Islamic University-Gaza

Deanship of Graduate Studies

Biological Sciences Master Program

The Antibacterial Potentials and Synergistic Effect of some Plant Extracts against Multidrug Resistant Clinical Pathogens

By

Wesam Soliman Abu Draz

Supervisor

Dr. Tarek Elbashiti — Assoc. Prof. of Biotechnology

Dr. Emad Abou El-Khair — Assoc. Prof. of Microbiology

October/2015

DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institutes, except where due acknowledgment has been made in the text.

Signature

Wesam S. I. Abu Draz

Copyright

Acknowledgment

My thanks first and foremost to Allah almighty, who has been the source of My energy throughout my study; without Allah none of this would be possible.

All thanks to my supervisors Dr. Tarek Elbashiti Assoc. Prof. of Biotechnology & Dr. Emad Abou El-Khair Assoc. Prof. of Microbiology

For their supervision, who didn't spare any effort or time to mentor me through providing his valuable advice and guidance.

In addition, I would like to thank the following persons who, without their help and support I would not have completed this study as desired.

Dr. Abdelraouf A. Elmanama

Dr. Abboud El kichaoi

Dr. Nedal Fayyad

Dr. Mohamed Abu Auda

Dr. Azmy Abu Dkah

Mr. Mahmoud El-Hindi

Mr. Mohamed Matter

Mr.Nasser Abu Shaaban

Mr. Mohamed Jouda

Mr. Mohamed Albayomi

Moreover, all the staff at the Biology & biotechnology Sciences Department, at the Islamic University of Gaza without exceptions.

Dedication

To my family especially my mother, my father and my wife who supported me all the way since the beginning of my life. To my brothers and sisters and my children who have been a great source of motivation and inspiration.

The Antibacterial Potentials and Synergistic Effect of some Plant Extracts against Multidrug Resistant Clinical Pathogens

Abstract

The present study was designed to screen *in vitro* antibacterial and synergistic activity of *Allium sativum, Ecballium elaterium, Pelargonium graveolens, Rosmarinus officinalis, Phagnalon rupestre & Ruta-graveolens* plants against multidrug resistant clinical pathogenic bacteria. The active compounds were extracted from the dried aerial parts of plants where successively extracted with aqueous, 80% ethanol and methanol solvents by using soxhlet extractor, and Essential Oils (EOs) which extracted from the fresh aerial parts of plant by using steam distillation. All extracts were screened for their antibacterial activity and synergistic effect in combination with known antimicrobial agents including. Ciprofloxacin (CIP), Ampicillin (AM), Cefotaxime (CTX), Nalidixic acid (NA), Norofloxacin (NOR), Cefuroxime (CXM), Cefaclor (CF or CEC), Ofloxacin (OFX), Cefalexin (CL or CN), Tetracycline (TE), Rifampicin (RIF), Amoxyclav (AMC), Gentamycin (GMN), Penicillin (P) & Oxacillin (OX), by using the disk diffusion method. The minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of the plant extracts were assessed by using micro-dilution technique. The microorganisms used for evaluation the antibacterial and synergistic activity were clinically isolates multi-drug-resistant (MDR) bacteria. *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae & Pseudomonas aeruginosa*.

Results:

The results revealed that, the average diameter of inhibition zones that resulting from the effect of plant extracts against the tested bacteria ranged from 7 to 14 mm, 7.33 to 16.66 mm, 7.66 to 17 mm and 7.33 to 12.66 mm for aqueous, ethanol, methanol & EOs extracts, respectively. The extracts showed antibacterial activity were subjected to minimum inhibitory concentration and minimum bactericidal concentrations assay; a micro-broth dilution assay was performed on 96-well plates using 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) as an indicator for bacterial growth. The average minimum inhibitory concentrations (MICs) values ranged from 1.562 to 100 mg/ml, 1.562 to 50 mg/ml, 1.562 to 50 mg/ml & 3.125 to 100 µl/ml for aqueous, ethanol, methanol & EOs extracts, respectively. While minimum bactericidal concentrations (MBCs) values

ranged from 25 to > 200 mg/ml, 25 to > 200 mg/ml, 25 to 200 mg/ml & 50 to > 200 µl/ml for aqueous, ethanol, methanol & EOs extracts, respectively. Synergistic activity of the plant extracts when combined with antibiotics had different degree of synergism against the selected microorganisms, where in case of aquatic extracts; *Rosmarinus officinalis* had the best synergism against *S. aureus*, *E. coli* & *K. pneumoniae*, while the best synergism against *P. aeruginosa* was observed with *Ruta-graveolens*. In case of ethanolic extracts, the best synergism was observed with *Rosmarinus officinalis* against *S. aureus* & *E. coli*, and with *Ruta-graveolens* & *Pelargonium graveolens* against *K. pneumoniae* & *P. aeruginosa* respectively. In addition, the best synergism was observed with methanolic extracts of *Rosmarinus officinalis*, *Ruta-graveolens*, *Ecballium elaterium* & *Pelargonium graveolens* against *S. aureus*, *E. coli*, *K. pneumoniae* & *P. aeruginosa* respectively. While EOs of screened plants had the best synergism against *S. aureus*, *E. coli* and *K. pneumoniae* with *Pelargonium graveolens* EO for all, and had the best synergism against *P. aeruginosa* with *Allium sativum* EO.

Conclusions:

The overall results of the present work provide baseline information for the possible use of the studied plant extracts and EOs in the treatment of bacterial infections involving MDR phenotypes.

In addition to these antibacterial activities, the results indicate that the possibility of concurrent use of these antimicrobial drugs and plant extracts in combination in treating infectious diseases caused by multi-drug-resistant *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* & *Pseudomonas aeruginosa* or at least the concomitant administration may not impair the antimicrobial activity of some of these antibiotics.

Key words.

Antibacterial, synergistic, aquatic extract, ethanolic extract, methanolic extract, essential oil, MIC & MBC.

التأثير الضد بكتيري المحتمل والتأثير التظافري لبعض المستخلصات النباتية ضد البكتيريا السريرية الممرضة المقاومة لعدة مضادات حيوية

الملخص

صممت هذه الدراسة لعمل فحوصات مخبرية للتأثير الضد بكتيري و التأثير التظافري لمستخلصات الثوم، فقوس الحمار (الضحاك) ، العطرة ، إكليل الجبل ، عشبة قديح و السذاب العطري ضد البكتيريا السريرة الممرضة المقاومة للمضادات الحيوية. تم إستخلاص المواد الفعالة من الأجزاء الهوائية المجففة للنباتات بإستخدام الماء المقطر، الإيثانول ٨٠%، الميثانول العالي النقاوة كمواد مذيبة من خلال إستخدام جهاز السوكسليت ، وتم فصل الزيوت العطرية من الأجزاء الهوائية الغضة للنباتات بواسطة التقطير بالبخار. تم فحص التأثير الضد بكتيري للمستخلصات بشكل منفرد وأيضا بواسطة دمجها مع المضادات الحيوية وهي السيبروفلوكساسين، الامبيسيلين، السيفوتوكسيم، اليوجرام، النوروفلوكساسين، الزيناكسين، السيكلور، الفلوكساسين، الجيفليكس، التيتراسيكلين، الريفامبسين، الأوجمنتين، الجنتاميسين، البنسلين والأوكسيسيلين بإستخدام تقنية الإنتشار في القرص، بينما تم تحديد أقل تركيز مثبط وأقل تركيز قاتل للمستخلصات النباتية بإستخدام تقنية التخفيف الجزئي الدقيق. الكائنات الدقيقة التي تم إستخدامها لتقييم التأثير الضد بكتيري و التأثير التظافري هي بكتيريا مقاومة لعدة مضادات حيوية تم عزلها سريريا وهي. المكورات العنقودية الذهبية، الإيشيريشيا القولونية، الكليبسيلا الرئوية و الزائفة الزنجارية.

