An-Najah National University
Faculty of Graduate Studies

Antibacterial Effect of Some Wild *Allium* Species in Palestine Compared with Cultivars *Allium cepa* and *Allium sativum*

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Dedication

To my dear parents, father and mother in law, husband, daughter, brothers, and friends with love and respect.

Acknowledgement

First of all, I am grateful to the God for the good health and wellbeing that were necessary to complete this thesis.

I wish to express my deepest gratitude to my thesis supervisor Dr. Ghadeer Omar for her supplement with literature, her advice and suggestions and great support through my study.

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الإقرار

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

Antibacterial Effect of Some Wild *Allium* Species in Palestine Compared with Cultivars *Allium cepa* and *Allium sativum*

تأثير بعض انواع البصل و الثوم البريه في فلسطين ضد البكتيريا ومقارنتها مع الاصناف الزراعية *Allium cepa* و *Allium sativum*

أقر بأن ما اشتملت عليه هذه الأطروحة إنما هو نتاج جهدي الخاص، باستثناء مـــا تمـــت الإشارة إليه حيثما ورد، وأن هذه الرسالة كاملة، أو أي جزء منها لم يقدم من قبل لنيل أي درجـــة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Abbreviations

MIC: Minimum Inhibitory Concentration

MRSA: Methicillin-Resistance *Satphylococcus aureus*

NA: Nutrient Agar

NB: Nutrient Broth

MHB: Mueller-Hinton Broth

MSA: Mannitol Salt Agar

MHA: Muellar Hinton Agar

DMSO: Dimethyl Sulfoxide

D.W.: Distilled Water

MBC: Minimum Bacterialcidal Concentration

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Abstract

Fourteen wild *Allium* species are collected, classified and extracted by three extraction methods (ethanol, water and fresh) and tested for their antimicrobial activity on five strains of Methicillin-Resistant *Staphylococcus aurues*. This is besides the cultivated one *Allium sativum* and *Allium cepa* by broth microdilution method. *Allium qasyunense* showed the highest antibacterial activity against MRSA strains by recording the lowest MIC and MBC values. Water leaf extract of *A. qasyunense s*howed the highest antibacterial activity against MRSA strain 5 at 1.56 mg/ml. Ethanolic leaf extract of the same species showed highest antibacterial activity against the same MRSA strain at 0.049 mg/ml. While, its fresh bulb extract showed the highest antibacterial activity at MIC= 0.49 mg/ml against MRSA strain 1. From this work, it was conclude that the part of plant and the extract type affected the antibacterial activity. For example leaf part when extracted by ethanol give higher antibacterial activity than water since ethanol helps in saving the stability of organosulfur compounds such as allicin. In case of plant part, Bulb part possessed higher antibacterial activity than the leaf part. The study revealed that wild *Allium* species have higher antibacterial activity than the cultivated one *A. cepa* and *A. sativum*.

Chapter One

Introduction

Chapter One
Introduction

Allium species are supposed to be one of the world's oldest cultivated vegetables as they have been largely reported. It is presumed that our predecessors discovered and consumed wild *Allium* species long before farming or writing was invented (Benkeblia, 2004). At the present time, the genus *Allium* is one of the largest genera in the world flora as about seven hundred *Allium* species can be found in the Northern Hemisphere. This genus in the past was classified under the Lily family (Liliaceae) but now with the advanced systematic research it is classified under the Aliaceae family. Most of *Allium* species are tall plants with umbrella like inflorescence and all their flower stalks emerge from one point. Bulbs are found in the entire genus *Allium,* which are used by the plant as a storage organ for nutrient and water, and as a reproductive organ, enabling it to survive during drought years and to bloom in different seasons (Fragman-Sapir, 1985). *Allium* was named by Romans for garlic (Wendelbo and Sturat, 1985). The most widely cultivated *Allium* species used are *A. cepa* (onion), *A. sativum* (garlic), and *A. porrum* (leeks) which are thought to be originated from the wild *A. vavilovii, A. longicuspis,* and *A. ampeloprasum* respectively. *A. schoenoprasum* (chives) and *A. ascalnicum* (shallots) are one of the cultivated species (Sengputa et al., 2004).

Although the genus *Allium* has more than seven hundred species, each one differs in appearance, color and taste, but close in biochemical, phytochemical and nutraceutical content. So that there are over one

hundred and twenty different studies documented the uses of the *Allium* plants (Benkeblia and Lanzotti, 2007).

Besides their remarkable medicinal power, *Allium* plants are generally consumed for their flavors, while their nutritive values have been recently appreciated. Alliums were recorded to possess antibacterial and antifungal activities, as they contain the powerful sulfur and other numerous phenolic compounds, which arouse great interest (Fenwick and Hanley,1990; Garlic and Health Group,2007).

In respect to *A. sativum* is thought to derive from the wild species *A. longicuspis* which is found in Turkey and central Asia (Maidment et al., 2001). It was known as one of the earliest plants used in diet of many Egyptians. It was fed particularly to the working class involved in heavy labor, as in the building of the pyramids. In ancient China and Japan, it was used as food and as a medicinal agent. The best estimate was that by or earlier 2000 BC, *A. sativum* was in wide use in China and formed part of the daily diet, and was also used as a food preservative. However, in ancient Chinese medicine, it was prescribed to aid respiration and digestion, especially in diarrhea and worm infestation. According to ancient Rome, the Romans perceived *A. sativum* as an aid in strength and endurance. In addition, it was prescribed for digestion, animal bites, arthritis and convulsions, and later was used for respiratory ailments and for parasites (Rivlin, 2001).

More recently, Alliums (Onion and garlic species) have been reported to be effective in various ailments such as cardiovascular diseases

as their extracts act on the blood coagulability risk factor for such diseases (Kendler, 1987). The effects of *A. sativum* on cardiovascular diseases were reviewed previously (Rahman and Lowe 2006). They showed that garlic reduces cholesterol, inhibits platelet aggregation, reduces blood pressure and increases antioxidant status. Moreover studies showed that alliums have a great ability to be an anticancer agent against different types of cancers, such as stomach, colon, esophagus, and perhaps breast cancer (Fleischauer et al., 2000; Fleischauer and Arab, 2001 and Sengupta et al., 2004). Information about other Alliums' medicinal effects was mentioned, such as, antioxidant as *A. cepa* was known for being a good natural source of flavonoids, which act as antioxidant against free radicals (Santas et al., 2009). Furthermore, they were known to be antifungal against different fungal species such as *Candida*, *Cryptococcus*, *Trichophyton*, *Epidermophyton*, *Microsporum*, and *Aspergillus* species (Yamada and Azuma, 1977; Yin and Tsao, 1999). Moreover, as antibacterial for different bacterial species, and also act as food preservative against many organisms (Benkeblia and Lanzotti, 2007). Furthermore, they have antiparasitic effects and antiviral effects against different parasites and viruses, respectively (Ankri and Mirelman 1999).

Objectives of the study

All previous studies concerning the medicinal effect of Alliums were conducted on the cultivated *Allium* species, mainly *A. sativum* and *A. cepa*. However, information about the medicinal uses of wild *Allium* species is

scarce. As the wild *Allium* species are needed to be collected from their natural habitat and to be identified and classified by a plant taxonomist. Therefore, due to lack of information about the effects of wild *Allium* species in Palestine as antibacterial agent, this research was conducted to study the antibacterial effect of water, fresh, and ethanolic extract of different *Allium* speices against Methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) strains using broth microdilution method.

Chapter Two
Literature Review

Chapter Two
Literature Review

2.1 Antimicrobial activity

The first evidence of *A. sativum* antimicrobial properties was established in France in 1721 by chance in Marseilles during plague. That is four men were employed to remove the dead bodies, none of them became infected with plague. The secret was due to a macerated *A. sativum* and wine tincture (Harris et al., 2001). Since investigating plant extracts for antibacterial activity, it was observed that a freshly prepared infusion of ground *A. sativum* bulblets bear high antibacterial activity when tested by cylinder plated method used for the assay of penicillin (Cavallito & Bailey, 1944). Several investigators have observed antibacterial activity of *A. sativum* extracts and have referred this activity to diallyl sulfide which is allicin derivative compound. Allicin isolated from *A. sativum* was characterized, physical properties and antimicrobial actions were studied. Allicin showed a sharp zone of inhibition with periphery accentuated by a line of heavy growth (Cavallito and Bailey, 1944). Later on it was reported that *A. sativum* showed an inhibitory effect against *Mycobacterium tuberculosis* (Raghunandana et al., 1946). The antibacterial effects of *A. sativum* and other *Allium* vegetables up to mid-1984 was reviewed (Fenwick and Hanley, 1985). Later, an *in vitro* mechanism of inhibition of bacterial cell growth by allicin was reported. It was shown that allicin exhibits its antimicrobial activity by delaying and inhibiting partially DNA and protein synthesis, while inhibition of RNA synthesis was immediate

and total, suggesting that this is the primary target of allicin action. In addition, it showed that the minimum inhibition concentration of allicin against *Salmonella typhimurium (S. typhimurium)* was at concentarion 0.2-0.5 mM (Feldberg et al., 1988).

