunibat 2008), antifungal, antiviral activity (Tamir, Satovic et al. 2011), and antibacterial activity was identified for *Escherichia coli* (IFO-3301), *Staphylococcus aureus* (IFO-14462). The method of extraction was done by extracting 200 gram of powdered aerial parts with hexane, ethyl acetate and ethanol successively using the Soxhlet apparatus for five hours for each solvent. The extracts were dried under reduced pressure (Al-Dabbas, Hashinaga et al. 2005). *C. iphionoides* also have hypoglycemic activity (Afifi, Saket et al. 1997).

Fig.4: *Chiliadenus iphionoides (Brullo).*

2.5 *Urginea Maritima*

Urginea Maritima have cytotoxic and antimalarial activities (Sathiyamoorthy, Lugasi-Evgi et al. 1999). The toxicity effect was reported (Tuncok, Kozan et al. 1995). In the past it was used to treat whooping cough, pneumonia, vipers bites, cough, bronchitis, diuretic, cardiac failures, jaundice, internal tumors and as an abortive agent (Benbacer, El Btaouri et al. 2012). It has antifungal activity against *Candida albicans* and has no activity against *Escherichia coli* (Hammoudi and Douali). Also it is used as a cardiotonic diuretic (Iizuka, Warashina et al. 2001). The method of extraction was done by using HPTLC and UV-Vis methods (Hammoudi and Douali).

Fig. 5: *Urginea Maritima (Baker)*

2.6 Screening methods of plant products to determine antimicrobial activity

The performance of antimicrobial susceptibility testing of significant bacterial isolates is an important task of the clinical microbiology laboratory. Detect compound or drug resistance for common pathogens and assure susceptibility of compound or drug for particular infections is the goals of testing. Manual methods that possibly saving money and provides flexibility include the disk diffusion and gradient diffusion methods. Each method has strengths and weaknesses. Some methods provide quantitative results as MIC, and most methods provide qualitative assessments using the categories susceptible, intermediate, or resistant as well or disk diffusion method (Reller, Weinstein et al. 2009).

The well or disk diffusion susceptibility method is simple and practical and has been well standardized. The zones of growth inhibition around each of the extracts disks are measured to the nearest millimeter. The diameter of the zone is related to the diffusion rate of the drug or extract through the agar medium and to the susceptibility of the isolate. The advantage of this method is the simplicity of test that does not require any special equipment. It is the least costly of all susceptibility methods (approximately $2.50–$5 per test for materials). The disadvantage of this test is the lack of mechanization or automation of the test in our university. Although not all fastidious or slow growing bacteria can be accurately tested by this method (Reller, Weinstein et al. 2009). Well diffusion method is a much more sensitive than the disc diffusion (Valgas, Souza et al. 2007, Jagessar, Mars et al. 2008).

Tube-dilution or micro dilution method is one of the earliest antimicrobial susceptibility testing methods (Ericsson and Sherris 1971). The tubes were examined for visible bacterial growth as evidenced by turbidity. The lowest concentration of antibiotic that prevented growth represented the MIC. The precision of this method was considered to be plus or minus ± 1 two-fold concentration. The advantage of micro dilution technique was including the generation of MICs; the generation of a quantitative result. While the principal disadvantages for this method were the possibility of errors in preparation of the solutions, the boring, manual task of preparing the solutions for each test, the relatively large amount of reagents and space required for each test (Reller, Weinstein et al. 2009).

MBC determine the proportion of survivors after 24 h of incubation in broth containing antibiotic, and the MBC is the concentration which kills at least 99.9% of the original inoculum (Taylor, Schoenknecht et al. 1983).

2.7 Thin Layer Chromatography

The principle of TLC based on separation depends on the relative affinity of components towards stationary and mobile phase, the components under the influence of mobile phase travel over the surface of stationary phase; during this movement the components with higher affinity to stationary phase travel slowly while others components travel faster. Thus separation of components in the mixture is completed (Sukhdev, Suman et al. 2008).

Chapter 3

Methodology

3.1 Collection and identification of *B. subovatum*

The plant was collected by Prof. Mahmoud Abu-hadid from Jerusalem area, between March and April 2014. This sample has all the plants parts (roots, stems, leaves, flowers and seeds), and we verified their online presence.

3.2 Extraction

All parts of the plant sample had been dried in the shade, at room temperature, until they became completely dry. They were cut into small pieces, and then were powdered in a mechanical grinder. Twenty five gram of the powdered plant were put in a bottle and suspended in 100 ml normal hexane which is relatively safe, largely unreactive, cheap and easily evaporated non-polar solvent, put in a bottle with continuous shaking (200 round per minute) at 25ºC for 72 hours (Making sure that the bottle was tightly covered and fully rapped with aluminum foil; as light may affect our sample and to avoid evaporation of hexane and active materials). After that, the mixture had been filtered by Whatman's No.1 filter paper. The liquid filtrate was organic extract and the plant materials that had been accumulated on the filter paper had been re-extracted again to have first aqueous extract. The organic extract was placed in a pre-weighed glass beaker, which was placed in the hood at room temperature with a cover with pores to allow evaporation of hexane, and to obtain the organic extract. The beaker with the organic extract had been weighed again after evaporation; the weight of the organic extract had been determined by calculated the difference of the weights. Then it was dissolved in dimethyl sulfoxide (DMSO), which is one of the most powerful organic solvents, the extract was dissolved at 100 mg/ml concentration and was kept in a sterile brown bottle at 4ºC in the refrigerator till further use (Jaradat and Abu-hadid 2014).

Another extraction had been done to have first aqueous extract, the plant materials that was accumulated on the filter paper after the first filtration were re-extracted again, by added 125 ml of 50% ethanol in triple distilled water, with continuous shaking for 72 hours in the shaking incubator at 25ºC as before. A second filtration for the mixture had been done by using Whatman's No.1 filter paper, this was first aqueous extract. Then the plant materials that accumulated on the filter paper after the

second filtration were re-extracted again, by adding 125 ml of 50% ethanol in triple distilled water, with continuous shaking for 72 hours in the shaking incubator at 25ºC as before. A third filtration for the mixture had been done by using Whatman's No.1 filter paper, this was second aqueous extract. Both aqueous extracts had been collected after filtration and kept in a volumetric flask in refrigerator. Then both aqueous extracts had been put separately in pre weighed freeze dryer bottles and place on the freeze dryer till they had been dried completely. Then the freeze dryer bottles had been reweighted again and the dried weight of both extracts had been calculated. Both extracts were dissolved in 30% ethanol in Phosphate-buffered saline (PBS) at a concentration of 100mg/ml. Then the aqueous extracts had been placed in refrigerator at 4ºC till we used them for the biological testing. This extraction was repeated two times for conformation (Jaradat and Abu-hadid 2014).

3.3 Antimicrobial assay

3.3.1 Test microorganisms and control

In vitro antimicrobial activities of the aqueous and organic extracts of *Bupleurum subovatum* were tested against three potentially human pathogenic bacterial strains, and against one fungus (Table 1).

Table 1: The tested microorganism and their sources. American Type Culture Collection (ATCC).

The microorganism	Category	ATCC reference no.
Staphylococcus aureus	Gram positive bacteria	25923
Escherichia coli	Gram negative bacteria	25922
Pseudomonas aeruginosa	Gram negative bacteria	27853
Candida albican	Yeast	10231

Imipenem (IPM) 10 µg/ml, a broad spectrum antibacterial antibiotic and nystatin (NS) 100 µg/ml an antifungal drug had been used as a positive control and the solvents (30% ethanol in PBS for the aqueous extracts and DMSO for the organic extract) had been used as a negative control.