النتائج:

أظهرت النتائج أن متوسط قطر مناطق التثبيط ضد البكتيريا المفحوصة الناتج عن تأثير المستخلصات النباتية تراوحت مابين ٧- ١٤ ملم، ٧.٣-١٦.٦ ملم، ٧.٦- ١٧.٧ ملم و ٧.٣- ١٢.٦ ملم بالنسبة للمستخلصات المائية، الإيثانولية، الميثانولية و الزيوت العطرية على التوالي. عندما يتعلق الأمر بتقييم أقل تركيز مثبط (MIC) و أقل تركيز قاتل (MBC) للبكتيريا فإن المستخلصات تظهر نشاطا واضحا ضد البكتيريا. حيث أن التقييم تم عمله بإستخدام التخفيف الجزئي الدقيق بإستخدام أطباق بلاستيكية معقمة تحتوي على ٩٦ حفرة و إستخدام كاشف التيترازوليوم كلورايد كمؤشر على نمو البكتيريا، حيث أن متوسط الMIC يتراوح مابين ١.٥٦٢- ١٠٠ ملجم / ملل، ١.٥٦٢- ٥٠ ملجم /ملل، ١.٥٦٢- ٥٠ ملجم / ملل و ٣.١٢٥- ١٠٠ ميكرولتر/ ملل لكل من المستخلصات المائية، الإيثانولية، الميثانولية و

الزيوت العطرية على التوالي. بينما متوسط ال MBC يتراوح مابين ٢٥- >٢٠٠ ملجم /ملل، ٢٥- >٢٠٠ ملجم/ ملل، ٢٥- ٢٠٠ ملجم/ ملل و ٢٠- >٢٠٠ ميكرولتر/ ملل لكل من المستخلصات المائية، الإيثانولية، الميثانولية و الزيوت العطرية على التوالي. كما وجد أن المستخلصات النباتية عند دمجها مع المضادات الحيوية لها نشاط تظافري متفاوت الدرجات ضد الكائنات الدقيقة التي تم إختبارها، حيث أنه في حالة المستخلص المائي وجد أن إكليل الجبل يملك أفضل تأثير تظافري ضد المكورات العنقودية الذهبية، الإيشيريشيا القولونية و الكليبسيلا الرئوية ، بينما كان أفضل تأثير تظافري ضد الزائفة الزنجارية تم ملاحظته مع السذاب العطري، بينما في حالة المستخلص الإيثانولي وجد ان أفضل تأثير تظافري كان مع إكليل الجبل ضد المكورات العنقودية الذهبية، الإيشيريشيا القولونية ، وأيضا مع السذاب العطري و العطرة ضد الكليبسيلا الرئوية و الزائفة الزنجارية على التوالي. بالإضافة إلى ذلك كان أفضل تأثير تظافري في المستخلصات الميثانولية مع إكليل الجبل، السذاب العطري، فقوس الحمار و العطرة ضد المكورات العنقودية الذهبية، الإيشيريشيا القولونية، الكليبسيلا الرئوية، الزائفة الزنجارية على التوالي. بينما الزيوت العطرية للنباتات المفحوصة تملك أفضل تأثير تظافري ضد المكورات العنقودية الذهبية، الإيشيريشيا القولونية و الكليبسيلا الرئوية، و أفضل تأثير تظافري لها ضد الزائفة الزنجارية كان مع زيت الثومة.

الإستنتاج:

توفر النتائج الكلية للدراسة معلومات أولية حول إمكانية إستخدام هذه المستخلصات النباتية والزيوت العطرية في علاج الأمراض والإلتهابات التي تسببها هذه البكتيريا المقاومة لعدة مضادات حيوية. بالإضافة إلى هذه الأنشطة المضادة للبكتيريا، تشير النتائج إلى إمكانية الإستخدام المتزامن لكلا من الأدوية المضادة للميكروبات و دمجها مع المستخلصات النباتية في علاج الأمراض والإلتهابات التي تسببها هذه البكتيريا المقاومة للمضادات الحيوية، أو على الأقل معرفة أن ذلك لا يؤثر سلبا على فعالية بعض هذه المضادات الحيوية.

الكلمات المفاتيح.

ضد بكتيري، التظافري، المستخلص المائي، المستخلص الإيثانولي، المستخلص الميثانولي، الزيوت العطرية، أقل تأثير مثبط و أقل تأثير قاتل.

List of Contents

List of Tables

List of Figures	
Figure 1.1. plant secondary metabolites as modifiers of multidrug resistance mechanisms	**3**
Figure 4.1.a,b. antibiotic susceptibility testing against *S. aureus*	**54**
Figure 4.2.a,b. antibiotic susceptibility testing against *E. coli*	**54**
Figure 4.3.a,b. antibiotic susceptibility testing against *K. pneumoniae*	**54**
Figure 4.4.a. antibiotic susceptibility testing against *P. aeruginosa*	**54**
Figure 4.5. *Allium sativum* extracts against *S. aureus*	**57**
Figure 4.6. *Ecballium elaterium* extracts against *S. aureus*	**57**
Figure 4.7. *Pelargonium graveolen* extracts against *S. aureus*	**57**
Figure 4.8. *Rosmarinus officinalis* extracts against *S. aureus*	**57**
Figure 4.9. *Phagnalon rupestre* extracts against *S. aureus*	**57**
Figure 4.10. *Ruta graveolen* extracts against *S. aureus*	**57**
Figure 4.11. *Allium sativum extracts against E. coli*	**60**
Figure 4.12. *Ecballium elaterium* extracts against *E. coli*	**60**
Figure 4.13. *Pelargonium graveolen* extracts against *E. coli*	**60**
Figure 4.14. *Rosmarinus officinalis* extracts against *E. coli*	**60**
Figure 4.15. *Phagnalon rupestre* extracts against *E. coli*	**60**
Figure 4.16. *Ruta graveolen* extracts against *E. coli*	**60**
Figure 4.17. *Allium sativum extracts against K. pneumoniae*	**63**
Figure 4.18. *Ecballium elaterium* extracts against *K. pneumoniae*	**63**
Figure 4.19. *Pelargonium graveolen* extracts against *K. pneumoniae*	**63**
Figure 4.20. *Rosmarinus officinalis* extracts against *K. pneumoniae*	**63**
Figure 4.21. *Phagnalon rupestre* extracts against *K. pneumoniae*	**63**
Figure 4.22. *Ruta graveolen* extracts against *K. pneumoniae*	**63**

List of Abbreviated Terms

MIC	Minimum Inhibitory Concentration
UTIs	Urinary Tract Infections
MBC	Minimum Bactericidal Concentration
CFU	Colony Forming Unit
BHI	Brain Heart Infusion
DMSO	Dimethyl sulfoxide
Ppm	parts per million
HIV	Human Immunodeficiency Virus
TM	traditional medicine
TB	Tuberculosis Bacterial
EO	Essential oil
IZ	zone of inhibition
MRSA	methicillin resistant *Staphylococcus aureus*
MSSA	methicillin sensitive *Staphylococcus aureus*
MDR	multi-drug-resistant

Chapter 1

INTRODUCTION

1.1. Overview:

Medicinal plants have been used as sources of medicine in virtually all cultures. During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (Tadeg *et al*., 2005).

Medicinal plants are an important source of current drugs; about 50% of available and common drugs are originated from plants. Herbal drugs are natural products with homolog continents and are well-tolerated agents with no side effects. Therefore, they can be used longer time and are more suitable for treating chronic illnesses (jalali *et al*., 2012). People use herbal remedies due to their efficacy, tradition and their low cost. However, they often do not inform their physicians about their use of medicinal plants (Alonso-Castro *et al*., 2012). Medicinal plants would be the best source to obtain variety of drugs. Approximately 80% of the word inhabitants rely on traditional medicine for their primary health care and play an important role in the health care system of the remaining 20% of the population, which has compounds derived from medicinal plants (Al-Sokari & El Sheikha., 2015; Ibrahim *et al*., 2011).

The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries for health programs (Ibrahim *et al*., 2011). Medicinal plants are important elements of the local medical systems in Palestine as well as in other developing countries. Complementary and alternative medicine utilization in Palestine is very common; some of the types of complementary and alternative medicine used in Palestine are common elsewhere, whereas other types were unique to this area (Elkhair *et al*., 2010).

It is well known that infectious diseases particularly those involving microorganisms like bacteria and fungi causes of premature deaths, killing almost 50000 people every day in the world (Ahmad & Beg., 2001). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases, which have led to the emergence of new bacterial

strains that are multi-resistant which have resulted in increase in morbidity and mortality and creates enormous health problems.

However, the situation is alarming in developing as well as developed countries due to misuse use of antibiotics (Al Sokari & El Sheikha., 2015; Elkhair., 2014; Davies & Davies., 2010; Parekh & Chanda., 2007; Ahmad & Beg., 2001). The development of drug resistance as well as the appearance of side effects of certain antibiotics has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages (Haddouchi *et al*., 2013).

The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants (Ahmad & Beg. 2001). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants (Ahmad & Beg. 2001).

The increase of microbial resistance to antibiotics threatens public health on a global scale as it reduces the effectiveness of treatments and increases morbidity, mortality and health care costs (Abd El-Kalek & Mohamed. 2012).