Furthermore, spectrophotmetric method for quantitative determination of allicin and total garlic thiosulfinates showed that the antibiotic activity of 1 mg of allicin has been equated to that of 15 IU of penicillin (Han *et al*. 1995). The first report of *Helicobacter pylori* (*H. pylori*) susceptibility to *A. sativum* extract of known thiosulfinate concentrations was reported. It was demonstrated in vitro that *H. pylori* was susceptible including some strains which were antibiotic-resistance (Sivam et al., 1997). Moreover, allicin and allyl-methyl plus methyl-allyl thiosulfinate extracted from *A. sativum* showed *in vitro* growth inhibition of *H. pylori*. The capacity and effectiveness of isolated natural thiosulfinates had been tested, and this has enabled the identification of the main compounds responsible for the bacteriostatic activity (Benkeblia and Lanzotti, 2007).

Other investigations have also documented an inhibitory effect agianst different bacterial species such as, *Bacillus subtilis* (*B. subtilis*), *Escherichia coli*(*E. coli*), *Flavobacterium* spp., *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella typhimurium* (*S. typhimurtium)*, and *Vibrio parahaemolyticus* (Abdou et al., 1979; Hsieh et al., 2001; Ward et al., 2002; Lai and Roy, 2004; Durmaz et al., 2006). However, these authors

reported different minimum inhibitory concentration (MIC) depending on the type of extract, concentration of effective ingredients of thiosulfinates in the extracts, methods used for the assessment of the inhibitory effect and the tested microorganisms.

Moreover, the antibacterial effect of *A. sativum* was reviewed showing that it exhibited a wide spectrum antibiotic effect against gram-positive bacteria, Enterotoxigenic *E. coli* (ETEC) strains and other pathogenic intestinal bacteria, which are responsible for diarrhea in humans. In addition, it is active against multi-drug resistant strains. Moreover, it gives partial or total synergism when combines with antibiotics. Also *A. sativum* oil preparation showed a good anti-tuberculosis activity in guinea pigs with an intraperitoneal dose of 0.5 mg/kg, in addition to complete lack of bacterial resistance to its extracts. It has a bactericidal activity; toxins production by bacteria is also prevented (Sivam, 2001).

The antimicrobial activity of essential oil (EO) extracts of *A. cepa* and *A. sativum* against two bacteria *S. aureus* and *Salmonella enteritidis* (*S. enteritidis*) was investigated. Results showed that inhibition zone depends on EO extracts concentration; as it increases the inhibition activity increases, recording that *S. enteritidis* is more sensitive at high concentration than *S. aureus* (Benkeblia, 2004).

It was reported that *A. cepa* essential oil extracts was effective *in vitro* against many bacteria species including *B. subtilis*, *Salmonella* spp,

and *E. coli* (Cavallito and Bailey, 1944; Yoshida et al., 1998; Yin and Cheng, 2003; Benkeblia, 2004; Azu et al., 2007). The sensitivity of these bacterial strains differs among different studies depending on the extraction methods of *A. cepa*. However, *A. cepa* is not as potent as *A. sativum,* since the sulfur compound in *A. cepa* is about one-quarter of that in *A. sativum*. Hughes and Lawson (1991) reported an antimicrobial effect of *A. sativum*, *A. ampeloprasum* and *A. cepa* against fungal species and two bacterial strains *E. coli*, and *S. aur*eus. They found out that *A. cepa* showed less antifungal and antibacterial activity than *A. sativum*. Another study investigated the antibacterial activity of *A. cepa* extracts (steam and chemicals treating) against oral pathogens, *Streptococcus mutans*, *Streptococcus sobrinus, Porphyromonas gingivalis* and *Prevotella intermedia*. Results showed that *A. cepa* extract possessed an inhibitoty effect on all bacterial strains (Kim, 1997). Durmaz et al. (2006) recorded an antibacterial activity of wild *A. vineale* species and other two wild plants against *B. subtilis*, *B. cereus*, *Micrococcus luteus* (M. luteus), *S. aureus, E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *S. enteritidis* and *S. typhimurium* using disc diffusion method. *Allium vineale* showed a higher antibacterial activity compared with the two other plant species.

On the same year, the effect of 15 medicinal wild plants species collected from Idris Mountain in Ankara-Turkey, one of which is *A. rotundum* was studied. They are used in traditional medicine in Turkey as antiseptic, antibacterial, wound healer laxative, diuretic etc.. In this study researchers used *S. aureus*, *E. coli*, *B. subtilis*, *Candida albicans* (*C.*

albicans), *C. krusei* and *C. glabrata* as test microorganisms. *Allium rotundum* showed moderate antibacterial activity on *B. subtilis*. Also the methanol extracts of *A. rotundum* showed antifungal activity on all tested candidas (Tosun et al., 2006).

Later, antimicrobial activity of *A. cepa* raw and aqueous extracts against *S. aureus* and *Pseudomonas aeruginosa* (*P. aeruginosa*) was examined. It showed an antibacterial effect against the two tested strains, with a widest zone of inhibition was against *P. aeruginosa* (Azu et al., 2007).

Furthermore, the antimicrobial and antioxidant activity of the crude *A. cepa* methanolic extracts were detected. The antimicrobial activity of flavonol standards and *A. cepa* extract was evaluated against some food spoiler microorganisms Gram positive bacteria (*B. cereus*, *S. aureus*, *M. luteus* and *L. monocytogenes*), and Gram negative bacteria (*E. coli* and *P. aeruginosa)*. Results showed that Gram negative bacteria more susceptible to *A. cepa* extract (Santas *et al.*, 2009).

Allium essential oils extract also has been considered as natural preservative or food additive, and can be used as supplementary methods of scheming pathogens (Whitemore and Naidu, 2000).

2.2 Organosulfur compounds in *Allium* species

Many studies have been made to identify the active compounds in the genus *Allium* which are responsible for its medicinal effects. It has been

shown that sulfur compounds such as allicin are the most important constitutes of Allium. These studies reveled that the observed medicinal effects are mainly because of the following organosulfer compounds:

1. Ajoene: the most active antiviral compound in Allium(Weber et. al., 1992).

2. Diallyl disulphide (DDS): this substance is active against yeast (Avato et. al., 2000).

3. Allicin: this compound appears in *Allium* after being crushed, as it occurs by the action of allinase enzyme (Benkeblia, 2004).

4. S-allylcysteine (SAC): the most abundant organosulfur compound found in aged *Allium* (Cruz et. al., 2007).

2.3 *Staphylococcus aureus*

Taxonomically, the genus *Staphylococcus* is in the bacterial family *Staphylococcaceae*. Staphylococci are Gram-positive spherical bacteria, which usually are arranged in grape-like clusters, facultative anaerobes, catalase-positive and oxidase-negative. The genus *Staphylococcus* has at least thirty species. The three main species of clinical importance are *S. aureus*, *S. epidemidis*, and *S. saprophyticus*. *Staphylococcus aureus* is coagulase positive which differentiates it from other species. It has a large, round, golden-yellow colonies, often with β-hemolysis when grown on blood agar (Brooks et al., 2007).

Staphylococcus aureus is an important pathogen due to a combination of toxin-mediated virulence, invasiveness, and antibiotic resistance. Approximately 20-30% of the human populations are *S. aureus* carriers (Wertheim et al., 2005).