3.3.2 Preparation of the bacterial and candidal suspensions

The bacterial and the candidal suspensions obtained from the ATCC had been standardized to 0.5 McFarland standard, and were comparable to a bacterial suspension density of 1.5 × 10ˆ8 CFU/ml. We prepared a test suspension into a sterile

glass tube, added 2 ml of normal saline, then using a sterile inoculating loop, picked 3-5 isolated colonies from an overnight growth on blood agar and suspended them in the saline. The bacterial suspension should be equal to 0.5 McFarland standards. Then will used spectrophotometer with a 1cm light path at wavelength of 625 nm, and the absorbance range from 0.08 to 0.10 should be used (McFarland 1907).

3.3.3 Screening for antibacterial and anticandidal activity of the plant extracts

Well diffusion method had been used for screening, by determining the zone of inhibition. The prepared bacterial and the candidal suspensions had been seeded into prepared plates of Muller Hinton agar. Then we made wells in the plate's using sterile straw of 6 mm diameter. Wells had been filled completely with the plant extracts (the first and second aqueous and the organic) and positive and negative controls with 100 µl in each well. Then the plates had been incubated at 37ºC for 24 h for the bacteria cultures, and 48 h for the candida cultures in an incubator. Controls also were set up using the solvents as negative control and imipenem and nystatin as positive control. After the incubation, the plates had been observed for inhibition zones, which had been measured in millimeters (Jaradat and Abu-hadid 2014).

3.3.4 Measuring the minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of an antimicrobial that inhibit the growth of microorganism or no visible growth. Serial broth dilution technique had been used to determine the MIC for all the tested microorganisms. A set of 4 tubes were prepared for each microorganism, 750 µl of nutrient broth was added in all tubes, then 200 µl of the aqueous extract of *B. subovatum* (with 100 mg/ml concentration) was added in first tube by micropipette and was mixed well. Then, from the solution of first tube, 200 µl were transferred to second tube, well mixed. Then, 200 µl of the solution in second were transferred to the third tube, then from third to fourth tube. Then 200 µl of the solution in fourth tube were discarded. Finally 50 µl of bacterial/candidal suspension standardized to 0.5 Mc-Farland Scale (1.5×10ˆ8 CFU/ml) was added to all four tubes after the dilution was done. The concentration of extract in first tube was 20 mg/ml and three times dilution was carried out. Positive control tubes were prepared, by using antibacterial and antifungal antibiotic instead of the plant extract as a positive control. And negative control tubes were prepared, by using 30 % ethanol

in PBS and DMSO of the plant extract as a negative control. The tubes were incubated at 37˚C for 24 h for the bacteria, and 48 h for the candida. After the incubation, the clear tubes (show inhibitory action) were observed for each microorganism and the least clear tube from each set was considered as the MIC. This test was repeated three times for confirmation and all the steps were carried out under sterile conditions, by working on hood, and sterilizing instruments in the autoclave (Jaradat and Abu-hadid 2014).

3.3.5 Minimum bactericidal/ fungicidal concentration (MBC/MFC)

Which is the minimum concentration that is required to kill the bacteria/fungi, had been tested after the results of the MIC. The tubes of the MIC that showed no growth (clear) of microbes have been sub-cultured into nutrient agar plates and incubated at 37ºC for 24 h for the bacteria, and 48 h for the candida with controls. The concentration of the extract that did not show any colony growth is label as the MBC/MFC (Jaradat and Abu-hadid 2014).

3.3.6 Thin layer chromatography

TLC was carried out by using silica plate, a drop of *Bupleurum subovatum* extracts (first, second aqueous and organic) and control were put on silica plate by capillary tube with controls, then the plate was placed in a covered beaker containing the solvents (-7, -3, 0, +3 and +7) according to the HEMWal. system #. These solvents use different percentages of hexane, ethyl acetate, methanol and water. Allow the extract to be separated. They were examined under the UV/Vis lamp and the spots were circled with a pencil (Jagessar, Mars et al. 2008). This test was repeated with different solvent concentrations.

3.3.7 Anticancer activity

This carried out by our tissue culture lab in Al-Quds University. We used breast cancer cells (MCF-7) (ATCC 67244), HOS (human osteosarcoma) (ATCC CRL-1547) and KHOS (ATCC CRL-1544) cell lines.

Chapter 4

Results

Our plant name is *Bupleurum subovatum*. Its common name is: False Thorow-wax.

4.1 Classification:

The identification process was done by expert group lead by Prof. Mahmoud Abu-hadid and my participation. Our plant classification was confirmed as follows:

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Rosidae

Order: Apiales

Family: Umbelliferae (apiaceae)

Genus: Bupleurum

Specie: Bupleurum subovatum

Twenty five grams of the *Bupleurum subovatum* plant powder were subjected to exhaustive extraction. The weights of the dried aqueous and organic extracts that were produced from the first and the second extractions are shown in Table 2. The total aqueous extract was 9200 mg (36.8 % of the total starting powder weight); the first extract was 25.24 % (6.31g) and the second extract was 11.56 % (2.89g) of the total starting powder weight, while the organic extract was 0.56 % (0.14g). All extracts were dissolved to 100 mg/ml concentration. The aqueous extracts were dissolved in 50 % ethanol in triple distilled water, the first in 63 ml and the second in 28 ml respectively. The organic extract was dissolved at 100 mg/ ml in DMSO (1.4 ml).

Fig 6: Closer look for the *Bupleurum subovatum* leaves (Abu-hadid 2014).

Fig 7: Closer look for the *Bupleurum subovatum* fruits (Abu-hadid 2014).

Fig 8: *Bupleurum subovatum* whole plant (Our sample) (Abu-hadid 2014).

Table 2: The weights of the resulted extracts

The extract	The weight in mg (g)	Percentage % of each extract to 25g	Percentage % of each extract to total extracts (9.34g)
The organic extract	140 (0.14 g)	0.56 %	1.5 %
The first aqueous	6310 (6.31 g)	25.24 %	67.6 %
The second aqueous	2890 (2.89 g)	11.56 %	30.9 %

4.2 Screening well diffusion method

It's screening for the antimicrobial activity, which was negative for all the test microorganisms (no zone of inhibition for all extracts except positive control).

4.3 Measuring the minimum inhibitory concentration (MIC)

The antimicrobial activities of *Bupleurum subovatum*, *Arum palaestinum*, *Nicotiana glauca*, *Chiliadenus iphionoides*, and *Urginea Maritima* extracts against the selected pathogens examined quantitatively by MIC and MBC/MFC, and were compared with the activity of a broad spectrum antibiotic, the imipenem antibacterial 10 µg/ml, and nystatin antifungal 100 µg/ml.

4.3.1 MIC/ *Bupleurum subovatum*

The antimicrobial activity for the aqueous and organic plant extracts for *Bupleurum subovatum* had been carried out by serial broth dilution. Positive results appeared for all the microorganisms. The MIC was tested at initial concentration 20 mg/ml with four fold dilution, in first and second aqueous extracts the lowest value was for *Pseudomonas aeruginosa:* 0.16 mg/ml, then for *Candida albican:* 0.8 mg/ml, for *Staphylococcus aureus*, and *Escherichia coli*: 4 mg/ml for all microorganisms for organic extract (Table 3). The result was repeated after six month of extraction and all extracts gave no biological activity. So we repeated the extraction and the above results were confirmed.

Table 3: The MIC for the test microorganisms for *Bupleurum subovatum*.