Bacterial infectious diseases represent a serious risk to the world population once they have been responsible for high morbidity and mortality through the times. However, this problem has been much more serious in the last years with the increase in the prevalence of infections caused by multi-drug-resistant (MDR) strains (Barreto *et al*., 2014). Emergence of MDR is a phenomenon occurring worldwide, due to the selective pressure exerted by extensive use of antibiotics and that has hindered the infectious illness therapy (Barreto *et al*., 2014). The problem of microbial resistance some time takes the shape of epidemic due to development of drug resistant microorganisms, thus in recent year lot of attentions diverted to discover new source

of natural antimicrobial agents. The potential of higher plants as source of new antimicrobial agents represent a vast untapped source for exploration of natural antimicrobial agent in modern medicine (Al-Sokari & El Sheikha., 2015; Elkhair., 2014 & Ibrahim *et al*., 2011).

Resistance to one antibiotic can mean that a whole class of antibiotics becomes ineffective, or even several classes. The development of antibiotic resistance can be natural (intrinsic) or acquired and this can be transmitted within same or different species of bacteria. Natural resistance is achieved by spontaneous gene mutation and the acquired resistance is through the transfer of DNA fragments like plasmids from one bacterium to another.

The mechanisms of resistance can be divided into different categories.

(i) Enzymes produced by the bacterium cause direct destruction or modification of the antibiotic (e.g. bacteria that produce beta lactamases (ESBLs) which cleave penicillin and related beta lactams);

(ii) Active site modification occurs so that there is inefficient binding of the antibiotic (e.g. MRSA where the target penicillin-binding protein is modified);

(iii) A reduced amount of antibiotic is present due to the removal, or efflux, from the cell (e.g. in Pseudomonas spp.) or

(iv) Production of an alternative target that is resistant to inhibition by the antibiotic (metabolic by-pass), e.g. an overproduction of the target enzyme in trimethoprim-resistant *E. coli*.

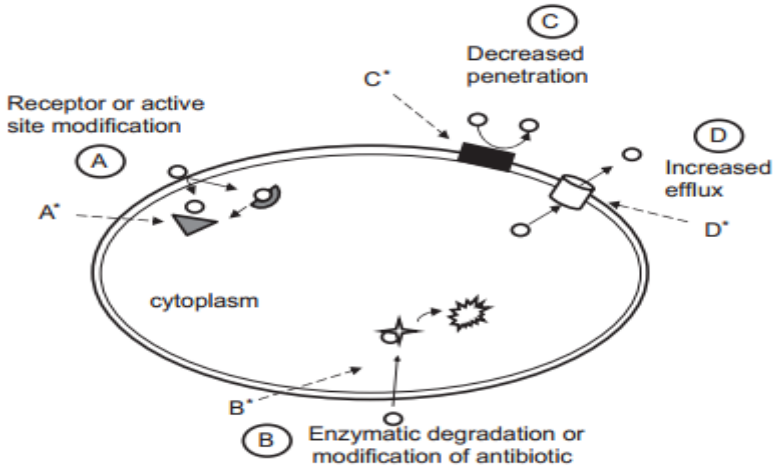


Fig. 1.1 Plant secondary metabolites as modifiers of multidrug resistance mechanisms. – antibiotic drug, – receptor, – modified receptor, – efflux pump, – enzyme, -degradation of the drug.

An inherent mechanism of resistance to antimicrobials in Gram-negative bacteria is the expression of efflux pumps, which actively remove certain toxic molecules from the cell, and renders them less susceptible to detergents and antibiotics (Langeveld *et al*., 2014 & Hemaiswarya *et al*., 2008).

One strategy employed to overcome these resistance mechanisms is the use of combination of drugs. The secondary metabolites from plant are good sources for combination therapy. As shown in Fig. 1.1, there are a wide ranges of phytochemicals which act as multidrug resistance modifiers depicted (Hemaiswarya *et al*., 2008).

Therefore, the search for new antimicrobial agents or new compounds able to potentiate the antimicrobial activity of old antibiotics against resistant microorganisms has become an important area of research.

In the present study, we have selected six medicinal plants (A. *Allium sativum*, B. *Ecballium elaterium*, C. *Pelargonium graveolens*, D. *Rosmarinus officinalis*, E. *Phagnalon rupestre* & F. *Ruta-graveolens*) to be screened for their antibacterial and synergistic effects against multi-drug resistant bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*.

1.2. Aim of the Study:

The aim of this study is to assess the antibacterial and synergistic effect of some medicinal plant extracts and essential oils with antibiotic drugs against multi-drug resistant clinical pathogens.

1.3. Specific objectives:

The following specific objectives will be achieved.

1. Collection and identification of plant Materials (*Allium sativum, Ecballium elaterium, Pelargonium graveolens, Rosmarinus officinalis, Phagnalon rupestre & Ruta graveolens*).
2. To obtain extracts from selected plants using different solvents such as methanol, ethanol & distilled water & to extract the EOs.
3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the plant extracts and essential oils.
4. Determination whether the type of solvent used in the extraction has any effect on the antibacterial activity of the plant extracts.

1.4. Significance:

- Due to development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents or a combination of drugs to be able to combat new resistant pathogenic bacteria.
- It has been observed in previous researches a synergistic effect of various plants extracts with antibiotic drugs against some resistant bacteria; therefore, we will check this possibility in our study by using Palestinian traditional plants.
- Confirm whether these traditionally used plants have antibacterial effect or not.
- Keep up with many of the research taking place in many countries around the world using plant extracts, and as in the Gaza strip there are a lot of rare medicinal plants, it is necessary to conduct such research in order to supplement those efforts.
- Misuse of antibiotics in the Gaza strip is very common because of the economic situation of the people, the spread of ignorance and lack of strict laws to divert drugs from pharmacies, where it led to a significant increase in pathogens resistance to presently available antibiotics has necessitated to find quick solutions to this phenomenon and this research is the starting point.

Chapter 2

Literature review

2.1. Medicinal Plants:

An alternative solution to allopathic medicine embodied with severe side effects, is the use of folk medicine plant preparations to arrest the insidious nature of the disease. Many herbs have been evaluated in clinical studies and are currently being investigated phytochemically to understand their anti-bacterial actions against various resistant microorganisms. Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity (Pandey & Chowdhry., 2006). Plants contain several phytochemicals, which possess strong antioxidant activities. The antioxidants may prevent and cure cancer and other diseases by protecting the cells from damage caused by 'free radicals' – the highly reactive oxygen compounds. Thus consuming a diet rich in antioxidant plant foods (e.g. fruits and vegetables) will provide a milieu of phytochemicals, nonnutritive substances in plants that possess health protective effects. Many naturally occurring substances present in the human diet have been identified as potential chemopreventive agents; and consuming relatively large amounts of vegetables and fruits can prevent the development of diseases. Some herbal combinations are more effective than the constituent herb used alone (Kennedy *et al.*, 2002). Positive beliefs about alternative medicines are not necessarily associated with their positive or negative effects. These findings suggest that, while placebo effects may be responsible for the therapeutic efficacies of some herbal products, those of other herbal products may arise from synergistic actions of herbal ingredients. Synergy occurs if two or more herbal ingredients mutually enhance each other's effect more significantly than the simple sum of these ingredients (Gilbert & Alves., 2003). To improve the efficacy of antibiotics it is necessary to find methods of improving diffusion of antibiotics across bacterial membranes and/or to hinder the efflux pumps that are a general resistance mechanism in Gram-negative bacteria. Mechanisms that can lead to pharmacological synergy are. (I) Multitarget effect in which compounds target different sites in the bacterial cell; (II) Pharmacokinetic or physicochemical effects (e.g. improvement of solubility or bioavailability); or (III) Targeting a specific resistance mechanism of bacteria (Langeveld, *et al*., 2014).

Combined antimicrobials are preferred as microbial tolerance is less likely to develop against substances having more than one type of modes of action. It was thus necessary to check the antimicrobial activities of these spices in combinations as used in conventional cooking or salad dressing (Gupta, *et al*., 2014).

2.2. Plants used in this study:

2.2.1. *Rosmarinus officinalis*:

- **Rosemary, Rosmarinus officinalis,** is an evergreen plant typical of the Mediterranean region. *Rosmarinus officinalis*, commonly known as rosemary, is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white, pink, purple, or blue flowers, native to the Mediterranean region. It is a member of the mint family Lamiaceae (Table 2.1), which includes many other herbs (Al-Sereiti, *et al*., 1999).

Table 2.1. Classification of *Rosmarinus officinalis* (Biology - Flora).

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	*Lamiaceae*
Genus	Rosmarinus
Species	*Rosmarinus officinalis*

Rosmarinus officinalis
Rosemary

- **Description and geographical distribution of *Rosmarinus officinalis*:**
  - *Rosemary* is an evergreen-branched bushy shrub, attaining a height of about one meter with upright stems, whitish-blue flowers and dark green leaves, which are small with edges turned over backward. Underneath these rolled edges are little glands containing aromatic oils. It grows wildly along the north and south coasts of the Mediterranean sea, and in the sub-Himalayan areas (Al-Sereiti, *et al*., 1999).