Staphylococcus aureus can cause a range of illnesses from minor skin infection, such as pimples, impetigo, boils, cellulitis, folliculitis, furuncles, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as astitis, pneumonia, meningitis, ostiomylitis, endocarditis, urinary tarct infections, toxic shock syndrome, and septicemia. Some *S. aureus* strains are able to produce staphylococcal food poisoning. This bacterium is a significant cause of nosocomial infections, as well as community-acquired diseases. This pathogen expresses many potential virulence factors: (1) surface proteins that promote colonization of host tissues; (2) invasions that promote bacterial spread in tissues (leukocidin kniases, hyaluronidase); (3) surface factors that inhibit phagocytic engulfment (capsule, protein A); (4) biochemical properties that enhance their survival in phagocytes (catalase production); (5) immunological antigens (protein A, coagulase, clotting factor); (6) membrane damging toxins that lyse eukaryotic cell membranes (hemolysis, leukotoxin, leukocidin; (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease (staphylococcal enterotoxins, the exofolative toxins, and toxic shock syndrome toxins) (8) inherent and acquired resistance (Dinges et al.,2000).

Chapter Three

Materials and Methods

Chapter Three
Materials and Methods

3.1 Media preparation

3.1.1 Nutrient agar (NA)

Nutrient agar was prepared according to the manufacturer's instructions labeled on the bottle (Acumedia). One L bottle containing 500 ml deionized water and 11.5 g of Nutrient Agar were boiled and stirred until the agar dissolved. The falsk was plugged with a piece of cotton, covered with aluminium foil, then autoclaved at 121 °C for 15 minutes and left to cool in 50ºC water bath. The agar was then poured into sterile Petri dishes 20ml each, covered and left overnight at room temperature. The following morning the Petri dishes were turned upside down and kept in refrigerator at 5ºC.

3.1.2 Nutrient broth (NB)

Nutrient broth was prepared according to the manufacturer's instructions labeled on the bottle (Acumedia). A 0.5 L bottle containing deionized water (250 mL) and 2 g of nutrient broth were mixed well and boiled. The broth was then poured into tubes 5-10 ml each, covered by cotton plug and aluminum foil, and autoclaved at 121°C for 15 minutes, allowed to cool and then kept in refrigerator at 5ºC.

3.1.3 Muellar hinton agar (MHA)

Muellar-Hinton agar was prepared according to the manufacturer's instructions labeled on the bottle (Acumedia). Two L bottle containing 1 L

of deionized water and 38 g of MHA and 20g NaCl were heated and stirred until the agar dissolved. The solution was allowed to boil for 1 minute. After that, the flask was plugged with cotton and covered with aluminum foil, and then autoclaved at 121 °C for 15 minutes. After that it was allowed to cool to about 50ºC, and the agar poured into sterile Petri dishes (25-30 ml) that covered and left overnight at room temperature. The following morning the Petri dishes were turned upside down and kept in refrigerator at 5ºC.

3.1.4 Mannitol salt agar (MSA)

Mannitol agar was prepared according to the manufacturer's instructions labeled on the bottle (Acumedia). A 0.5 L bottle containing deionized water (250 mL) and 27.75 g of MSA were heated and stirred until the agar dissolved. The solution was allowed to boil for 1 minute. After that, the flask was plugged with cotton and covered with aluminum foil, and then autoclaved at 121°C for 15 minutes. After that it was allowed to cool at about 50ºC, and the agar poured into sterile Petri dishes (20 ml) that was covered and left overnight. The following morning the Petri dishes were turned upside down and kept in refrigerator at 5ºC.

3.1.5 Blood agar

One L bottle containing 500 ml of deionized water and 20 g of blood agar base (Himedia) were heated and stirred until the agar dissolved. The flask was plugged with cotton and covered with aluminum foil. Then, the

solution was autoclaved at 121ºC for 15 minutes and allowed to cool to about 50ºC. After that, 25 ml of sterile defibrinated blood was added aseptically and mixed well. The agar then was poured into Petri dishes to have 20-25 ml each, then covered and left overnight at room temperature. The following morning the Petri dishes were turned upside down and stored at 5ºC.

3.2 Bacterial preparation

3.2.1 Bacterial strains

Five MRSA strains were isolated from clinical specimens collected from hospitals in North Palestine (n=1; wound swab from Specialized Arab Hospital, n=2; nasal swab and diabetic patient wound swab from Rafedia Hospital, and n=2; urine and blood samples from Specialized Nablus Hospital). Strains identification was confirmed in Microbiology laboratories at An-Najah National University-Nablus, Palestine, according to colonial and microscopic morphology, growth on Mannitol Salt agar, 5% blood sheep agar, positive catalase, and coagulase production. Methicillin resistance was carried out in the microbiology laboratories at An-Najah National University, Palestine, using the disk diffusion method (Bauer et al., 1966). Oxacillin (1μg) disks (Oxoid) were used, and inhibition zones were determined in accordance with procedures described by Clinical and Laboratory Standards Institute (CLSI, 2011). According to CLSI, *S. aureus* isolates were considered resistant to oxacillin if inhibition zones were ≤ 10 mm after incubation on 2% NaCl MHA at 35ºC for 18-24 hr. Oxicillin

resistance control strains from our department collection and susceptible refrance strain of *S. aureus* (ATCC 25923) were used.

3.3 Identification of *S. aureus*

3.3.1 Gram staining

Gram staining of bacteria was performed from nutrient broth by placing 1-2 drops of fresh bacterial broth on a clean glass slide. The slide was air-dried and heat fixed. After that, the slide was flooded with crystal violet stain for 1 min, then washed by tap water and flooded again with potassium iodine for 30 secondes. This was followed by washing the slide with tap water and then by aceton alcohol decolorizer for 30 second. Then, the slide was flodded with safranin counter stain for 1 min. After that the slide was washed with tap water, then dried and examined under oil immersion lens of light microscope, as described by Cappuccino and Sherman (1996).

3.3.2 Catalase test

Catalase test was carried out by addition of 1-2 drops of 3% hydrogen peroxide on bacterial colony cultured on NA (Cappuccino and Sherman, 1996). Positive catalase test was indicated by development of air bubbles, while absence of air bubbles was recorded as negative catalase test.

3.3.3 Mannitol fermentation

Aseptically a single line of inoculation of test organism was cultured on MSA. The plate culture was incubated for 24 hours at 37ºC (Cappuccino and Sherman, 1996). *Staphylococcus aureus* was able to ferment manitol and produced yellow halo around the colonies on MSA.

3.3.4 Slide coagulase test

Using sterile flame inoculating loop, part of suspected colony from NA was emulsified with one drop of diluted 1:4 fresh citerated Plasma (1 ml citrated plasma with 3 ml sterile normal saline) on a slide with continuous mixing. Agglutination or clumping within 1 minute was considered as positive (Cappuccino and Sherman, 1996). Negative samples were further tested by tube-coagulase test method.

3.3.5 Tube coagulase test

Using inoculating loop, a loopful from slide coagulase negative colony was inoculated in 1 ml of 1:4 diluted fresh citrated human plasma. The tube was incubated for 18-24 hours at 37ºC and inspected for clot formation at hourly intervals. The absence of coagulation after 24 hours of incubation is a negative result. (Cappuccino and Sherman, 1996).

3.4 Plant collection and extraction preparation

3.4.1 Plant specimens

The field survey was conducted over different regions in the West Bank, Palestine starting in April 2011 till June 2012, and 2013 to collect

different wild *Allium* species during their vegetative growing season. Fourteen plant species of *Allium* were collected. Collected wild and cultivated *Allium* plant species were identified and classified by Dr. Ghadeer Omar according to Flora Palestina (Feinburn-Dothan, 1986). Then identified plant specimens were pressed till drying, treated chemically and after then mounted on herbarium sheets. The representative plant specimens of the studied taxa were deposited at An-Najah National University Herbarium, Department of Biology and Biotechnology, Faculty of Science. The collected *Allium* species were:

1. *Allium ampeloprasum* L.
2. *Allium artemisietorum* Eig & Feinbrun.
3. *Allium desertorum* Forssk.
4. *Allium hierochuntnium* Boiss.
5. *Allium neapolitanum* Cyr.
6. *Allium orientale* Boiss.
7. *Allium pallens* L.
8. *Allium paniculatum* L.
9. *Allium phanerantherum* Boiss & Hausskn.
10. *Allium qasyunense* Mout.
11. *Allium schubertii* Zucc

12. *Allium stamineum* Boiss.

13. *Allium trifoliatum* Cyr.

14. *Allium truncatum* (Feinbrn.) Kollmann & D. Zohary

Table (3.1): The plant specimen's area of collection.