Microorganism	Positive control	MIC at Initial Conc. 20 mg/ml for 1st tube			Negative control for aqueous extracts (30% ethanol in PBS)	Negative control for organic extract (DMSO)
		The first aqueous	The second aqueous	The organic extract		
Staphylococcus aureus	IPM 10 µg/ml	4 mg/ml	4 mg/ml	4 mg/ml	No effect	No effect
Escherichia coli	IPM 10 µg/ml	4 mg/ml	4 mg/ml	4 mg/ml	No effect	No effect
Pseudomonas aeruginosa	IPM 10 µg/ml	0.16 mg/ml	0.16 mg/ml	4 mg/ml	No effect	No effect
Candida albican	NS 100 µg/ml	0.8 mg/ml	0.8 mg/ml	4 mg/ml	No effect	No effect

4.3.2 MIC/ *Arum palaestinum*

The antimicrobial activity for the aqueous extracts for *Arum palaestinum* had been carried out by serial broth dilution. Positive results appeared for all the microorganisms. The MIC was tested at initial concentration 20 mg/ml with four fold dilution, the value for all microorganisms: 4 mg/ml for first and second aqueous extract (Table 4).

Table 4: The MIC for the test microorganisms for *Arum palaestinum*

Microorganism	MIC at Initial Conc. 20 mg/ml for 1st tube		Positive control	Negative control for aqueous extracts (30% ethanol in PBS)
	The first aqueous	The second aqueous		
Staphylococcus aureus	4 mg/ml	4 mg/ml	IPM 10 µg/ml	No effect
Escherichia coli	4 mg/ml	4 mg/ml	IPM 10 µg/ml	No effect
Pseudomonas aeruginosa	4 mg/ml	4 mg/ml	IPM 10 µg/ml	No effect
Candida albican	4 mg/ml	4 mg/ml	NS 100 µg/m	No effect

4.3.3 MIC/ *Nicotiana glauca*

The antimicrobial activity for the aqueous extracts for *Nicotiana glauca* had been carried out by serial broth dilution. Positive results appeared for all the microorganisms. The MIC was tested at initial concentration 20 mg/ml with four fold dilution, the lowest value for *Staphylococcus aureus* for first and second aqueous extracts: 0.8 mg/ml, then for *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albican* for first and second aqueous extracts: 4 mg/ml (Table 5).

Table 5: The MIC for the test microorganisms for *Nicotiana glauca*

Microorganism	MIC at Initial Conc. 20 mg/ml for 1st tube		Positive control	Negative control for aqueous extracts (30% ethanol in PBS)
	The first aqueous	The second aqueous		
Staphylococcus aureus	0.8 mg/ml	0.8 mg/ml	IPM 10 µg/ml	No effect
Escherichia coli	4 mg/ml	4 mg/ml	IPM 10 µg/ml	No effect
Pseudomonas aeruginosa	4 mg/ml.	4 mg/ml	IPM 10 µg/ml	No effect
Candida albican	4 mg/ml	4 mg/ml	NS 100 µg/ml	No effect

4.3.4 MIC/ *Chiliadenus iphionoides*

The antimicrobial activity for the aqueous extracts for *Chiliadenus iphionoides* had been carried out by serial broth dilution. Positive results appeared for all the microorganisms. The MIC was tested at initial concentration 20 mg/ml with four fold dilution, the lowest value for *Staphylococcus aureus* and *Escherichia coli* for first and

second aqueous extracts: 0.8 mg/ml, then for *Pseudomonas aeruginosa* and *Candida albican* for first and second aqueous extracts: 4 mg/ml (Table 6).

Table 6: The MIC values for the test microorganisms for *Chiliadenus iphionoides*

Microorganism	**MIC at Initial Conc. 20 mg/ml for 1st tube**		**Positive control**	**Negative control for aqueous extracts (30% ethanol in PBS)**
	The first aqueous	**The second aqueous**		
Staphylococcus aureus	0.8 mg/ml	0.8 mg/ml	IPM 10 µg/ml	No effect
Escherichia coli	0.8 mg/ml	0.8 mg/ml	IPM 10 µg/ml	No effect
Pseudomonas aeruginosa	4 mg/ml	4 mg/ml	IPM 10 µg/ml	No effect
Candida albican	4 mg/ml	4 mg/ml	NS 100 µg/ml	No effect

4.3.5 MIC/ *Urginea Maritima*

The antimicrobial activity for the aqueous extracts for *Urginea Maritima* had been carried out by serial broth dilution. Positive results appeared for all the microorganisms. The MIC was tested at initial concentration 20 mg/ml with four fold dilution, the value for *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*: 4 mg/ml, and *Candida albican*: 0.8 mg/ml for first and second aqueous extracts (Table 7).

Table 7: The MIC values for the test microorganisms for *Urginea Maritima*

Microorganism	**MIC at Initial Conc. 20 mg/ml for 1st tube**		**Positive control**	**Negative control for aqueous extracts (30% ethanol in PBS)**
	The first aqueous	**The second aqueous**		
Staphylococcus aureus	4 mg/ml	4 mg/ml	IPM 10 µg/ml	No effect
Escherichia coli	4 mg/ml	4 mg/ml	IPM 10 µg/ml	No effect
Pseudomonas aeruginosa	4 mg/ml	4 mg/ml	IPM 10 µg/ml	No effect
Candida albican	0.8 mg/ml	0.8 mg/ml	NS 100 µg/ml	No effect

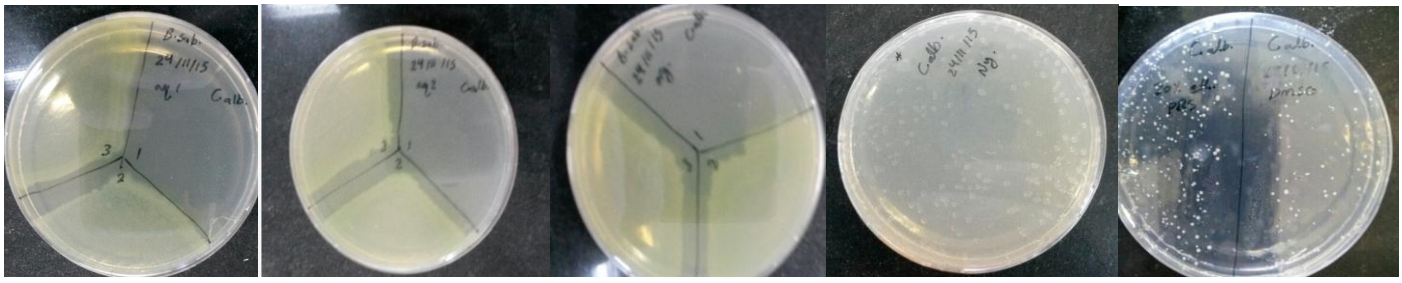
4.4 Measuring the minimum bactericidal/ fungicidal concentration (MBC/MFC)

4.4.1 MBC/MFC - *Bupleurum subovatum*

Bupleurum subovatum extracts had showed bactericidal activity against *Pseudomonas aeruginosa* for first and second aqueous extracts: 20 mg/ml and fungicidal activity against *Candida albican* for all (first, second aqueous and organic) extracts: 20 mg/ml, as no growth occurred in these sub-cultured pure tubes. And it has inhibitory effect (bacteriostatic) activity for *Staphylococcus aureus* for all extracts and *Escherichia coli* for first aqueous and organic at 20 mg/ml (Table 8).