- **Traditional uses of *Rosmarinus officinalis*:**
  - Rosemary plant is cultivated for its aromatic oil, which is called "rosemary oil" and is obtained by steam distillation of the fresh leaves and flowering tops of the plant. It is an ingredient for Eau-de-cologne, hair infection, cold cream etc. The leaves are used for flavouring foods as condiment (Marin *et al*., 2006).
  - Since the ancient days rosemary has been used in folk medicine for manifold conditions, some of which may be enumerated as follows. It has been used as an Antispasmodic in renal colic and dysmenorrhoea and in relieving respiratory disorders. It has also been used as an analgesic, antirheumatic, carminative, cholagogue, diuretic, expectorant, antiepileptic and for effects on human fertility. Other uses are as a general tonic in case of excessive physical or intellectual works and in heart diseases; and as an insecticide and herbicide. Externally, it is a rubefacient, and is used to stimulate the growth of hair and treatment of head infections (Al-Sereiti *et al*., 1999).
  - The plant and extracts possess antibacterial and antioxidant activity and it is considered good source of nectar for bees. As a medicinal plant, rosemary has been used as an external stimulant and as a relaxant for nervousness, muscle spasms, and headaches. It has also been used in the treatment of cancer, and is categorized today as a therapeutic emmenagogue (Marin *et al*., 2006).

2.2.2. *Pelargonium graveolens*:

- *Pelargonium graveolens* belongs to the Geraniaceae family **(Table 2.2)** (Hsouna, & Hamdi., 2012).
- The large family Geraniaceae comprised approximately 750–800 species including three principal genera. Erodium, Geranium and Pelargonium **(**Boukhris *et al*., 2013**).**
- Rose-scented geranium (Pelargonium species) is a multi-harvest high value, aromatic plant cultivated for its EO, which is widely used in cosmetic industry, and as flavouring for foods. It is widely distributed in South Africa. In recent years, it has been introduced into Europe, Asia and North Africa. In all Mediterranean regions, it is cultivated in gardens and parks as an ornamental species (Boukhatem *et al*., 2013).

Table 2.2. Classification of *Pelargonium graveolens* (Biology - Flora)**:**

Kingdom	**Plantae**	
Subkingdom	Tracheobionta	
Superdivision	Spermatophyta	
Division	Magnoliophyta	
Class	Magnoliopsida	
Subclass	Rosidae	
Order	Geraniales	
Family	*Geraniaceae*	
Genus	Pelargonium	***Pelargonium graveolens***
Species	*Pelargonium graveolens*	

- **Traditional uses of *Pelargonium graveolens*:**
  - This species have much value, and it is used in pharmaceutical industries. Its flower water was extracted from the aerial organs (flowers, leaves and stems), used in gastronomy, and popular medicine. Moreover, its essential oil is extracted and utilized in perfumery and cosmetic industries (Boukhatem *et al*., 2013 & Ko *et al*., 2007).
  - Its oil has been used for many years in traditional medicine as antiasthmatic, antiallergic, antioxidant, antidiarrhoeic, antihepatotoxic, diuretic, tonic, haemostatic, stomachic and antidiabetic (Boukhris *et al*., 2012).
  - Its leaves are popularly used as flavouring, insect repellent, in perfume and in aromatherapy for the treatment of gastrointestinal diseases, throat infections, and bleeding (Hsouna, & Hamdi., 2012).

2.2.3. *Allium sativum*:

- *Allium sativum*; commonly known as garlic, is a species in the onion family *Alliaceae* (**Table 2.3**) (Saravanan *et al*., 2010 & Akintobi *et al*., 2013).
- Garlic (*Allium sativum* L.) belongs to the Liliaceae genus, which includes a number of commercially important vegetables. It was domesticated at least 3000 years ago, and it is thought to have evolved in southwestern Asia (Ovesná *et al*., 2011).

Table 2.3. **Classification of *Allium sativum*** (Biology - Flora)**:**

Kingdom	**Plantae**	
Subkingdom	Tracheobionta	
Superdivision	Spermatophyta	
Division	Magnoliophyta	
Class	Liliopsida	
Subclass	Liliidae	
Order	Liliades	
Family	*Alliaceae*	
Genus	Allium	***Allium sativum***
Species	*Allium sativum*	

- **Traditional uses of *Allium sativum*:**
  - Therapeutical applications of garlic have been known for many ages. The plants are broadly used as antibiotics and are effective against diabetes, artherosclerosis and cancer. This plant is also known to reduce blood plasma cholesterol and blood pressure. It also inhibits platelet mass formation & had revealed that garlic stimulates the activity of the defensive cells of the body such as the lymphocytes and macrophages. These blood cells protect us from pathogens. They are also able to destroy cancerous cells in the initial stage of cancer formation. Garlic is currently used with some degree of success, as a complement in the treatment of AIDS (Akintobi *et al*., 2013).
  - *Allium sativum* is natural plant being used as a food as well as folk medicine for centuries in all over the world, the garlic plant have various biological properties like antimicrobial, anti-cancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic, and anticardiovascular effects. As well as different properties such as antiviral, antifungal, expectorant, antiseptic, antihistamine (Hannan *et al*., 2011).
  - In this century, more than 3000 publications have provided evidences for the efficacy of this herb in the prevention and treatment of a variety of diseases and for validating its traditional uses. It has been shown that garlic has

different applications as antimicrobial (Ghazanfari *et al*., 2006), antitumor (Sundaram & Milner., 1996), antithrombotic, hypolipidaemic, antiarthritic and hypoglycemic agent (Kumar *et al*., 2003). Garlic extract and a garlic protein fraction were shown to augment the oxidative burst in peritoneal macrophages of *Balb*/*c* mice. Garlic enhances delayed type hypersensitivity (DTH), T lymphocyte proliferation and NK cell activity. In addition, the garlic enhances the T helper-1 (Th1) type cytokine response in an animal model of leishmaniasis (Ghazanfari *et al*., 2006).

2.2.4. *Ecballium elaterium*:

- The squirting cucumber, *Ecballium elaterium*, belongs to the Cucurbitaceae family **(Table 2.4)** (Adwan *et al*., 2011 & Preedy *et al*., 2011).
- Although *Ecballium elaterium* is the official Latin name, it is derived from Greek, due to its various medicinal uses in Greece. The word *Ecballium*, or, more precisely Ekballein, means "I expel". This refers to the mode of dispersion of the seeds, which is characteristic to the plant. *Elaterium*, which also means "to cast out," refers to the purgative action of the plant (Preedy *et al*., 2011).

Table 2.4. **Classification of *Ecballium elaterium*** (Biology - Flora)**:**

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Violales
Family	Cucurbitaceae
Genus	Ecballium
Species	*Ecballium elaterium*

Ecballium elaterium

- **Description and geographical distribution:**
  - *Ecballium elaterium*, the *squirting cucumber or spitting cucumber*, is a decumbent, perennial herb with a tuberous root restricted to the Mediterranean

Basin and cultivated in central Europe and England. This plant has hairy vine, cordate to triangular, and rather fleshy (4-10 cm) leaves. The flowers are unisexual, with the male flowers having a yellowish corolla. The fruit is ovoid, fleshy, approximately 4 cm in length. The unripe fruit is of a pale green color, and covered with numerous, uniseriate glandular hairs, which eject dark seeds and juice after maturity in response to light pressure. The seeds are ovoid in shape, coffee-brown in color, with a size of 4 - 5 mm. It is common throughout the Mediterranean area as a medicinal plant (Adwan *et al*., 2011; Preedy *et al*., 2011 & Kavalci *et al*., 2007).

- **Traditional usage of *Ecballium elaterium*:**
  - *Ecballium elaterium* is of interest today because its fruits extracts are still used in Mediterranean region as a remedy for many ailments. The uses of this plant are relatively ancient. The diluted aqueous extract of *Ecballium elaterium* fruits is a traditional anti-inflammatory and analgesic for chronic sinusitis. It also possesses other uses especially the treatment of fever, cancer, liver disorders, jaundice, constipation, hypertension, dropsy, rheumatic diseases, and fungicidal (Adwan *et al*., 2011 & Uslu *et al*., 2006).
  - In Arabic folk medicine, the use of the *elaterium* as a laxative, and the juice as a treatment for otitis and as a remedy to purge the brain. The Hindus used the fresh and dried fruit juice in a similar way. In Georgian popular medicine, the plant was used as a remedy for malarial fever. In Turkish folk medicine, the elaterium has been used in treating jaundice and headache. The powdered *elaterium* (precipitate from the fruit juice) mixed with milk used to be applied in the nostrils to clear icterus and cure persistent headaches. It has also been used in the treatment of sinusitis. *Ecballium elaterium* was popular in Maltese folk medicine, as a cathartic and in the treatment of jaundice. The *elaterium* can also be used to remove edema, which is the accumulation of excessive water in the body tissues. At high doses, the elaterium can cause vomiting; hence, it may have been used in the treatment of poisoning where induced vomiting was necessary (Adwan *et al*., 2011 & Preedy, *et al*., 2011).