Plant number	Plant scientific name	Area of collection
1	*Allium ampeloprasum*	Nablus, Ramallah, Tamoun, Yammoun
2	*Allium artemisietorum*	Dead Sea Valley, Annasarieah
3	*Allium desertorum*	Jericho
4	*Allium hierochuntnium*	Jericho
5	*Allium neapolitanum*	Nablus
6	*Allium orientale*	Nablus
7	*Allium pallens*	Nablus
8	*Allium paniculatum*	Nablus
9	*Allium phanerantherum*	Nablus
10	*Allium qasyunense*	Upper Jordan Valley, Tamoun
11	*Allium schubertii*	Nablus
12	*Allium stamineum*	Nablus, Tamoun, Yamoun, Yaseed, Ramallah.
13	*Allium trifoliatum*	Nablus, Tamoun, Upper Jordan valley
14	*Allium truncatum*	Nablus, Toulkarm, Ramallah, Taloza, Yaseed, Tamoun, Yamoun

3.4.2 Plant extraction

3.4.2.1 Water leaf extraction

Five grams of dry ground *Allium* leaves were soaked in 25 ml of sterilized water and incubated in shaking incubator at room temperature for 48 hour. The extracts were filtered by centrifugation and dried by the incubator at 37ºC to obtain stock solutions of 100 mg/ml of D.W. The

extracts were kept in the refrigerator at 5ºC till use (Jabar and Al-Mossawi, 2007).

3.4.2.2 Ethanolic leaf extraction

Five grams of dry ground *Allium* leaves were soaked in 25 ml of ethanol and incubated in shaking incubator at room temperature for 48 hour. The extracts were then filtered out by centrifugation and dried using Rotary evaporator, to obtain stock solutions of dried *Allium* leaves of about 100 mg/ml of Dimethyl sulfoxide (DMSO) (10%). Stock solutions were kept in the refrigerator till used (Jabar and Al-Mossawi, 2007).

3.4.2.3 Fresh bulbs extraction

Allium bulbs were skinned, chopped, blended (Moulinex Blender) and finally freeze-dried. The freeze-dried bulbs were ground with a mortar and pistil to obtain fine powders which dissolved in sterile D.W to achieve 100 mg/ml solution (Santas et al., 2009). Stock solutions were refrigerated until use.

3.5 Antimicrobial activity tests

3.5.1 Preparation of starter cultures

A flame sterilized inoculating loop was used to scrape a 3-5 colonies from the nutrient agar plate and then transferred into a tube containing 5 ml of nutrient broth. The loop was rotated numerous times to ensure that the tip of the loop came in contact with the bottom of the vial. The inoculated

broth was incubated at 37 °C for 4-6 h and gently agitated approximately every half an hour. These cultures were used to inoculate 2% NaCl-MHA to detect MRSA strains.

3.5.2 Preparation of McFarland turbidity standard No. 0.5

McFarland 0.5 turbidity standard was prepared by adding 50 µl of a 1.175% (wt/vol) barium chloride dihydrated ($BaCl_2.2H_2O$) solution to 9.95 ml of 1% (vol/vol) sulfuric acid. McFarland standard tube was then sealed with Parafilm to prevent evaporation and stored in the dark at room temperature. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer with a 1-cm light path. For the 0.5 McFarland standards, the absorbance at a wavelength of 625 nm and water as a blank was 0.08 to 0.13. The 0.5 McFarland standard was vigorously agitated on a vortex mixer before use. As with the barium sulfate standards, a 0.5 McFarland standard is comparable to bacterial suspension of 1.5 X 10^8 colony-forming units (CFU/ml) (Andrews, 2006).

3.5.3 Antibacterial activity (broth microdilution method)

Antibacterial activity was measured using Broth microdilution method in accordance with procedures described by Clinical and Laboratory Standard Institute (CLSI, 2010). The plant extract was serially diluted in Mueller Hinton broth, and then bacterial inoculums size of 10^5 CFU/ml was added to each well. Controls wells containing either bacterial suspension only, plant extract suspension only or sterile nutrient broth only were included in the microdilution plate. Each plant extract was run in

duplicate. Then test plates were incubated at 37ºC for 18 hour. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test microorganism. The minimum bactericidal concentration (MBC) was determined following the method described previously by (Irobi and Daramola, 1994). Well, with no visible growth MIC assays were subcultured using a 10 µl inoculating loop on nutrient agar and incubated at 37ºC for 18-24 hours. If no growth seen on NA, the tested dilution was considered as MBC. The MBC was defined as lowest concentration of the extract at which bacteria are killed. The average of two replicates for each extract was calculated.

Ampicillin antibiotic 100 µg/ml and each plant extract were serially diluted in duplicated and bacterial strains were included. A reference strain [*Staphylococcus aureus* ATCC 25923] was also included in this study.

3.6 Statistical analysis

Mean (average) and standard deviation (σ) of MIC values (mg/ml) of each species against five MRSA strains was computed by formulas (µ= sum of x values/ N (numbers of values), $\sigma=\sqrt{(X-\mu)/(N}$). T-value was computed by paired t-test formula $t=\frac{\bar{d}}{\sqrt{s^2/n}}$ where d bar: is the mean difference between two samples (MIC average of plant and MIC average of Ampicillin), s^2: is the sample variance, n: is the sample size. P-value was taken from the t-value by SPSS Software. Results were considered significant when $p< 0.05$.

Chapter Four

Results

Chapter Four
Results

4.1 Identification of MRSA isolates

Gross colony morphology was medium-sized yellow colonies on MSA. Gram stain of the isolates showed gram positive cocci in clusters, single, short chain and diploid. All isolates were catalase positive, coagulase positive and mannitol fermentation positive. All five strains of *S. auerus* were resistant to oxacillin using disk diffusion method and Inhibition zones were ≤10 mm.

4.2 Detection of antibacterial effect of water leaf extract of studied *Allium* species using microdilution method

Statistical analysis of the obtained results showed variation among the water leaf extracts of the studied *Allium* species. Among of which five species have had significant antibacterial activity against MRSA strains, under investigation. Those species were *A. orientale, A. truncatum, A. stamineum, A. schubertii* and *A. trifoliatum*.

However, statistical analysis confirmed that there were variations among examined MRSA strains. Therefore, they cannot be considered as one sample.

Results indicated that *A. qasyunense* had a lower MIC value against strain 5 which was 1.56 mg/ml. As a result, it can be considered as the most effective wild *Allium* plant species against strain 5 and strain 1. While,

Allium neapolitanum showed the lowest antibacterial activity against all studied MRSA strains by having the highest MIC of >50 mg/ml.

Allium sativum had the highest antibacterial activity against strain 1 at MIC =2.3425 mg/ml, and *A. cepa* at MIC =9.375 mg/ml against the same strain. Minimum inhibitory concentrations values of water leaf extract of studied *Allium* species against the 5 MRSA strains are presented in table 4.1.

Table (4.1) MIC values (mg/ml) for water leaf extract of studied *Allium* species against different strains of MRSA.

Plant number	Plant species	MIC (mg/ml) for leaf water extract										
		Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	MICs average	Σ	t-value	p-value	Significance	Reference strain*
1	*A. ampeloprasum*	50	>50	50	50	50	>50					50
2	*A. neapolitanum*	>50	>50	>50	>50	>50	>50	0.00				50
3	*A. orientale*	6.25	6.25	6.25	3.125	12.5	6.88	3.42	3.369	0.028	significant	6.25
4	*A. pallens*	37.5	12.5	3.125	NT	6.25	11.88	15.05	1.509	0.206	Not sig	3.125
5	*A. hierochuntnium*	50	50	>50	25	>50	41.6	14.43	4.794	0.041	significant	25
6	*A. truncatum*	12.5	6.25	3.125	12.5	6.25	8.13	4.19	3.417	0.027	significant	0.78
7	*A. stamineum*	25	12.5	25	12.5	25	20.00	6.85	5.971	0.004	significant	25
8	*A. schubertii*	6.25	3.125	6.25	6.25	4.6875	5.31	1.40	5.751	0.005	significant	3.125
9	*A. qasyunense*	3.125	NT	NT	NT	1.56	2.34	1.11	0.798	0.571	Not sig	0.78
10	*A. trifoliatum*	6.25	12.5	12.5	6.25	3.125	8.13	4.19	3.417	0.027	significant	3.125
11	*A. phanerantherum*	NT	NT	25	NT	NT						25
12	*A. artemisietorum*	50	50	50	NT	50	50.00	0.00				25
13	*A. desertorum*	50	50	50	NT	50	50.00	0.00				25
14	*A. paniculatum*	NT	NT	NT	NT	NT						50
15	*A. sativum*	2.3425	9.375	25	12.5	9.375	11.72	8.31	2.693	0.055	Not sig	18.75
16	*A. cepa*	9.375	18.75	25	12.5	18.75	16.88	6.09	5.564	0.005	Sig	18.75
	Ampiciline (100µg/ml)	3.125	0.780	3.125	0.780	0.780	1.718					

*** NT: Not tested, ****S. aureus*** **ATCC 25923, * Not sig: not significant, * Sig,:significant**

4.3 Detection of antibacterial effect of ethanolic leaf extract of studied *Allium* species using microdilution method

The highest antibacterial activity of the obtained ethanolic leaf extract of the examined *Allium* species was observed for *A. qasyunense* against MRSA strain 5 as having the lowest MIC value of 0.049 mg/ml. while, the lowest antibacterial activity of the ethanolic leaf extract was recorded for *A. ampeloprasum, A. pallens, A. trumcatum, A. stamineum, A. phanerantherum* and *A. paniculatum* against certain MRSA strains as they had MIC of 50 mg/ml. On the other hand, the cultivated *Allium* species *A. sativum* and *A. cepa* have had their highest antibacterial activity against the MRSA strains 1 and 2 at MIC of 18.75 mg/ml and 12.5 mg/ml, respectively. Minimum inhibitory concentration values of ethanolic leaf extract of studied *Allium* species against 5 MRSA strains are presented in table 4.2.