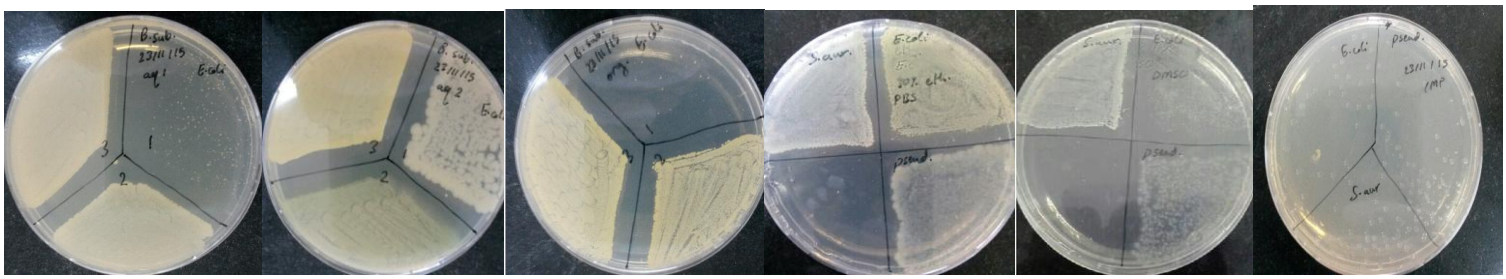
Table 8: The MBC/MFC values for the test microorganisms for *Bupleurum subovatum*.

Microorganism	MBC/MFC at Initial Conc. 20 mg/ml for 1st tube			Positive control	Negative control for aqueous extracts (30% ethanol in PBS)	Negative control for organic extracts (DMSO)
	The first aqueous	The second aqueous	The organic extract			
Staphylococcus aureus	Bacteriostatic at 20 mg/ml	Bacteriostatic at 20 mg/ml	Bacteriostatic at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth	Growth
Escherichia coli	Bacteriostatic at 20 mg/ml	Growth at 20 mg/ml	Bacteriostatic at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth	Growth
Pseudomonas aeruginosa	20 mg/ml (no growth)	20 mg/ml (no growth)	Growth at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth	Growth
Candida albican	20 mg/ml (no growth)	20 mg/ml (no growth)	20 mg/ml (no growth)	NS 100 µg/ml (no growth)	Growth	Growth



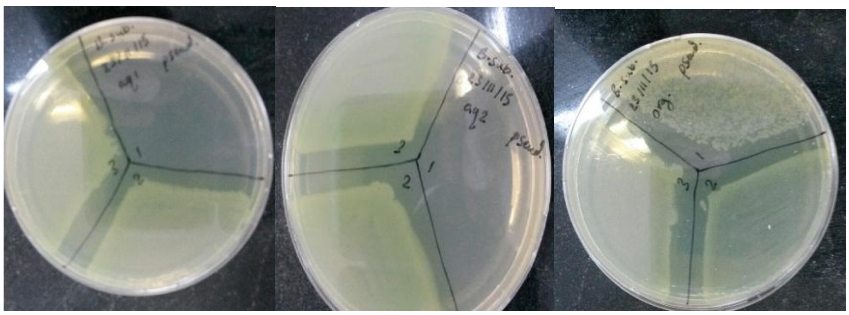
(A) (B) (C) (D) (E)

Fig. 9: MFC for Bupleurum subovatum for Candida albican, (A) show no growth in tube 1 for 1st aqueous extract at concentration 20 mg/ml, (B) show no growth in tube 1 for 2nd aqueous extract at concentration 20 mg/ml, (C) show no growth in tube 1 for organic extract at concentration 20 mg/ml, (D) show nystatin positive control and (E) show negative control 30% ethanol in PBS for aqueous extracts and DMSO for the organic extract.



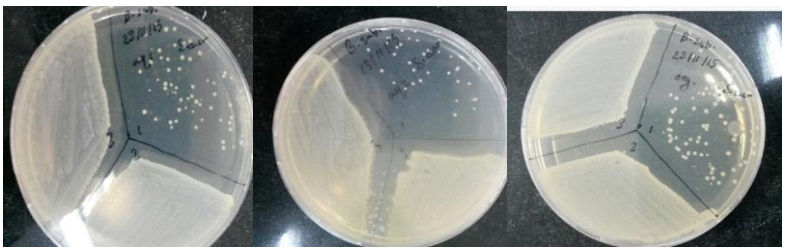
(A) (B) (C) (D) (E) (F)

Fig. 10: MBC for Bupleurum subovatum for Escherichia coli, (A) show few growth in tube 1 for 1st aqueous extract at concentration 20 mg/ml, (B) show many growth in tube 1 for 2nd aqueous extract as many growth at concentration 20 mg/ml, (C) show few growth in tube 1 for organic extract at concentration 20 mg/ml, (D) show negative control 30% ethanol in PBS for aqueous extracts for Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa, (E) show negative control DMSO for the organic extract for Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa, and (F) show imipenem positive control.



(A) (B) (C)

Fig. 11: MBC for Bupleurum subovatum for Pseudomonas aeruginosa, (A) show no growth in tube 1 for 1st aqueous extract at concentration 20 mg/ml, (B) show no growth in tube 1 for 2nd aqueous extract as at concentration 20 mg/ml, (C) show many growth in tube 1 for 2nd aqueous extract as many growth at concentration 20 mg/ml



(A) (B) (C)

Fig. 12: MBC for Bupleurum subovatum for Staphylococcus aureus, (A) show few growth in tube 1 for 1st aqueous extract at concentration 20 mg/ml, (B) show few growth in tube 1 for 2nd aqueous extract at concentration 20 mg/ml, (C) show few growth in tube 1 for organic extract at concentration 20 mg/ml.

4.4.2 MBC/MFC - *Arum palaestinum*

Arum palaestinum extracts had been showed inhibitory effect (Bacteriostatic) activity for *Staphylococcus aureus* at 20 mg/ml and has many growths for *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albican* at 20 mg/ml for first and second aqueous extracts (Table 9).

Table 9: The MBC/MFC values for the test microorganisms for *Arum palaestinum*

Microorganism	MBC/MFC at Initial Conc. 20 mg/ml for 1st tube		Positive control	Negative control for aqueous extracts (30% ethanol in PBS)
	The first aqueous	The second aqueous		
Staphylococcus aureus	Bacteriostatic at 20 mg/ml	Bacteriostatic at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth
Escherichia coli	Growth at 20 mg/ml	Growth at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth
Pseudomonas aeruginosa	Growth at 20 mg/ml	Growth at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth
Candida albican	Growth at 20 mg/ml	Growth at 20 mg/ml	NS 100 µg/ml (no growth)	Growth

4.4.3 MBC/MFC - *Nicotiana glauca*

Nicotiana glauca extracts had been showed bactericidal activity against *Staphylococcus aureus* at 20 mg/ml and fungicidal activity against *Candida albican* at 20 mg/ml for first and second aqueous extracts, as no growth occurred in these sub-cultured pure tubes. And it has many growths for *Escherichia coli* and *Pseudomonas aeruginosa* for first and second aqueous extracts at 20 mg/ml (Table 10).