2.1.5. *Ruta-graveolens*:

- *Rutagraveolens* (*Ruta*) is a medicinal plant of family *Rutaceae* (**Table 5**) (Arora &Tandon., 2015 & Harat, *et al*., 2008).

Table 2.5. **Classification of *Ruta-graveolens*** (Biology - Flora)**:**

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rpsiae
Order	sapindales
Family	rutaceae
Genus	ruta
Species	*Ruta- graveolens*

Ruta-graveolens

- *Rutagraveolens* (*Ruta*) is native to mediterranean area and south-western Asia, *R. graveolens* L. (Rutaceae) is a perennial, medicinal plant that has been used in Europe and Asia for more than 1500 years (Tang & Ren., 2011).

- **Description and geographical distribution:**
  - *Rutagraveolens* (Rutaceae) commonly known as rue, is a strongly scented medicinal and aromatic plant of Europe, mostly grown in the Mediterranean region as an ornamental because of its yellow-cupped beautiful flowers (Ahmad, *et al*., 2010).
  - The plant is now available allover the world, though preferably grown in Mediterranean climates (Raghav, *et al*., 2007).
  - It is a hardy, evergreen shrub of up to 1 m tall, with a characteristic grayish color and a sharp unpleasant odor. Its flowers are relative big, yellow and in clusters during spring and early summer (Tang & Ren., 2011).
  - The leaves are small, oblong, deeply divided, pinnate, glandular dotted. The stems are much ramified. Its flowers are small, yellow and in clusters during spring and summer. They have four petals, except for the central flower, which

has five petals. The fruits are round, brown, small and lobulated. The taste is slightly stinging but is masked by the strong bitter odor (Harat, *et al*., 2008).

- **Traditional uses of *Ruta-graveolens*:**
  - *Ruta graveolens* is a medicinal plant, which has been traditionally used as a sedative and antihelmintic to relieve menstrual and gastrointestinal disorders. Also hypotensive, antifertility, anti-inflammatory effects have also been claimed as further analgesic actions of this plant (Saieed, *et al*., 2006 & Harat, *et al*., 2008), and known to prevent the attacks by fleas and other noxious insects (Lievre, *et al*., 2005 & De Feo, *et al*., 2002).The plant is also used as a flavouring agent for spirits and foods (De Feo, *et al*., 2002). Its documented medicinal uses include anti-ulcer, anti-inflammatory, anti-fungal, antimicrobial, antitumor and cytotoxic activities (Arora &Tandon., 2015 & Harat, *et al*., 2008).
  - *Ruta graveolens* has been used for a long time as a folklore medicine for treatment of various conditions such as eye problems, rheumatism, dermatitis, pain and many inflammatory diseases and as a rubefacient, applied in poultices for rheumatic pain, dislocations, tendon strains, varicose veins and skin conditions such as psoriasis and eczema (Al-Sokari & El Sheikha., 2015).
  - Many Ruta species are sources of diverse classes of natural products with biological activities including anti-fungal & herbicide activities (Meepagala, *et al*., 2005 & Harat, *et al*., 2008).
  - *Ruta graveolens* has been traditionally used in treatment of leucoderma, vitiligo, psoriasis, multiple sclerosis, cutaneous lymphomas, rheumatic arthritis. Recently its extracts were shown to have potent anti-cancer activity (Diwan & Malpathak., 2009). Rue is an important remedy for deep aching pain and rheumatism besides being used for eyestrain-induced headache and dermatitis (Raghav, *et al*., 2007). It has also been used as a remedy for gastric disorders, stiff neck, dizziness, headache (Raghav *et al*., 2006).
  - *Ruta graveolens* L. is an herbal plant which is traditionally used as an energizer and an anti-bleeding to heal injuries (Azizi & Karouei *et al*., 2007), and as Antiseptic, stimulant, emmenagogue, abortifacient, used against rheumatic pain, hysteria, worms, colics, atonic, amenorrhea and menorrhagia (Ivanova *et al*., 2005).

2.2.5. *Phagnalon rupestre*:

- *Phagnalon rupestre* is belongs to the family *Asteraceae* & is one of the Euro-Mediterranean genera of the worldwide-distributed **(Table 2.6)** (Góngora**,** *et al*., 2002).

Table 2.6. **Classification of *Phagnalon rupestre*** (Biology - Flora)**:**

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	*Phagnalon*
Species	*Phagnalon rupestre*

Phagnalon rupestre

- **Description and geographical distribution:**
  - The genus Phagnalon is distributed throughout Northeastern tropical Africa, the Macaronesian region, the Mediterranean basin, the Irano-Turanian region and the Saharo-Arabian region, but its greatest diversity is found in the Arabian Peninsula; it comprises about 36 species. Phagnalon species are suffruticose shrubs or subshrubs and grow mainly in rocky areas, in a wide range of habitats. Species are morphologically characterized as having heterogamous disciform capitula; female florets outnumbering the hermaphrodite ones; involucral bracts with undivided stereome; receptacle flat; anthers provided with long or medium tails; style bifid with obtuse or acute sweeping hairs; achenes with duplex hairs and monomorphic and uniseriate pappus composed by barbellate bristles. Species of Compositae exhibit most of the life forms, including annuals, pyrophytes, hemicryptophytes, trees, succulent plants, halophytes, lianas and also epiphytes and aquatics, although the majority of genera are subshrubs, shrubs or perennial herbs (Moreno, *et al*., 2013).

- **Traditional uses of *Phagnalon rupestre*:**
  - *Phagnalon rupestre* have antimicrobial and skin clearing activities and showing anti-allergic and anti-inflammatory activities (Olmos, *et al*., 2002 & Góngora, *et al*., 2005).
  - *Phagnalon rupestre* was used in the past to make deliberate burns, to treat asthma, anesthetic for toothache, to treat headachea & is had antimicrobial activities (Ali-Shtayeh, *et al*., 1998).

2.3. Microorganisms and bacterial resistance:

Microbes are living organisms that reproduce, thrive, and spread quickly and efficiently increasing their numbers. Microbes include bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa and Escherichia coli* & *S. aureus*, viruses such as colds and influenza, which causes the "flu", Fungi such as Candida albicans, which causes some yeast infections, and parasites such as Plasmodium falciparum, which causes malaria.

Antibacterial is a general term given to medicines that kill or slow the growth of microbes. Antibacterial drug resistance is the ability of a microbe to grow in the presence of a chemical that would normally kill it or limit its growth.

The antibiotics initiated when Paul Ehrlich first coined the term ‘magic bullet’, or chemotherapy, to designate the use of antimicrobial compounds to treat microbial infections (Gensini *et al*., 2007).

At the beginning of the 1910s, Ehrlich discovered the first antibiotic drug, Salvarsan, which was used against syphilis and was used to treat trypanosomiasis (Oldfield & Feng., 2014). Ehrlich was followed by Alexander Fleming, who discovered penicillin by accident in 1928 (Quirke., 2007). Then, in the 1935, Gerhard Domagk discovered the sulfa drugs, thereby paving the way to the discovery of the anti-TB drug Isoniazid. Then, in 1939, René Dubos became the first scientist to discover an antibiotic after purposely looking for it in soil microbes. Dubos discovered Gramicidin, which is still used today to treat skin infections. Finally, in 1943, the first TB drug, Streptomycin, was discovered by Selman Waksman and Albert Schatz. Waksman was also the one who coined the term ‘antibiotics’. Thus, antibiotics have been used to treat bacterial infections since the 1940s (Rubin., 2007).

After more than 50 years of widespread use, evolution of disease-causing microbes has resulted in many antimicrobials losing their effectiveness. As microbes evolve, they adapt to their environment. If something stops them from growing and spreading-such as an antimicrobial-they evolve new mechanisms to resist the antimicrobials by changing their genetic structure. Changing the genetic structure ensures that the offspring of the resistant microbes are also resistant (Johnson., 2006).

Antimicrobial resistance makes it harder to eliminate infections from the body. As a result of a microbe's ability to survive in spite of antimicrobials, some infectious diseases are now more difficult to treat than they were just a few decades ago. In fact, antimicrobials have helped people so effectively that humans e hurting the protective value of medicines through overuse and misuse. acquires a resistance gene (Nascimento *et al*., 2000).