Table (4.2) MIC values (mg/ml) for ethanolic leaf extract of studied *Allium* species against different strains of MRSA.

Plant number	Plant species	MIC (mg/ml) for leaf ethanol extract										
		Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	MICs average	Σ	t-value	p-value	Significance	Reference strain*
1	*A. ampeloprasum*	50	50	>50	50	50	50					50
2	*A. neapolitanum*	12.5	25	12.5	6.25	12.5	13.750	6.847	3.93	0.017	Sig	6.25
3	*A. orientale*	1.56	0.78	1.56	0.39	0.195	0.897	0.641	-2.86	0.046	Sig	0.39
4	*A. pallens*	50	50	50	NT	12.5	40.625	18.750	4.15	0.025	Sig	25
5	*A. hierochuntnium*	12.5	12.5	25	25	12.5	17.500	6.847	5.15	0.007	Sig	6.25
6	*A. truncatum*	50	50	25	25	25	35.000	13.693	5.44	0.006	Sig	25
7	*A. stamineum*	50	25	50	18.75	37.5	36.250	14.252	5.42	0.006	Sig	25
8	*A. schubertii*	>50	1.56	0.78	3.125	0.195	1.415	1.270	-0.47	0.666	Not sig	0.78
9	*A. qasyunense*	1.17	0.098	0.098	NT	0.049	0.354	0.545	-5.01	0.015	Sig	0.78
10	*A. trifoliatum*	1.56	0.78	NT	NT	NT	1.170	0.552	-1.41	0.394	Not sig	0.78
11	*A. phanerantherum*	25	50	25	50	12.5	32.500	16.771	4.10	0.015	Sig	3.125
12	*A. artemisietorum*	12.5	25	25	25	25	22.500	5.590	8.31	0.001	Sig	12.5
13	*A. desertorum*	12.5	12.5	25	12.5	12.5	15.000	5.590	5.31	0.006	Sig	6.25
14	*A. paniculatum*	25	50	25	25	25	30.000	11.180	5.66	0.005	Sig	25
15	*A. sativum*	18.75	18.75	12.5	25	25	20.000	5.229	7.82	0.001	Sig	12.5
16	*A. cepa*	12.5	12.5	12.5	25	12.5	15.000	5.590	5.31	0.006	Sig	18.75
	Ampiciline (100µg/ml)	3.125	0.780	3.125	0.780	0.780	1.718					

*** NT: Not tested, *_S. aureus_ ATCC 25923, * Not sig: not significant, * Sig,:significant**

4.4 Detection of antibacterial effect of fresh bulb extract of studied *Allium* species using microdilution method

The lowest MIC for the fresh bulb extract of the studied *Allium* species was recorded for *A. qasyunense*. It had MIC of 0.4875 mg/ml against MRSA strain 1. However, the highest MIC value of 50 mg/ml was shown by *A. am*peloprasum, *A. neapolitanum, A. pallens, A. truncatum* and *A. phanerantherum.* The cultivated *A. cepa* fresh bulb extract antibacterial activity was highest on MRSA strain 2 of MIC = 9.375 mg/ml. While, *A. sativum* highest activity was against MRSA strains 1 and 5 of MIC= 18.75. the represented results reveled the higher antibacterial activity of *A. cepa* as it had lower MIC value. Minimum inhibitory concentrations values for fresh bulb extract of the studied *Allium* species against 5 MRSA strains are presented in table 4.3.

Table (4.3) MIC values (mg/ml) for bulb fresh extract of studied *Allium* species against different strains of MRSA

Plant number	Plant species	MIC (mg/ml) fresh bulb extract										
		Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	MICs average	Σ	t-value	p-value	Significance	Reference strain *
1	*A. ampeloprasum*	50	50	50	50	50	50.00					50
2	*A. neapolitanum*	25	50	50	12.5	25	32.50	16.77	4.10	0.015	Sig	12.5
3	*A. orientale*	25	50	6.25	50	50	36.25	19.96	3.87	0.018	Sig	0.78
4	*A. pallens*	12.5	25	50	NT	18.75	26.56	16.44	3.02	0.057	Not sig	12.5
5	*A. hierochuntnium*	12.5	25	12.5	6.25	25	16.25	8.39	3.88	0.018	sig	3.125
6	*A. truncatum*	25	50	50	50	>50	43.75	12.5	6.725	0.007	Sig	25
7	*A. stamineum*	50	50	50	37.5	50	47.50	5.59	18.31	0.00	Sig	50
8	*A. schubertii*	25	>50	>50	>50	>50	>50					25
9	*A. qasyunense*	0.4875	0.78	1.56	NT	3.125	1.49	1.18	-0.39	0.723	Not sig	0.098
10	*A. trifoliatum*	0.78	12.5	6.25	6.25	6.25	6.41	4.15	2.53	0.065	Not sig	1.17
11	*A. phanerantherum*	25	50	50	50	50	45.00	11.18	8.66	0.001	Sig	3.125
12	*A. artemisietorum*	18.75	18.75	12.5	18.75	25	18.75	4.42	8.62	0.001	Sig	12.5
13	*A. desertorum*	18.75	25	12.5	25	12.5	18.75	6.25	6.09	0.004	Sig	12.5
14	*A. paniculatum*	12.5	12.5	12.5	12.5	25	15.00	5.59	5.31	0.006	Sig	12.5
15	*A. sativum*	18.75	25	25	25	18.75	22.50	3.42	13.58	0.00	Sig	9.375
16	*A. cepa*	12.5	9.375	18.75	18.75	18.75	15.63	4.42	7.04	0.002	Sig	4.6875
	Ampiciline (100µg/ml)	3.125	0.780	3.125	0.780	0.780	1.718					

*** NT: Not tested, *_S. aureus_ ATCC 25923, * Not sig: not significant, * Sig,:significant**

4.5 Detection of minimum bactericidal concentrations (MBC)

All tested plants showed specific MIC were subjected to MBC assay by subculturing 10µl from well showed no visible growth on nutrient agar plate. Three wild *Allium* species showed a bactericidal effect, those were: *A. orientale, A. schubertti,* and *A. qasyunense.* Water leaf extract of *A. orientale* killed bacterial strains (1, 2, 3) at MBC=12.5 mg/ml. While, ethanolic leaf extract of the same species killed MRSA strain 5 at MBC=3.125 mg/ml. Ethanolic leaf extract of *A. schubertti* killed bacterial strain 3 and strain 4 at MBC=12.5 mg/ml. Ethanolic leaf extract and fresh bulb extract of *A. qasyunense* killed MRSA strain 3 and 1 respectively at MBC= 0.39 mg/ml.

4.6 Evaluation of antibacterial activity depending on plant part used and extracts type

The obtained results of the antibacterial activity of different wild *Allium* species and cultivated species against different five MRSA strains reveled that their bioactivity varied depending on the plant species, the plant part from which extraction has been obtained and the used extraction solvent. For example, the ethanolic and water leaf extract of *A. qasyunense* showed different antibacterial activity. As the ethanolic extract was higher than the water one by having MIC= 0.049 mg/ml and 1.56 mg/ml respectively on the same MRSA strain 5. On the other hand, the fresh bulb extract of the same plant species showed different antibacterial activity on MRSA strain 5 being the lowest among the other two extracts of MIC

3.125 mg/ml. However, this antibacterial behavior was different on another MRSA strain. That the highest antibacterial activity on MRSA strain 1 was for the fresh bulb extract of MIC= 0.4875 mg/ml.Therefore, each plant extraction method of a specific plant part of a specific plant species showed a particular MIC value indicating their antibacterial activity against the different five MRSA strains under examination. A comparison between MIC values (mg/ml) for different *Allium* species using water leaf extract, ethanolic leaf extract and fresh bulb extract against different MRSA were shown on table 4.5.