Table 10: The MBC/MFC values for the test microorganisms for *Nicotiana glauca*

Microorganism	MBC/MFC at Initial Conc. 20 mg/ml for 1st tube		Positive control	Negative control for aqueous extracts (30% ethanol in PBS)
	The first aqueous	The second aqueous		
Staphylococcus aureus	20 mg/ml (no growth)	20 mg/ml (no growth)	IPM 10 µg/ml (no growth)	Growth
Escherichia coli	Growth at 20 mg/ml	Growth at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth
Pseudomonas aeruginosa	Growth at 20 mg/ml	Growth at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth
Candida albican	20 mg/ml (no growth)	20 mg/ml (no growth)	NS 100 µg/ml (no growth)	Growth

4.4.4 MBC/MFC - *Chiliadenus iphionoides*

Chiliadenus iphionoides extracts had been showed bactericidal activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* for first and second aqueous extracts at 20 mg/ml and fungicidal activity against *Candida albican* for first and second aqueous extracts at 20 mg/ml, as no growth occurred in these sub-cultured pure tubes (Table 11).

Table 11: The MBC/MFC values for the test microorganisms for *Chiliadenus iphionoides*

Microorganism	**MBC/MFC at Initial Conc. 20 mg/ml for 1st tube**		**Positive control**	**Negative control for aqueous extracts (30% ethanol in PBS)**
	The first aqueous	**The second aqueous**		
Staphylococcus aureus	20 mg/ml (no growth)	20 mg/ml (no growth)	IPM 10 µg/ml (no growth)	Growth
Escherichia coli	20 mg/ml (no growth)	20 mg/ml (no growth)	IPM 10 µg/ml (no growth)	Growth
Pseudomonas aeruginosa	20 mg/ml (no growth)	20 mg/ml (no growth)	IPM 10 µg/ml (no growth)	Growth
Candida albican	20 mg/ml (no growth)	20 mg/ml (no growth)	NS 100 µg/ml (no growth)	Growth

4.4.5 MBC/MFC - *Urginea Maritima*

Urginea Maritima extracts had been showed fungicidal activity against *Candida albican* for first and second aqueous extracts at 20 mg/ml, as no growth occurred in these sub-cultured pure tubes. And it has many growths for *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* at 20 mg/ml for first and second aqueous extracts(Table 12).

Table 12: The MBC/MFC values for the test microorganisms for *Urginea Maritima*

Microorganism	MBC/MFC at Initial Conc. 20 mg/ml for 1st tube		Positive control	Negative control for aqueous extracts (30% ethanol in PBS)
	The first aqueous	The second aqueous		
Staphylococcus aureus	Growth at 20 mg/ml	Growth at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth
Escherichia coli	Growth at 20 mg/ml	Growth at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth
Pseudomonas aeruginosa	Growth at 20 mg/ml	Growth at 20 mg/ml	IPM 10 µg/ml (no growth)	Growth
Candida albican	20 mg/ml (no growth)	20 mg/ml (no growth)	NS 100 µg/ml (no growth)	Growth

Table 13: Comparison between plants for MIC values for aqueous extracts.

Organisms / Plants	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albican
Bupleurum subovatum	4 mg/ml	4 mg/ml	0.16 mg/ml	0.8 mg/ml
Arum palaestinum	4 mg/ml	4 mg/ml	4 mg/ml	4 mg/ml
Nicotiana glauca	0.8 mg/ml	4 mg/ml	4 mg/ml	4 mg/ml
Chiliadenus iphionoides	0.8 mg/ml	0.8 mg/ml	4 mg/ml	4 mg/ml
Urginea Maritima	4 mg/ml	4 mg/ml	4 mg/ml	0.8 mg/ml

Table 14: Comparison between plants for MBC/MFC values for aqueous extracts.

Organisms / Plants	**Staphylococcus aureus**	**Escherichia coli**	**Pseudomonas aeruginosa**	**Candida albican**	**Negative control (30% ethanol in PBS)**
Bupleurum subovatum	Bacteriostatic at 20 mg/ml	Bacteriostatic at 20 mg/ml for first aqueous and growth for second aqueous	20 mg/ml (no growth)	20 mg/ml (no growth)	Growth
Arum palaestinum	Bacteriostatic at 20 mg/ml	Growth at 20 mg/ml	Growth at 20 mg/ml	Growth at 20 mg/ml	Growth
Nicotiana glauca	20 mg/ml (no growth)	Growth at 20 mg/ml	Growth at 20 mg/ml	20 mg/ml (no growth)	Growth
Chiliadenus iphionoides	20 mg/ml (no growth)	20 mg/ml (no growth)	20 mg/ml (no growth)	20 mg/ml (no growth)	Growth
Urginea Maritima	Growth at 20 mg/ml	Growth at 20 mg/ml	Growth at 20 mg/ml	20 mg/ml(no growth)	Growth

4.5 Thin layer chromatography (TLC)

The separation of extracts did not occurred with different concentration of solvent materials.

4.6 Anti-cancer activity

We tested many cancer cell lines and no any anti-cancer activity was observed for *Bupleurum subovatum* extracts (done by our tissue culture lab in Al-Quds University).

Chapter 5

Discussion

Compounds derived from plants have recently attracted much attention with regard to human health, due to their broad availability, limited toxicity and low cost. Thus, plant based antimicrobial compounds have large therapeutically potential as they can do the purpose with minimal side effects that are often linked with synthetic drugs (Husein, Ali-Shtayeh et al. 2014).

During normal plant life, plant growth is dynamic so plant chemical composition can be expected to vary. Different chemical composition of plants in different growth area depending on the relative availability of nutrients and light. Concentration of nitrate and amino acids were high in plants grown with a relative excess of nutrients. Concentrations of starch were high in plants grown with a relative excess of carbon (Waring, McDonald et al. 1985).

In our study, extraction was carried out where organic components were extracted first then followed by extracting the aqueous components extracts twice and producing 25.24 % in the first time and 11.56 % in the second time, which is a significant amount. So multiple extractions are needed in order to get most of the plant components extracted.

Bupleurum subovatum aqueous and organic extracts were tested against gram positive and gram negative bacteria, in addition to *Candida albican.* These extracts differ significantly in their activity against tested microorganisms.

5.1 Screening by well diffusion method

Well diffusion method was performed for our plant *Bupleurum subovatum* as screening for the antimicrobial activity, which was negative for all the test microorganisms. It showed that, well diffusion method could not always be a dependable method for screening the antimicrobial activity of plant extract, and the lack of inhibition zone did not necessarily mean that the extract was not effectual, especially for the low polar compounds which diffuse more slowly into the medium. In addition to that, in the diffusion method, the amount of the plant extracts used is limited, and that may be less than the amount needed to show the antimicrobial activity.

5.2 MIC- MBC/MFC

5.2.1 MIC- MBC/MFC *Bupleurum subovatum*

The minimum inhibitory concentration (MIC) and Minimum bactericidal/ fungicidal concentration (MBC/MFC) were performed for our plant extracts as well as for some other plants extracts for comparison purposes in this study. *Bupleurum subovatum* has antimicrobial activity against *Pseudomonas aeruginosa* and *Candida albican* the result in a repeat that we have done after six months was weak for all microorganisms as well as to *Pseudomonas aeruginosa* and *Candida albican* that the plant extract has a strong activity against. So these indicate that biological activity of the extracts decrease with time up to six month after extraction. To confirm this fresh extracts were obtained from the dry powdered plant and the results that we got from the fresh extracts were the same in kind and magnitude to the first plant extraction. These results may suggest that the antimicrobial activity is due to different chemical agents in the extract, which could include the compounds with phenolic structure as carvacrol which cause damages to the cell membrane of *Pseudomonas aeruginosa* and inhibits their proliferation (Cox and Markham 2007), and thymol which have the ability to alter the hyphal morphology of fungi and cause aggregation for hypha, resulting in reduced diameters of hypha and lyses the hyphal wall. In addition to that, thymol is lipophilic, enabling it to interact with the cell membrane of fungal cells, altering the permeability of cell membrane permitting the loss of macromolecules (Numpaque, Oviedo et al. 2011). The aqueous extracts may have different active compound to kill or inhibit the gram negative bacteria rather than acting on the bacterial cell wall, it may inhibit a bacterium ability to turn glucose into energy, inhibit nucleic acid, protein, or cell membrane synthesis (Olano, Paz et al. 1996). So in our study the aqueous and organic extracts from the same plant showed different activities, the aqueous extracts showed greater activity than the organic extracts, these results suggest that the interesting active compounds in this plant have solubility in water and are expected to be polar, hydrophilic compounds.