2.3.1. *Klebsiella pneumoniae*:

- *Klebsiella* are Gram-negative, nonmotile, usually encapsulated rod-shaped bacteria, belonging to the family Enterobacteriaceae **(Table 2.7)** (Drancourt *et al*., 2001)**.**

Table 2.7. Classification of *Klebsiella pneumoniae*:

Scientific classification	
Kingdom.	Bacteria
Phylum.	Proteobacteria
Class.	Gammaproteobacteria
Order.	Enterobacteriales
Family.	Enterobacteriaceae
Genus.	*Klebsiella*
Species.	***K. pneumoniae***
Binomial name	
Klebsiella pneumoniae	

These bacteria produce lysine decarboxylase but not ornithine decarboxylase and are generally positive for Voges-Proskauer test, Oxidase negative and catalase positive. Members of the Enterobacteriaceae family are generally

facultatively anaerobic, and range from 0.3 to 1.0 µm in diameter and 0.6 to 6.0 mm in length and dome-shaped, glistering colonies of varying degrees of stickiness. *Klebsiella* spp. often occurs in mucoid colonies (Drancourt *et al*., 2001).

- *Klebsiella* have been identified as important common pathogens for nosocomial pneumonia, septicaemia, urinary tract infection, wound infections, intensive care unit (ICU) infections, and neonatal septicaemias. *Klebsiella* spp. can also cause bacteremias and hepatic infections, and have been isolated from a number of unusual infection, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, fascial space infections of the head and neck, and septic arthritis (Tortora *et al*., 2010)**.**

2.3.2. *Pseudomonas aeruginosa*:

- *Pseudomonas aeruginosa* is an aerobic, gram-negative rod that is motile by polar flagella. Under certain conditions, particularly in weakened hosts, this organism can infect the urinary tract, burns, and wounds, and can cause blood infections (sepsis), abscesses, and meningitis (Tortora *et al*., 2010)**.**

Table 2.8. Classification of *P. aeruginosa*:

Scientific classification	
Kingdom.	Bacteria
Phylum.	Proteobacteria
Class.	Gamma Proteobacteria
Order.	Pseudomonadales
Family.	Pseudomonadaceae
Genus.	*Pseudomonas*
Species.	***P. aeruginosa***
Binomial name	
Pseudomonas aeruginosa	

- In general, *P. aeruginosa* is naturally less susceptible than other gram-negative bacilli to many antibiotics, such as ampicillin (Principen), most cephalosporins, and the macrolides. This is because of its relatively impermeable outer membrane and its ability to actively transport some antibiotics out of the cell, preventing accumulation. *P. aeruginosa* also harbors an inducible chromosomally encoded beta-lactamase, referred to as AmpC beta-lactamase, which is capable of degrading many beta-lactams even though it is naturally expressed at very low levels (Hauser *et al*., 2005).

2.3.3. *Escherichia coli*:

- *E. coli* are facultatively anaerobic, gram-negative rods that are peritrichously flagellated. Its presence in water or food is an indication of fecal contamination. *E. coli* is not usually pathogenic. However, it can be a cause of urinary tract infections, and certain strains produce enterotoxins that cause traveler's diarrhea and occasionally cause very serious foodborne disease (Bhunia and Ray, 2008).

Table 2.9. Classification of *Escherichia coli*:

Scientific classification	
Kingdom.	Bacteria
Phylum.	Proteobacteria
Class.	Gammaproteobacteria
Order.	Enterobacteriales
Family.	Enterobacteriaceae
Genus.	*Escherichia*
Species.	***E. coli***
Binomial name	
Escherichia coli	

- *E. coli* is a common kind of bacteria that lives in the intestines of animals and humans and most are harmless. The most dangerous strain of *E. coli* is called 0157.H7 because it produces a very powerful poison and can make you very

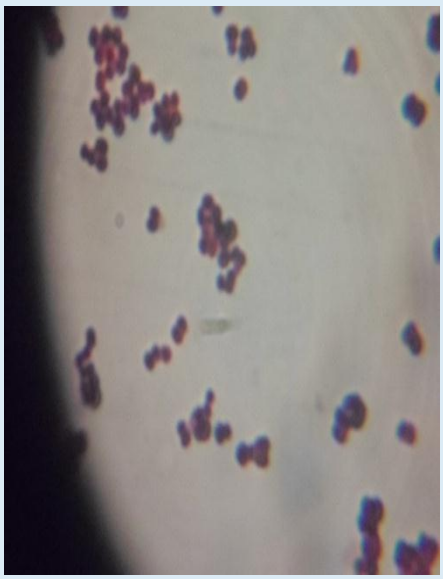
sick if it is in your food or drink. Bloody diarrhea and stomach pains are the most common symptoms for people infected with *E. coli*. Eating unwashed greens such as spinach, lettuce or green onions or undercooked beef can cause the infection. People can spread the disease through contact with one another if they do not wash their hands after using the bathroom. The drinking of unpasteurized milk can be spread the disease because the *E. coli* can be on the cows utters (Nelson, 2008).

2.3.4. *Staphylococcus aureus*:

S. aureus is a facultatively anaerobic, Gram-positive coccus. *S. aureus* produces many toxins that contribute to the bacterium's pathogenicity by increasing its ability to invade the body or damage tissue. The infection of surgical wounds by *S. aureus* is a common problem in hospitals. Either its ability to develop resistance quickly to such antibiotics as penicillin contributes to its danger to patients in hospital environments. *S. aureus* produces the toxin responsible for toxic shock syndrome, a severe infection characterized by high fever and vomiting, sometimes even death. *S. aureus* also produces an enterotoxin that causes vomiting and nausea when ingested; it is one of the most common causes of food poisoning (Bhunia and Ray, 2008).

Table 2.10 Classification of *S. aureus*:

Scientific classification	
Domain.	Bacteria
Kingdom.	Eubacteria
Phylum.	Firmicutes
Class.	Coccus
Order.	Bacillales
Family.	Staphylococcaceae
Genus.	*Staphylococcus*
Species.	***S. aureus***
Binomial name	
Staphylococcus aureus	



- It produces pustules, carbuncles, boils, and impetigo; it is also a frequent cause of septicaemia, osteomyelitis، bacteraemia, and otitis (Uwaezuoke and Aririatu, 2004).

2.4. Previous Studies:

2.4.1. *Rosmarinus officinalis*:

- In 2015, **Barbosa *et al*** evaluated in vitroAntibacterial and Chemical Properties of EOs Including native plants from brazil against pathogenic and resistant bacteria. aimed to examine the chemical characterization (GC-MS) of EO from seven plants and measure antibacterial activities against bacterial strains isolated from clinical human specimens (methicillin-resistant *Staphylococcus aureus* (MRSA) and MSSA, *E. coli, P. aeruginosa, S. Typhimurium*) and foods (*Salmonella Enteritidis*). Assays were performed using the minimal inhibitory concentration (MIC and MIC 90%) (mg/mL) by agar dilution and time kill curve methods (log CFU/mL) to aiming synergism between EO. EO chemical analysis showed a predominance of terpenes and its derivatives. The results revealed that, the *Rosmarinus officinalis* EO had antibacterial activity against MRSA, MSSA, *S. aureus*, *E. coli* & *P. aeruginosa* with MIC values of 8.60±0.72 , 8.60±0.90, 8.60±0.85, 79.35±2.96 & 79.91±3.40 mg/ml respectively.
- In 2014, **Irshaid *et al*** evaluated the Phenol content, antioxidant capacity and antibacterial activity of methanolic extracts derived from four Jordanian medicinal plants (*Artemisia sieberi, Peganum harmala, Rosmarinus officinalis* (Green-Flowered) and *Sarcopterium spinosium*)**.** Four Gram-negative stains [*E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *P. mirabills* (ATCC 426), *Enterobacter cloacae* (ATCC 29004)] and one Gram-positive [*S. aureus* (ATCC 25923)] were used for antimicrobial activity studies**.** Broth dilution used to determine the MICs and disc diffusion assays were performed to measure the antibacterial activity of these extracts against available bacterial strains. The results revealed that, the methanolic extract**s** (extracted in Soxhlet apparatus) were found to posses antibacterial activity against all tested Gram positive and Gram negative bacteria in dose dependent manner. The values of inhibition zones of these methanolic extracts were found to be ranged from 8 to 30 mm. The highest values of inhibition were obtained for methanolic extract derived from *S. spinosium* against *P. mirabilis* (zone of inhibition. 30±2.5 mm), a Gram negative bacterium and *S. aureus* (zone of inhibition.

29±2.6), a Gram positive bacterium, as well as for methanolic extract derived from *Rosmarinus officinalis* against *S. aureus* (zone of inhibition. 30±2.8 mm), *E. coli* (zone of inhibition. 21±2.3 mm) & *P. aeruginosa* (zone of inhibition. 18±2.0 mm), using the extract concentration of 0.4mg/ml. Conversely, weak inhibitory effects were observed against *E. coli, P. aeruginosa, E. cloacae* and *P. mirabilis* using methanolic extract derived from *A. sieberi* at concentration of 400 μg mL^{-1} when compared to the other tested methanolic extracts. The MIC of methanolic extracts derived from *Rosmarinus officinalis* against *S. aureus* (1.0mg/ml), *E. coli* (1.2mg/ml) & *P. aeruginosa* (2.2mg/ml).