Table (4.4) A comparison between MIC values (mg/ml) for studied *Alliums* species using water leaf extract, ethanolic leaf extract, and fresh bulb extract against different strains of MRSA.

Plant species	Extraction type	MIC (mg/ml)							
		Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Reference Strain	MIC average	p-value
A. ampeloprasum	Leaf water	50	>50	50	50	50	50	>50	
	Leaf ethanol	50	50	>50	50	50	50	>50	
	Bulb fresh	50	50	50	50	50	50	50	
A. neapolitanum	Leaf water	>50	>50	>50	>50	>50	50	>50	
	Leaf ethanol	12.5	25	12.5	6.25	12.5	6.25	13.75	0.017
	Bulb fresh	25	50	50	12.5	25	12.5	32.5	0.015
A. orientale	Leaf water	6.25	6.25	6.25	3.125	12.5	6.25	6.25	0.028
	Leaf ethanol	1.56	0.78	1.56	0.39	0.195	0.39	0.897	0.046
	Bulb fresh	25	50	6.25	50	50	0.78	36.25	0.018
A. pallens	Leaf water	37.5	12.5	3.125	NT	6.25	3.125	14.844	0.206
	Leaf ethanol	50	50	50	NT	12.5	25	40.625	0.025
	Bulb fresh	12.5	25	50	NT	18.75	12.5	26.56	0.057
A. hierochuntnium	Leaf water	50	50	>50	25	>50	25	41.67	0.041
	Leaf ethanol	12.5	12.5	25	25	12.5	6.25	17.5	0.007
	Bulb fresh	12.5	25	12.5	6.25	25	3.125	16.25	0.018

Plant species	Extraction type	MIC (mg/ml)							
		Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Reference Strain	MIC average	p-value
A. truncatum	Leaf water	12.5	6.25	3.125	12.5	6.25	0.78	8.125	0.027
	Leaf ethanol	50	50	25	25	25	25	35	0.006
	Bulb fresh	25	50	50	50	>50	25	43.75	0.007
A. stamineum	Leaf water	25	12.5	25	12.5	25	25	20	0.004
	Leaf ethanol	50	25	50	18.75	18.75	25	36.25	0.006
	Bulb fresh	50	50	50	37.5	50	50	47.5	0.00001
A. schubertii	Leaf water	6.25	3.125	6.25	6.25	4.6875	3.125	5.313	0.005
	Leaf ethanol	>50	1.56	0.78	3.125	0.195	0.78	1.415	0.394
	Bulb fresh	25	>50	>50	>50	>50	25	>50	
A. qasyunense	Leaf water	3.125	NT	NT	NT	1.56	0.78	2.343	0.571
	Leaf ethanol	1.17	0.098	0.098	NT	0.049	0.78	0.354	0.015
	Bulb fresh	0.4875	0.78	1.56	NT	3.125	0.098	1.488	0.723
A. trifoliatum	Leaf water	6.25	12.5	12.5	6.25	3.125	3.125	8.125	0.027
	Leaf ethanol	1.56	0.78	NT	NT	NT	0.78	1.17	0.314
	Bulb fresh	0.78	12.5	6.25	6.25	6.25	1.17	6.406	0.065
A. phanerantherum	Leaf water	NT	NT	25	NT	NT	25	25	
	Leaf ethanol	25	50	25	50	12.5	3.125	32.5	0.015

Plant species	Extraction type	MIC (mg/ml)							
		Strain 1	**Strain 2**	**Strain 3**	**Strain 4**	**Strain 5**	**Reference Strain**	**MIC average**	**p-value**
	Bulb fresh	25	50	50	50	50	3.125	3.125	0.001
A. artemisietorum	Leaf water	50	>50	>50	NT	>50	25	>50	
	Leaf ethanol	12.5	25	25	25	25	12.5	22.5	0.001
	Bulb fresh	18.75	18.75	12.5	18.75	25	12.5	18.75	0.001
A. desertorum	Leaf water	50	50	>50	NT	>50	25	>50	
	Leaf ethanol	12.5	12.5	25	12.5	12.5	6.25	15	0.006
	Bulb fresh	18.75	25	12.5	25	12.5	12.5	18.75	0.004
A. paniculatum	Leaf water	NT	NT	NT	NT	NT	50	NT	
	Leaf ethanol	25	50	25	25	25	25	30	0.005
	Bulb fresh	12.5	12.5	12.5	12.5	25	12.5	15	0.006
A. sativum	Leaf water	2.3425	9.375	25	12.5	9.375	18.75	11.75	0.055
	Leaf ethanol	18.75	18.75	12.5	25	25	12.5	20	0.0001
	Bulb fresh	18.75	25	25	25	18.75	9.375	22.5	0.00001
A. cepa	Leaf water	9.375	18.75	25	12.5	18.75	18.75	18.75	0.005
	Leaf ethanol	12.5	12.5	12.5	25	12.5	18.75	15	0.006
	Bulb fresh	12.5	9.375	18.75	18.75	18.75	4.6875	15.625	0.002

*** NT: Not tested, *_S. aureus_ ATCC 25923.**

Chapter Five

Discussion

Chapter Five
Discussion

Allium species possess an antibacterial activity against different bacterial species, as well as against fungi (Yamada and Azuma,1977; Yin and Tsao,1999), viruses and parasites (Ankari and Mirelman 1999). This is due to the powerful sulfur and other numerous phenolic compounds (Benkeblia & Lanzotti, 2007). These observations have helped in identifying the active principle responsible for such activities and in developing drugs for the therapeutic use in human beings. All these studies were carried out on using the cultivated *Allium* species. However, reports on the antifungal or antibacterial property of wild *Allium* species are scare.

In the present study, *Allium* species exhibit different inhibition concentrations activity against five bacterial strains of MRSA. The effect varies from species to other according to the species itself, the extraction method (solvent used), and the plant part.

Different statistical analysis trials have been applied during the interpretation of the obtained results in this study. Those trials revealed that such statistical analysis cannot be relied on to explain the obtained data. This conflict could be due to the variations among the examined MRSA strains. Therefore the MIC average of the five MRSA strains cannot be considered. Moreover, the statistical analysis using the p-value for the determination whether the results are significant or not considers the remoteness of the MIC value from the average. In the case of this study, this will lead to an opposite point of view. As here the more close the MIC

value to the average the more significant result it will be and vise versa. So the obtained results were considered and discussed depending on the MIC values themselves.

According to this observation *A. qasyunense* showed the highest antibacterial activity against different strains of MRSA. This antibacterial activity was affected by the plant part and the extract type. The ethanolic leaf extract of *A. qasyunense* had higher antibacterial activity against strain 5 at MIC= 0.049 mg/ml which was lower than MIC value of water leaf extract (1.56 mg/ml). This observation confirmed that the type of extract affected the antibacterial activity, and ethanolic extracts were better than water extract. Many studies (Momeni and Zamanzad, 2010; Azu et al., 2006; Durmaz et al., 2006; Martin, 1995; Paz et al., 1995; Vlientinck et al., 1995) agreed with being the ethanolic extraction of *Allium* species is more effective against different bacterial organisms than the aqueous extraction method. This could be explained by that the aromatic or saturated organic compounds are more soluble in methanol and ethanol (Cowan, 1999). However, aqueous extraction makes allicin to react with water and form diallyl disulphide which does not exhibit the same level of antibacterial activity as does allicin alone (Block, 1992; Lawson and Wang, 1996; Hughes and Lawson, 1991).

The plant part also affected the antibacterial activity. This work showed that bulb fresh extract of *A. qasyunense* possessed higher antibacterial activity against the same strain (strain 1) than the leaf water

extract. Strain 1 was inhibited at 3.125 mg/ml by *A. qasyunense* water leaf extract, while the fresh bulb extract inhibited it at 0.4875 mg/ml. This difference between MIC values was clear enough to confirm that the bulb fresh extract was better than the water leaf extract. This coincide with that a freshly prepared infusion of ground *Allium* cloves possessed high antibacterial activity (Benkeblia and Lanzotti, 2007).