5.2.2 MIC - MBC/MFC *Arum palaestinum*

Arum palaestinum show bacteriostatic activity against *Staphylococcus aureus* and has many growths for *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albican* (these results were repeated twice to confirm), previous studies were showed that

Arum palaestinum plant have many compounds as vitexin, isovitexin, orientin, and isoorientin (Farid, Hussein et al. 2014), these compounds give the plants antimicrobial activity against many microorganisms as *Staphylococcus aureus*, *Escherichia coli* and *Candida albican* (Gumgumjee, Khedr et al. 2012). Although *Arum palaestinum* contain these compounds in our hand it is effect on the microorganisms that we tested were minimal. These results can be explained either by presence of low concentration of these compounds in our sample or *Arum palaestinum* might contain compounds that block the antimicrobial activities of these compounds.

5.2.3 MIC- MBC/MFC *Nicotiana glauca*

Nicotiana glauca have antimicrobial activity against *Staphylococcus aureus*, and *Candida albican*, and have no activity against gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* (these results were repeated twice to confirm), may be explained by the structural difference of the cell wall in gram positive bacteria and gram negative bacteria while the later have a cell wall with the complex structure. Phytochemical constituents of *Nicotiana glauca* such as flavonoids, alkaloids, steroid and glycosides and several other aromatic compounds are metabolites that serve as defense mechanisms against many microorganisms and have been shown to be responsible for the antimicrobial activities (Rahman, Abd-Ellatif et al. 2013).

5.2.4 MIC- MBC/MFC *Chiliadenus iphionoides*

Chiliadenus iphionoides have antimicrobial activity against all microorganisms tested in this study (these results were repeated twice to confirm), the essential oil was found to contain borneol, carvacrol and thymol and many others compounds (Tamir, Satovic et al. 2011) responsible for the antimicrobial activities.

5.2.5 MIC- MBC/MFC *Urginea Maritima*

Urginea Maritima has antifungal activity against *Candida albican* at 20 mg/ml and does not have activity against others bacteria tested in this study. A phenolic compound which functions as antifungal (Bernays, Cooper-Driver et al. 1989) previous data showed the antifungal activity against the growth of *Candida albican (Hammoudi and Douali).* It is expected that the compounds that produced those activities are different, due to the structural differences between prokaryotic and eukaryotic cells- as the membranes of fungi contain sterols, whereas the bacteria do not have- the antifungal compounds make a pore in the membrane of fungi by binding

to membrane sterols and the leakage out of contents occur. Prokaryotic cells are not inhibited by polyenes or bind to it (Bhardwaj, Saraf et al. 2009).

This difference in activity of plant extracts tends to show that the plant extracts are different in biological activities from one plant to another.

5.3 TLC

Preliminary results from TLC did not show any components separation, so we may need a quantitative method as high performance thin layer chromatography (HPTLC) to separate the components.

5.4 Limitations

- The plant is not always available because it grows only in spring
- Lack of necessary laboratory equipment in one location.
- Lack of financial support from the program and the university.

5.5 Conclusion

In our study, we can conclude that we confirmed the identity of our sample to be *Bupleurum subovatum* and established a new modified exhaustive extraction method for most plants including *Bupleurum subovatum* that preserve the biological activity of most plants constituents against bacteria and fungi and perhaps against viruses, protozoa and cancer cells. Additionally, it was concluded that *Bupleurum subovatum* has antimicrobial activity against *Pseudomonas aeruginosa* and *Candida albican;* this may help in discovering new antimicrobial agents, which will be an important addition to antibacterial and anticandidal drugs.

5.6. Future study:

Fractionation and identification of all extracted component from a plant grown in chemically defined environment and determine their biological activity individually and in combination.

References:

Abu-hadid, M. M. (2014). Photo Bupleurum subovatum. jpg.

Abu-Shanab, B., G. M. Adwan, N. Jarrar, A. Abu-Hijleh and K. Adwan (2007). "Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against methicillin-resistant Staphylococcus aureus." Turkish Journal of Biology **30**(4): 195-198.

Afifi, F., E. Khalil and S. Abdalla (1999). "Effect of isoorientin isolated from Arum palaestinum on uterine smooth muscle of rats and guinea pigs." Journal of Ethnopharmacology **65**(2): 173-177.

Afifi, F., M. Saket, M. Jaghabir and D. Al-Eisawi (1997). "Effect of Varthemia iphionoides on blood glucose level of normal rats and rats with streptozocin-induced diabetes mellitus." Current therapeutic research **58**(11): 888-892.

Al-Dabbas, M. M., F. Hashinaga, S. A. Abdelgaleil, T. Suganuma, K. Akiyama and H. Hayashi (2005). "Antibacterial activity of an eudesmane sesquiterpene isolated from common Varthemia, Varthemia iphionoides." Journal of ethnopharmacology **97**(2): 237-240.

Al-Mustafa, A. H. and O. Al-Thunibat (2008). "Antioxidant activity of some Jordanian medicinal plants used traditionally for treatment of diabetes." Pak J Biol Sci **11**(3): 351-358.

Ali-Shtayeh, M., R. M.-R. Yaghmour, Y. Faidi, K. Salem and M. Al-Nuri (1998). "Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area." Journal of Ethnopharmacology **60**(3): 265-271.

Ali-Shtayeh, M. S., Z. Yaniv and J. Mahajna (2000). "Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants." Journal of Ethnopharmacology **73**(1): 221-232.

Baker, L. "Urginea maritima (L.) Baker." Retrieved 28 August 2009, from http://luirig.altervista.org/schedenam/fnam.php?taxon=Urginea+maritima.

Begue, W. J. and R. M. Kline (1972). "The use of tetrazolium salts in bioauthographic procedures." Journal of Chromatography A **64**(1): 182-184.

Benbacer, L., H. El Btaouri, H. Morjani, M. Attaleb, M. El Mzibri, N. Merghoub, S. Amzazi and S. Gmouh (2012). Antiproliferative effect and induction of apoptosis by Inula viscosa L. and Retama monosperma L. extracts in human cervical cancer cells, INTECH Open Access Publisher.

Benthin, B., H. Danz and M. Hamburger (1999). "Pressurized liquid extraction of medicinal plants." Journal of Chromatography A **837**(1): 211-219.

Bernays, E. A., G. A. Cooper-Driver and M. Bilgener (1989). Herbivores and plant tannins, Academic Press.

Bhardwaj, N., S. Saraf, P. Sharma and P. Kumar (2009). "Syntheses, evaluation and characterization of some 1, 3, 4-oxadiazoles as antimicrobial agents." Journal of Chemistry **6**(4): 1133-1138.

Bromilow, C. (2003). Problem Plants of South Africa, Briza Publications.