- In 2013, **Qabaha** evaluated the Antimicrobial and free radical scavenging activities of five Palestinian medicinal plants. Extracts from five indigenous Palestinian medicinal plants including *Rosmarinus officinalis, Pisidium guajava, Punica granatumpeel, grape seeds* and *Teucrium polium* were investigated for antimicrobial and free radical scavenging activities against eight microorganisms, using well diffusion method. The microorganisms included six bacterial isolates (i.e. *S. aureus* & MDR. (*E. coli, P. aeruginos, K. pneumoniae*), *B. subtilis and Micrococcus luteus*) and two fungal isolates (i.e. *Candida albicans and Aspergillus niger*). A standard antioxidant assay was performed on the plant extracts to assess their capability in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH). Of the five tested plant extract, only *Rosmarinus offcinalis* ethanolic extract exhibit significant antimicrobial activity against all eight microbial isolates, with inhibition zone diameter of 20.3±0.6, 9.0±1.7 & < 7 (resestant) mm/12.5mg ml^{-1}/well against *S. aureus, E. coli, P. aeruginos, K. pneumonia*e respectively, but *K. pneumoniae* was sensetive against *Rosmarinus offcinalis* ethanolic extract at concentration of 25mg/ml with inhibition zone diameter of 9.7±0.6 mm. Extracts from other four plants exhibited a variable antimicrobial activity against all microorganisms, except *P.aeruginosa*. Significant antioxidant activity was detected in all plant extracts. However, extracts from *Pisidium guajava* leaves contained significantly higher antioxidant activity compared to the other extracts tested. The antimicrobial and scavenging activities detected in this in vitro study in extracts from the five Palestinian medicinal plants suggest that further study is needed to identify active compounds to target diseases caused by a wide-spectrum pathogens.

- In 2013, **Hosn**i ***et al*** evaluated the chemical composition and antimicrobial activity of *Rosmarinus officinalis*. The EO from *Rosmarinus officinalis* was tested against three Gram-positive bacteria. *S. aureus* (ATCC 6538), *Enterococcus feacium* (ATCC 19434) and *S. agalactiae*. Two Gram-negative bacteria (*E. coli* ATCC 8739 and *Salmonella typhimurium* ATCC 14028) and the yeast *Candida albicans* ATCC 10231 were also used in this bioassay. The disk diffusion assay indicated that the different essential oils inhibited the growth to variable extent, depending on the essential oil and the microbial strains. The maximum activity was noted against *E. coli* (24 mm), *Salmonella typhimurium* (23 mm), *Candida albicans* (15mm), *S. agalactiae* (20mm), *S. aureus* (32mm), *Enterococcus feacium* (11mm).
- In 2013, **Hosn**i ***et al*** evaluated the Enzyme-assisted extraction of EOs from *thyme* (*Thymus capitatus* L.) and *rosemary* (*Rosmarinus officinalis* L.). Impact on yield, chemical composition and antimicrobial activity. The results indicated that the *Rosmarinus officinalis* EO had antimicrobial activities against 6 food-borne pathogens (*E. coli, S. aureus, Salmonella typhimurium, Streptococcus agalactiae, Enterococcus feacium and Candida albicans*). Which had inhibition zone diameter of 19 ± 1.0 & 9 ± 0.0 mm/10µl/disk of 200mg/ml of *Rosmarinus officinalis* EO against of *E. coli* & *S. aureus* respectively.
- In 2013, **Jordan *et al*** evaluated the Effect of the phenological stage on the chemical composition, and antimicrobial and antioxidant properties of *Rosmarinus officinalis* L essential oil and its polyphenolic extract. *Rosmarinus offcinalis* plants were collected at the full bloom and fruit maturation phenological stages. The results obtained from both bioclimatic areas revealed that the EOs and the polyphenolic extracts from plants harvested at the fruit maturation phase provide better antimicrobial and antioxidant activities than those collected at the full bloom stage. These improvements could be explained by higher concentrations of δ -terpinene, α-terpinene, terpinolene and caryophyllene oxide determined in the EOs and of rosmarinic acid, hesperidin, and carnosol in the polyphenolic extracts. The results obtained from full bloom and fruit maturation phenological stages revealed that the EOs had no effect

against *E. coli* or *L. monocytogenes* (inhibition zone <12 mm/15µl/disk), whereas moderate activity was observed against *Salmonella Typhimurium* and *S. aureus* (inhibition zone <20–12 mm/15µl/disk).

- In 2012, **Jordan *et al*** evaluated the chemical constituents and antibacterial activities of *Rosmarinus officinalis* against two food-borne pathogens of Gram negative strains (*S. typhimurium* and *E. coli*) and two Gram positive strains (*L. monocytogenes* and *S. aureus*). The results indicated the aqueous extract of *Rosmarinus officinalis* showed strong activity (inhibition zone ≥ 20 mm) against *S. typhimurium, S. aureus, L. monocytogenes,* and *E. coli*.
- In 2010, **Hussain *et al*** evaluated the *Rosmarinus officinalis* essential oil. antiproliferative, antioxidant and antibacterial activities. The disc diffusion and modified resazurin microtitre-plate assays were used to evaluate the inhibition zones (IZ) and minimum inhibitory concentration (MIC) of *Rosmarinus officinalis* EO, respectively. It is concluded from the results that *Rosmarinus officinalis* EO exhibited antiproliferative, antioxidant and antibacterial activities. The antibacterial activities of *Rosmarinus officinalis* EO estimated against eight bacterial strains including within of it *S. aureus*, *P. aeruginosa* & Amphicilin resistant *E. coli* strain. The results revealed that, the *Rosmarinus officinalis* EO exhibited antibacterial activities with inhibition zone of 22.0 ± 1.0 mm/ 15µL of EO/disc & MIC of 0.30 ± 0.01mg/ml against *S. aureus*, and inhibition zone of 12.8 ± 0.5 mm/ 15µL of EO/disc & MIC of 1.72 ± 0.04 mg/ml against *E. coli*, and inhibition zone of 17.0 ± 1.0 mm/ 15µL of EO/disc & MIC of 1.26 ± 0.03 mg/ml against *P. aeruginosa*.
- In 2010, **Zaoual *et al*** investigated the essential oils composition of *Rosmarinus officinalis* and the antimicrobial and antioxidant activities. antimicrobial activities were tested against *E. coli* ATCC10536, *P. aeruginosa* ATCC 9027, *K. pneumoniae* ATCC 10031, *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633, *B. cereus* ATCC 11778, *S. epidermis* ATCC 12228 and *Streptococcus feacalis* ATCC 10541. The results indicated the highest activity was observed against *E. coli* ATCC 10536 with the strongest inhibition zones (16.5 mm/10µl/disk), *P. aeruginosa* ATCC 9027 was have a weak inhibition zone (7.5 mm/10µl/disk) or resistant to the EOs of *Rosmarinus officinalis*. *K. pneumoniae* ATCC 10031 is sensitive to oils (growth inhibition zone

16 mm/10µl/disk). Against *B. subtilis* ATCC 6633 and *B. cereus* ATCC 11778, oils exhibited a slight to moderate antimicrobial activity. Against *S. aureus* ATCC 6538 moderate activities (13.5 mm/10µl/disk) were observed for oils. *S. feacalis* ATCC 10541 was relatively resistant (7.5 to 11.5 mm). All tested bacteria were more susceptible to Gentamycin (12–21 mm) than to the EOs tested; *S. feacalis* and *P. aeruginosa* being the most resistant to both Gentamycin and oils. The Results indicate a high variation of MICs and MBCs among *R.officinalis* oils and bacteria strains. *P. aeruginosa* was found to be resistant to *R.officinalis* oils. All oils exhibited a low antibacterial activity (MIC = 10 µl/ml) against *S. epidermis* and *S. feacalis* (MICs ≤11.5 µl/ml). Against *E. coli* all oils showed a low minimum inhibitory concentration (MICs = 1.25 and 2.5µl/ml). The oils exhibiting a moderate activity (MIC value was observed for the essential oil EO1 of var.typicus 2.5 & 1.25 µl/ml against *K. pneumoniae & S. aureus* respectively). Oils from var. typicus showed a low bactericidal effect (MBCs = 10µl/ml). However, oils EO5 and EO6 from var.troglodytorum, showed a moderate bactericidal activity (MBC = 5µl/ml) restricted to the gram-negative bacteria *E. coli and K. pneumoniae. P. aeruginosa, S. epidermis* and *S. feacalis* were resistant for all oils.