According to minimum bactericidal concentration (MBC), three wild *Allium* plant species possessed a bactericidal effect against MRSA strains. Those species were *A. orientale, A. schubertii* and *A. qasyunense*. Water leaf extract of *A. orientale* was bactericidal against MRSA strains 1, 2, and 3 at MBC= 12.5 mg/ml and bacteriostatic at 6.25 mg/ml against the same bacterial strains. Ethanolic leaf extract of *A. schubertii* was bactericidal against MRSA strains 3 and 4 at MBC= 12.5 mg/ml for both strains and bacteriostatic at 0.78 mg/ml and 3.125 mg/ml respectivley. MIC values of *A. orientale* and *A. schubertii* were lower than their MBC values suggesting that the plant extracts (regardless of the extract types) were bacteriostatic at lower concentrations and bactericidal at higher concentrations.

If comparing between the cultivated *Allium* species and the wild *Allium* species, it was clear that the wild one had possessed higher antibacterial activity against MRSA strains. For example, *Allium qasyunense* inhibited strain 5 growth at MIC value = 1.56 mg/ml by its water leaf extract, which was lower than both *A. sativum* and *A. cepa* (MIC= 9.375 mg/ml, 18.75 mg/ml respectively) for the same extract type

against the same strain. Also *A. qasyunense* ethanolic leaf extract inhibited strain 5 growth at 0.049 mg/ml that is still lower than *A. sativum* (MIC =25 mg/ml) and *A.cepa* (MIC = 12.5 mg/ml) of the same extraction type against the same strain. This study revealed that wild *Allium* species was more effective than the cultivated one. This may be due to irrigation process that diluted the concentration of active ingredients such as alicin.

Finally, it is concluded from the results of this investigation that the antibacterial activity is affected by the extraction type and plant part used. Ethanol extraction of leaf part of studied *Allium* species gave higher antibacterial activity than that of water extracts of the same plant part. On the other hand, the bulbs of *Allium* species gave higher antibacterial activity than the leaf when both extracted by water. The reason after that could be explained by being the bulbs are the storage organ of Alliums and they have higher concentrations of the active organosulfer compounds than the leaf parts. The wild Alliums had higher antibacterial activity against MRSA strains since the MIC averages lower than the cultivated one.

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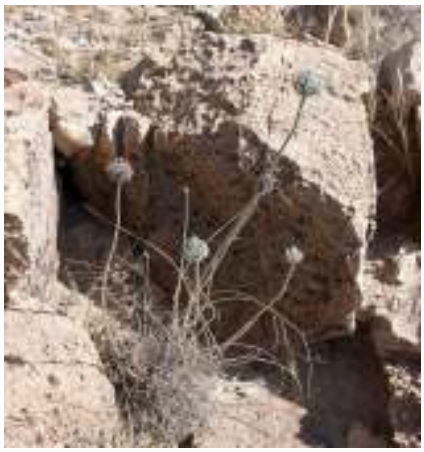
Appendix

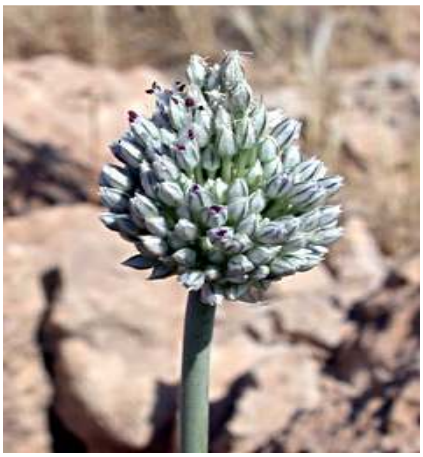
Plate 1: Photographs of wild *Allium* species

1. *Allium ampeloprasum*

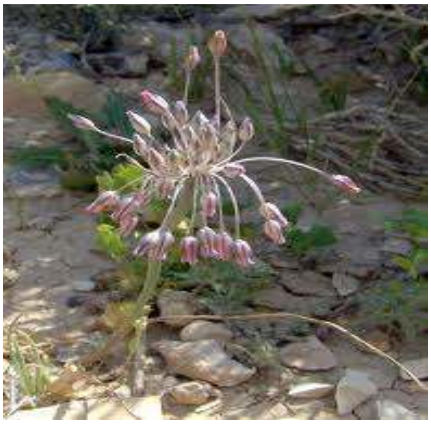
2. *Allium ampeloprasum*



3. *Allium artemisietorum*



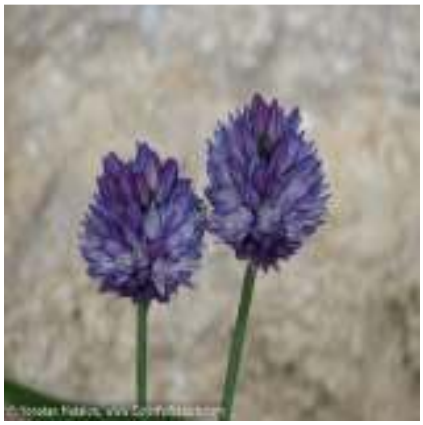
4. *Allium artemisietorum*



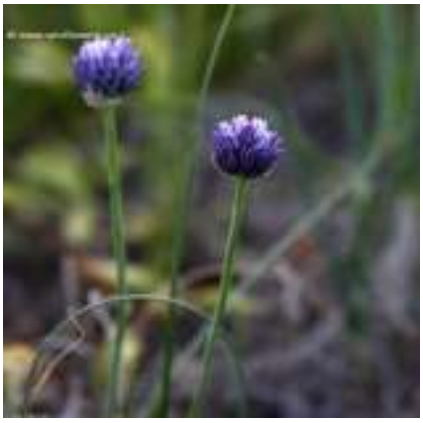
5. *Allium desertorum*

6. *Allium desertorum*

Plate 2: Photographs of wild *Allium* species

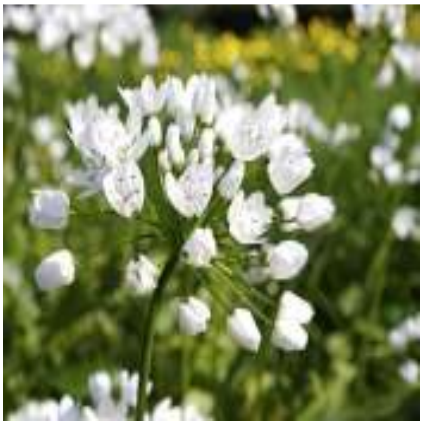


7. *Allium hierochuntinum*



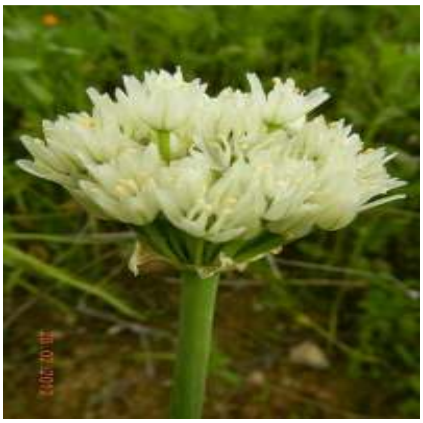
8. *Allium hierochuntinum*

9. *Allium neapolitanum*



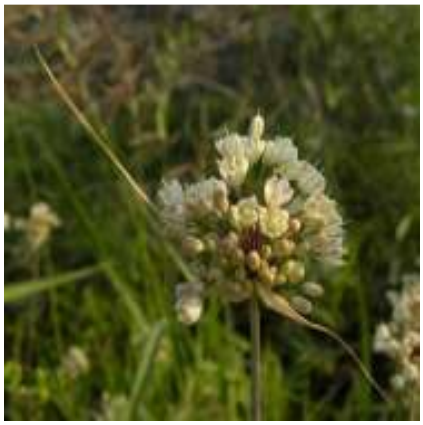
10. *Allium neapolitanum*

11. *Allium orientale*

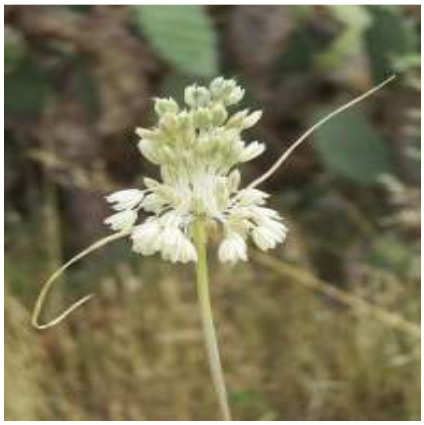


12. *Allium orientale*

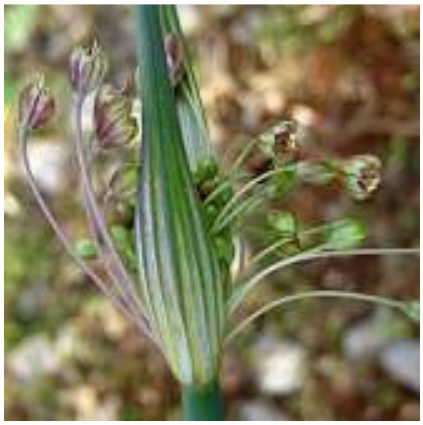
Plate 3: Photographs of wild *Allium* species