Brullo, N. A."Chiliadenus iphionoides(Boiss. & Blanche)."Retrieved October 12, 2012., from https://commons.wikimedia.org/wiki/Category:Chiliadenus_iphionoides#/media/File:Chiliadenus_iphionoides_1.jpg.

Burgess, J. G., E. M. Jordan, M. Bregu, A. Mearns-Spragg and K. G. Boyd (1999). "Microbial antagonism: a neglected avenue of natural products research." Journal of Biotechnology **70**(1): 27-32.

Calisher, C. H. (2007). "Taxonomy: What's in a name? Doesn'ta rose by any other name smell as sweet?" Croatian medical journal **48**(2): 268.

Chase, M. W., J. L. Reveal and M. F. Fay (2009). "A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae." Botanical Journal of the Linnean Society **161**(2): 132-136.
Conceiçao, L. F., F. Ferreres, R. M. Tavares and A. C. Dias (2006). "Induction of phenolic compounds in Hypericum perforatum L. cells by Colletotrichum gloeosporioides elicitation." Phytochemistry **67**(2): 149-155.
Cox, S. and J. Markham (2007). "Susceptibility and intrinsic tolerance of Pseudomonas aeruginosa to selected plant volatile compounds." Journal of applied microbiology **103**(4): 930-936.
David, C. N. (1973). "A quantitative method for maceration of hydra tissue." Wilhelm Roux'Archiv für Entwicklungsmechanik der Organismen **171**(4): 259-268.
El-Desouky, S., K. H. Kim, S. Y. Ryu, A. F. Eweas, A. M. Gamal-Eldeen and Y.-K. Kim (2007). "A new pyrrole alkaloid isolated fromArum palaestinum Boiss. and its biological activities." Archives of pharmacal research **30**(8): 927-931.
El Astal, Z., A. Ashour and A. Kerrit (2005). "Antimicrobial activity of some medicinal plant extracts in Palestine." Pakistan Journal of Medical Sciences **21**(2): 187-193.
Ericsson, H. M. and J. C. Sherris (1971). "Antibiotic sensitivity testing. Report of an international collaborative study." Acta Pathologica Microbiologica Scandinavica(Suppl. 217).
Farid, M. M., S. R. Hussein, L. F. Ibrahim, M. A. El Desouky, A. M. Elsayed and M. M. Saker (2014). "Shoot regeneration, biochemical, molecular and phytochemical investigation of Arum palaestinum Boiss." African Journal of Biotechnology **13**(34): 3522-3530.
Feinbrun Dothan, N. (1978). "Flora Palaestina: Part 3. Ericaceae to Compositae." Jerusalem, Israel Academy of Sciences and Humanities **2**.
Gibernau, M., D. Macquart and G. Przetak (2004). "Pollination in the genus Arum–a review." Aroideana **27**: 148-166.
Ginsbuk, J. M. (1935). "Comparative toxicity of anabasine and nicotine sulphates to insects'." Journal of Agricultural Research **51**(4).
Giordana,F."43777-Nicotianaglauca."from http://luirig.altervista.org/pics/display.php?pos=43777.
Grene, M. and D. Depew (2004). The philosophy of biology: An episodic history, Cambridge University Press.
Gumgumjee, N. M., A. Khedr and A. Hajar (2012). "Antimicrobial activities and chemical properties of Tamarindus indica L. leaves extract." African Journal of Microbiology Research **6**(32): 6172-6181.
Hamburger, M. O. and G. A. Cordell (1987). "A direct bioautographic TLC assay for compounds possessing antibacterial activity." Journal of Natural Products **50**(1): 19-22.
Hammoudi, K. and S. Douali "HPLC, UV characterisation of some heterosides extracted from urginea maritima (algeria) and determination of some biological properties."
Handa, S. S. (2008). An Overview of Extraction Techniques for Medicinal and Aromatic Plants. EXTRACTION TECHNOLOGIES FOR MEDICINAL AND AROMATIC PLANTS. S. S. Handa, S. P. S. Khanuja, G. Longo and D. D. Rakesh**:** 21 -54.
Hostettmann, K., J. Wolfender and S. Rodriguez (1997). "Rapid detection and subsequent isolation of bioactive constituents of crude plant extracts." Planta medica **63**(1): 2-10.
Husein, A. I., M. S. Ali-Shtayeh, R. M. Jamous, S. Y. A. Zaitoun, W. J. Jondi and N. A.-A. Zatar (2014). "Antimicrobial activities of six plants used in Traditional Arabic Palestinian Herbal Medicine." African Journal of Microbiology Research **8**(38): 3501-3507.

Iizuka, M., T. Warashina and T. Noro (2001). "Bufadienolides and a New Lignan from the Bulbs of Urginea maritima." Chemical and pharmaceutical bulletin **49**(3): 282-286.
Jagessar, R., A. Mars and G. Gomes (2008). "Selective Antimicrobial properties of Phyllanthus acidus leaf extract against Candida albicans, Escherichia coli and Staphylococcus aureus using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method." Nature and Science **6**(2): 24-38.
Jaradat, N. and M. Abu-hadid (2014). "Exhaustive extraction and screening the biological activities of Heliotropium hirsutissimum (Hairy heliotrope): a member of palestinian flora." Asian Journal of Pharmaceutical and Clinical Research **7**(5): 207-210.
Kaileh, M., W. V. Berghe, E. Boone, T. Essawi and G. Haegeman (2007). "Screening of indigenous Palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity." Journal of ethnopharmacology **113**(3): 510-516.
Martins, M. D., M. M. Marques, S. K. Bussadori, M. A. T. Martins, V. C. S. Pavesi, R. A. Mesquita-Ferrari and K. P. S. Fernandes (2009). "Comparative analysis between Chamomilla recutita and corticosteroids on wound healing. An in vitro and in vivo study." Phytotherapy Research **23**(2): 274-278.
McFarland, J. (1907). "The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines." Journal of the American Medical Association **49**(14): 1176-1178.
Mizrachi, N., S. Levy and Z. Goren (2000). "Fatal poisoning from Nicotiana glauca leaves: identification of anabasine by gas-chromatography/mass spectrometry." Journal of forensic sciences **45**(3): 736-741.
Molinari, G. (2009). Natural products in drug discovery: present status and perspectives. Pharmaceutical Biotechnology, Springer**:** 13-27.
Numpaque, M. A., L. A. Oviedo, J. H. Gil, C. M. García and D. L. Durango (2011). "Thymol and carvacrol: biotransformation and antifungal activity against the plant pathogenic fungi Colletotrichum acutatum and Botryodiplodia theobromae." Tropical plant pathology **36**(1): 3-13.
Olano, I., E. A. Paz, M. Cerdeiras, J. Fernandez, F. Ferreira, P. Moyna, M. Soubes, A. Vazquez, S. Vero and M. Bassagoda (1996). "Screening of Uruguayan medicinal plants for antimicrobial activity. Part II." Journal of ethnopharmacology **53**(2): 97-104.
Pascual-Villalobos, M. J. (2002). "Anti-insect activity of bufadienolides from Urginea maritima." Trends in New Crops and New Uses. ASHS Press, Alexandria, VA: 564-566.
Rahman, S. M. A., S. A. Abd-Ellatif, S. F. Deraz and A. A. Khalil (2013). "Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment." African Journal of Biotechnology **10**(52): 10733-10743.
Rane, S. T. a. S. (2008). Decoction and Hot Continuous Extraction Techniques. Extraction Technologies for Medicinal and Aromatic Plants. S. S. Handa, S. P. S. Khanuja, G. Longo and D. D. Rakesh**:** 93 - 106.
Regasini, L. O., J. C. R. Vellosa, D. H. S. Silva, M. Furlan, O. M. M. de Oliveira, N. M. Khalil, I. L. Brunetti, M. C. M. Young, E. J. Barreiro and V. S. Bolzani (2008). "Flavonols from Pterogyne nitens and their evaluation as myeloperoxidase inhibitors." Phytochemistry **69**(8): 1739-1744.
Reller, L. B., M. Weinstein, J. H. Jorgensen and M. J. Ferraro (2009). "Antimicrobial susceptibility testing: a review of general principles and contemporary practices." Clinical infectious diseases **49**(11): 1749-1755.

Sarker, S. D., Z. Latif and A. I. Gray (2005). Natural products isolation, Springer Science & Business Media.
Sathiyamoorthy, P., H. Lugasi-Evgi, P. Schlesinger, I. Kedar, J. Gopas, Y. Pollack and A. Golan-Goldhirsh (1999). "Screening for cytotoxic and antimalarial activities in desert plants of the Negev and Bedouin market plant products." Pharmaceutical biology **37**(3): 188-195.
Seidel, V. (2012). Initial and Bulk Extraction of Natural Products Isolation. Natural Products Isolation. L. N. Satyajit D. Sarker.
Silver, L. and K. Bostian (1993). "Discovery and development of new antibiotics: the problem of antibiotic resistance." Antimicrobial agents and chemotherapy **37**(3): 377.
Singh, J. (2008). Maceration, Percolation and Infusion Techniques for the Extraction of Medicinal and Aromatic Plants. Extraction Technologies for Medicinal and Aromatic Plants. S. S. Handa, S. P. S. Khanuja, G. Longo and D. D. Rakesh.
Snogerup, S. and B. Snogerup (2001). "Bupleurum L.(Umbelliferae) in Europe: 1. The Annuals, B. sect. Bupleurum and Sect. Aristata." Willdenowia: 205-308.
Souza, S. M. d., F. D. Monache and A. Smânia (2005). "Antibacterial activity of coumarins." Zeitschrift fuer Naturforschung C **60**(9-10): 693-700.
Steenkamp, P., F. Van Heerden and B.-E. Van Wyk (2002). "Accidental fatal poisoning by Nicotiana glauca: identification of anabasine by high performance liquid chromatography/photodiode array/mass spectrometry." Forensic science international **127**(3): 208-217.
Stökl, J., A. Strutz, A. Dafni, A. Svatos, J. Doubsky, M. Knaden, S. Sachse, B. S. Hansson and M. C. Stensmyr (2010). "A deceptive pollination system targeting drosophilids through olfactory mimicry of yeast." Current Biology **20**(20): 1846-1852.
Sukhdev, S., P. Suman, L. Gennaro and D. Dev (2008). "Extraction technologies for medicinal and aromatic plants." International Center for Science and High Technology: 196-198.
Sun, J., H. Lou, S. Dai, H. Xu, F. Zhao and K. Liu (2008). "Indole alkoloids from Nauclea officinalis with weak antimalarial activity." Phytochemistry **69**(6): 1405-1410.
Tabassum, N. and M. Hamdani (2014). "Plants used to treat skin diseases." Pharmacogn Rev **8**(15): 52-60.
Tamir, H., Z. Satovic, J. Gorelick, A. Danin, R. Fischer, D. Chaimovitsh and N. Dudai (2011). "Intraspecific variation of Chiliadenus iphionoides essential oil in Israel." Chemistry & biodiversity **8**(6): 1065-1082.
Tandon, S. (2008). Distillation Technology for Essential Oils. Extraction Technologies for Medicinal and Aromatic Plants. S. S. Handa, S. P. S. Khanuja, G. Longo and D. D. Rakesh**:** 118 -127.
Taylor, P., F. Schoenknecht, J. Sherris and E. Linner (1983). "Determination of minimum bactericidal concentrations of oxacillin for Staphylococcus aureus: influence and significance of technical factors." Antimicrobial Agents and Chemotherapy **23**(1): 142-150.
Tshikalange, T. E., J. J. Meyer and A. A. Hussein (2005). "Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases." J Ethnopharmacol **96**(3): 515-519.
Tuncok, Y., O. Kozan, C. Cavdar, H. Guven and J. Fowler (1995). "Urginea maritima (squill) toxicity." Journal of Toxicology: Clinical Toxicology **33**(1): 83-86.
Valgas, C., S. M. d. Souza, E. F. Smânia and A. Smânia Jr (2007). "Screening methods to determine antibacterial activity of natural products." Brazilian Journal of Microbiology **38**(2): 369-380.

Van Wyk, B. and N. Gericke (2000). "People's Plants; Briza Publ." Arcadia, Pretoria, South Africa.
Waksmundzka-Hajnos, M., A. Petruczynik, A. Dragan, D. Wianowska, A. L. Dawidowicz and I. Sowa (2004). "Influence of the extraction mode on the yield of some furanocoumarins from Pastinaca sativa fruits." Journal of Chromatography B **800**(1): 181-187.
Waring, R., A. McDonald, S. Larsson, T. Ericsson, A. Wiren, E. Arwidsson, A. Ericsson and T. Lohammar (1985). "Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition." Oecologia **66**(2): 157-160.
Watt, J. and M. Breyer Brandwijk (1962). "Medicinal and poisonous plants of southern and eastern Africa. 2nd edn. 1457 pp. E. & S. Livingstone Ltd.: Edinburgh & London." S. & E. Africa.[At Museum.] Poisonous plants Toxic plants, geog, General article Review article, Materia medica Pharmacognosy Drug plants Medicinal plants (PMBD, 185304874).

استخلاص شامل وفحص النشاط الحيوي ضد الميكروبات لنبتة ببيليريوم سبوفاتوم: عضو من النباتات الفلسطينية

اعداد: ايه علي أحمد القاضي

إشراف: الاستاذ الدكتور محمود ابو حديد

الملخص

العديد من الأبحاث تتجه اليوم الى استخلاص مركبات جديدة من النباتات التي ستستخدم بعلاج الامراض المختلفة. القدرة العلاجية لمستخلصات لنبتتنا ببيليريوم سبوفاتوم لا تزال غير معروفة. لقد تم استخلاص المركبات العضوية والغير عضوية من نبتتنا من خلال تطوير طريقة استخلاص قادرة على استخلاص جميع المركبات تقريبا من هذا النبات. استخدمنا البكتيريا موجبة غرام ستافيلوكوكس اوريس والبكتيريا سالبة غرام ايشريشيا كولي و سيودومونس اريجنوزا والفطر الخميرة (الكانددا البكن). وقد تم استخلاص مستخلصين مائيين ومستخلص عضوي .وكانت نسبة المستخلص العضوي للاجمالي الكلي 0.56% والمستخلص الاول المائي 25.24% والمستخلص الثاني 11.56%. والبكتيريا الاكثر حساسية لمستخلص النباتنا المائي كانت سيدومونس اريجينوزا 0.16 ملغ / مل ثم الفطر الخميرة (الكانددا البكن) 0.8 ملغ/ مل ثم الايشريشا كولي والستاف اوريس 4ملغ/ مل . وقد تم الحصول على مستخلص قاتل لبكتريا على تركيز 20 ملغ/ مل السودمونس اريجنوزا وفطر الكانددا البكن. وتمت مقارنة نبتتي مع اربع نباتات اخرى في مختبرنا استخلصت بنفس الطريقة. وثبت ان هذا الاستخلاص فعال وكذلك يحافظ على النشاط البيولوجي.