13. *Allium pallens*



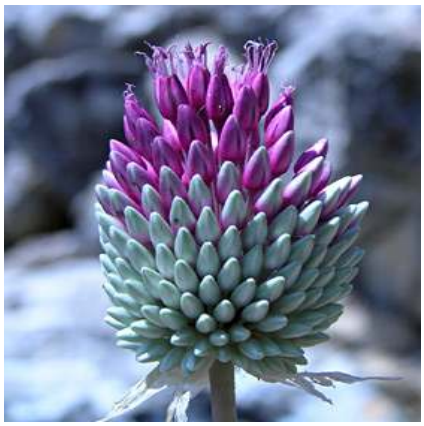
14. *Allium pallens*



15. *Allium paniculatum*

16. *Allium paniculatum*

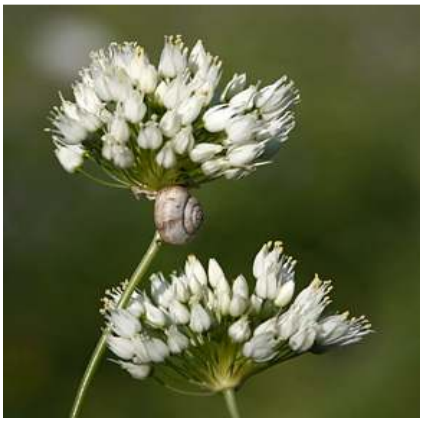
17. *Allium phanerantherum*



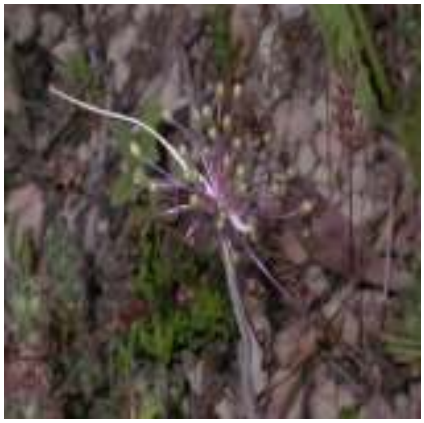
18. *Allium phanerantherum*

Plate 4: Photographs of wild *Allium* species

19. *Allium qasyunense*

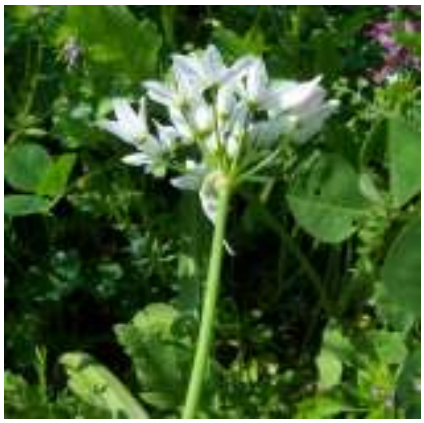


20. *Allium qasyunense*

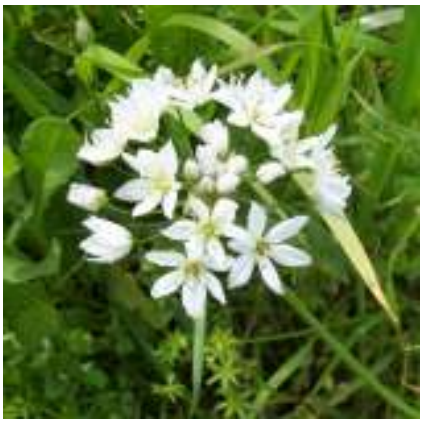


21. *Allium stamineum*

22. *Allium stamineum*



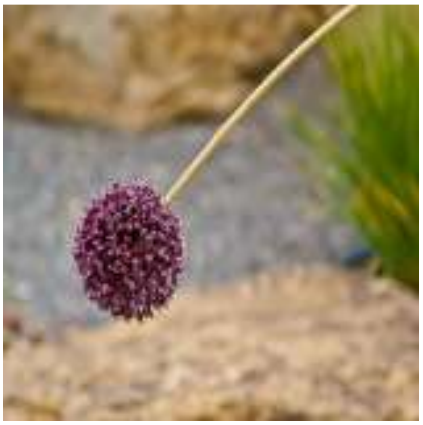
23. *Allium trifoliatum*



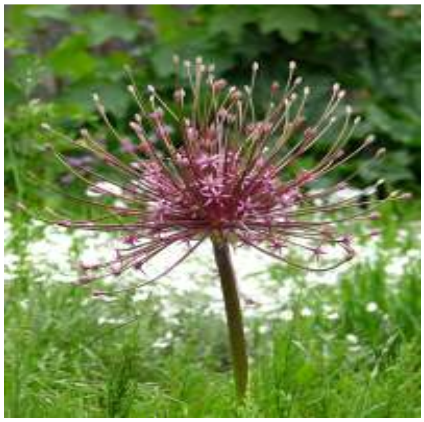
24. *Allium trifoliatum*

Plate 5: Photographs of wild *Allium* species

25. *Allium truncatum*



26. *Allium truncatum*



27. *Allium schubertii*

28. *Allium schubertii*

جامعة النجاح الوطنية
كلية الدراسات العليا

تأثير بعض انواع البصل و الثوم البريه في فلسطين ضد البكتيريا ومقارنتها مع الاصناف الزراعية *Allium cepa* و*Allium sativum*

إعداد
ضحى ياسر فائق أبو صفية

إشراف
د. غدير عمر
د. غالب عدوان

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.
2016م

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إعداد

ضحى ياسر فائق أبو صفية

إشراف

د. غدير عمر

د. غالب عدوان

الملخص

تم جمع وتصنيف أربعة عشر نوعاً من البصل والثوم البرية، والتي استخلصت بثلاث طرق هي (الايثانول، والماء والخام)، واختبرت فعاليتها ضد خمس سلالات من البكتيريا الكروية العنقودية الذهبية المضاده للمشيلين (Methicillin-Resistance*Staphylococcus aurues*) بالإضافة إلى نوع آخر من البصل المزروع (*Allium sativum*) والثوم المزروع (*Allium Cepa*) بطريقة التخفيف (Broth Microdilution). وقد تبين أن *A. qasyunense* و هو من الانواع البرية قد سجلت اعلى فعالية ضد السلالت البكتيرية.بحيث عند استخراج اوراقها باستخدام الماء اعطت فعالية ضد سلالة البكتيريا رقم 5 على تركيز=1.56مغ/مل. وعند استخراج اوراقها ب استخدام الايثانول اعطت فعاليه على تركيز= 0.049 مغ/مل ضد السلالة البكتيريه ذاتها. اما في حاله استخراج الماده الفعاله من جزء البصيله باستخدام الطريقه الخام، اعطت هذه النبته فعاليه ضد سلاله البكتيريا رقم 1 على التركيز التالي 0.49 مغ/مل. من جهة اخرى، اظهرت الدراسة أن الجزء المستخدم من النبته و الماده المستخدمه في الاستخراج يؤثرا على كفائة فعاليتها ضد البكتيريا،على سبيل المثال عند استخراج اوراق البصل والثوم باستخدام الاثانول كمذيب، اظهر الايثانول فعاليه اعلى من الماء كان لذلك تفسير بان الإيثانول يساعد في توفير الاستقرار لمركبات الكبريت العضوية مثل الأليسين. بالنسبه للجزء المستخدم من النبته، البصيلات اعطت نتيجه بكفائه اعلا من الاوراق.عند المقارنه بين الانواع البرية من البصل و الثوم مع الانواع المزروعه منها، اعطت الانواع البرية منها نتيجه اعلى في الفعالية ضد السلالات البكتيرية (MRSA) المستخدمه في البحث.