- In 2010, **Okoh *et al*** evaluated of the antibacterial activities of the essential oils of *Rosmarinus officinalis* against two gram-positive and two gram negative bacterial species. The bacteria species including within of it. *E. coli* (ATCC 8739); *B. subtilis* (ATCC 10702); *K. pneumoniae* (ATCC 10031) and *S.aureus* (ATCC 6538). The results indicated that the EOs of *R. officinalis* have shown broad spectra of activity against the tested microorganisms (*E. coli*; *B. subtilis*; *K. pneumoniae* and *S. aureus*) at 100µl/well of 10 mg/ml. The oils inhibited the growth of both the gram-positive and gram-negative bacteria at MIC values of 3.75, 7.50, 0.49 & 1.88 mg/ml against *S. aureus, E. coli, K. pneumoniae* & *B. subtilis* respectively. The oils are lethal against both the gram-positive and gram-negative bacteria at MBC values of 7.50 mg/ml against both *S. aureus & E. coli* & > 7.50 mg/ml against both *K. pneumoniae & B. subtilis*.
- In 2009, **Oskay *et al*** evaluated the Activity of Some Plant Extracts against Multi-Drug Resistant Human Pathogens. Extracts of *Liquidambar orientalis,*

Vitis vinifera, Rosmarinus officinalis, Punica granatum, Cornus sanguinea, Euphorbia peplus, Ecballium elaterium, Inula viscosa and Liquidambar orientalis showed broad-spectrum antibacterial activity with inhibition zones ranging from 8 to 26 mm. The results indicated that the *MRSA*, *E. coli*, *K. pneumoniae* & *P. aeruginosa* was susceptible for the ethanolic extracts of *Rosmarinus officinalis* & ethanolic extracts of *Ecballium elaterium*, with inhibition zone of (24 & 14), (16 & 14), (16 & 8) & (18 & 20) mm/100µl/disk of 4mg/ml concentration respectively, while the MIC values for *Rosmarinus officinalis* was 8.6 & 12.6 mg/ml against MRSA & *E. coli* respectivly, while the MIC against both *K. pneumoniae & P. aeruginosa* not determained at this concentration (4mg/ml).

- In 2009,**Van Vuuren *et al*** evaluated the The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. The results revealed that, the *Rosmarinus officinalis* EO displayed moderate antimicrobial activity with MIC values in the range (3.3–8.0mg/ml), which had MIC of 6.0 & 8.0 mg/ml against *S. aureus* & *K. pneumoniae*. Interactions of the EOs (*Melaleuca alternifolia, Thymus vulgaris, Mentha piperita and Rosmarinus officinalis*) when combined with ciprofloxacin against *S. aureus* indicate mainly antagonistic profiles at the ratio of 5.5. When tested against *K. pneumoniae* the isobolograms show antagonistic, synergistic and additive interactions depending on the combined ratio. The *R. officinalis* / ciprofloxacin combination against *K. pneumoniae* displayed the most favourable synergistic pattern. The interactions of *R. officinalis* (rosemary) essential oils with amphotericin B indicate mainly antagonistic profiles when tested againstCandida albicans.
- In 2007, **Celiktas *et al*** evaluated the Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. The goal of this work was to test the antimicrobial activity of the essential oils and methanolic extracts of *R. officinalis* collected from three different regions at four different time intervals of the year against *Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus feacalis, E. coli, S. epidermidis, B. subtilis* and *C. albicans*. Essential oils were obtained from the aerial parts of the plant by

using a Clevenger apparatus, for 4 h. After distillation, the distillates were filtered, air-dried and then extracted by using a Soxhlet apparatus for 9 h to obtain the methanolic extracts. The antimicrobial activities of the methanolic extracts were tested by the disc diffusion technique (20µl/disc at concentration ranging from 125 to 15.6 mg/ml of the extracts). The antimicrobial activities of the essential oils obtained from *R. officinalis* were determined by minimum inhibitory concentration (MIC).The results indicated that the tested bacteria were sensitive to the essential oils (MIC/MBCfor *R. officinalis* EO harvested in December 10/20, 20/20, 20/20 & 10/10 mg/ml against *S. aureus*, *E. coli*, *K. pneumonia* & *P. aeruginosa* respectively) and the methanolic extracts exhibited very low antimicrobial activities compared to the essential oils. The results of the antimicrobial screening for the methanolic extracts showed low activity against *S. aureus* whereas the rest of the extracts were inactive against other microorganisms at concentration ranging from 125 to 15.6 mg/ml of the extracts. The antimicrobial activities of the essential oils against the tested bacteria differed, depending on location and seasonal variations.

- In 2004, **Abu-Shanab *et al*** evaluated the Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. The dried water, methanol and ethanol extracts of *Syzyium aromaticum* (Myrtaceae) (seed), *Cinnamomum cassia* (Lauraceae) (cassia bark, Chinese cinnamon) (bark), *Salvia officinalis* (Lamiaceaea) (leaf), *Thymus vulgaris* (Lamiaceaea) (leaf) and *Rosmarinus officinalis* (Labiatae) (leaf) were tested in vitro against 4 bacterial species by well diffusion and microdilution methods. The patterns of inhibition varied with the plant extract, the solvent used for extraction, and the organism tested. The results indicated that the wateric, ethanolic & methanolic extracts of Rosmarinus officinalis showed various inhibitory effects (10-13 mm/50 µl /disk of 100 mg/ml concentration) against MRSA with inhibition zone diameter of 12, 22 & 17 mm respectively. No effects were detected for *EHE. coli* or *P. aeruginosa* at concentration of 100mg/ml (inhibition zone diameter of 6 mm for wateric, ethanolic & methanolic extracts). The MIC of the ethanol extracts of *Rosmarinus officinalis* was 1.5 mg/ml against MRSA.While against *EHE.coli* & *P. aeruginosa* not active at concentration range from 6.25 to 0.781 mg/ml

- In 1999, **Mangena & Muyima** made comparative evaluation of the antimicrobial activity of essential oils of *Artemisia afra, Pteronia incana* and *Rosmarinus officinalis* on selected bacetria and yeast strains. The bacterial strains included within of it *S. aureus, E. coli & P. aeruginos*. The results indicated that, the neat essential oils of *R. officinalis* had a lower antibacterial activity against *S. aureus & E. coli* (streptomycin resistant) with inhibition zone of 12 ± 2.3 & 12 ± 0.0 mm /15µl / disk respectively, while *Pseudomonas aeruginos* showed no sensitivity against the neat essential oils of *R. officinalis* (with inhibition zone of 6.0 ± 0.0 mm /15µl / disk).
- In 1998, **Baratta *et al*** evaluate the chemical composition, antimicrobial and ant-ioxidative activity of laurel, sage, rsemary, oregano and coriander essential oils. Twenty-five different genera of bacteria including within of it *S. aureus, E. coli, K. pneumoniae & P.* aeruginosa, and one fungal species were used in this study as test organisms. The oils showed a high degree of inhibition against all the microorganisms tested. In addition, the results revealed that, the *Rosmainus officinalis* EO exhibit antibacterial activity against *S. aureus, E. coli, K. pneumoniae & P.* aeruginosa with inhibition zones diameter of 46.00, 10.00, 8.70 & 8.60 mm/ 10 µL of the undiluted oils/well respectively.

2.4.2. *Pelargonium graveolens*:

- In 2013, **Boukhatem *et al*** evaluated the essential oil of algerian *rose-scented geranium* (*Pelargonium graveolens*). Chemical composition and antimicrobial activity against food spoilage pathogens, (23 food spoilage pathogens)**.** The results indicated that the oil exhibited promising antibacterial effect against Gram-positive more than Gram-negative bacteria and provides a good inhibitory effect against *Candida* strains. Among the Gram-negative bacteria, the EO was more effective against *E. coli* and *Enterobacter aerogenes* ATCC 13043 with inhibition zones measured at 15 and 14 mm/10µl/disk respectively (at the concentration of 10µl per disc). In contrast, *P. aeruginosa* and *K. pneumoniae* were the most resistant strains to the oils while *P. vulgaris*, an important food pathogen, shown a modest sensibility. Among the Gram-positive bacteria, *S. aureus* and *E. faecalis* ATCC 29212 (DIZ 12.83 & 21.17 mm/10µl/disk respectively (at the concentration of 10µl per disc) were the most sensitive strains to the EO, followed by *B. subtilis* ATCC

6051(20.5 mm) and *S. epidermidis* (16.17 mm). Of the yeasts, it was interesting to note that the EO exhibited the strongest inhibitory effect against *Candida* strains among all the tested microorganisms in comparison with the Amphetorecin B (positive reference standard for yeast).

- In 2013, **Boukhris *et al*** evaluated the chemical composition and biological activities of essential oil of *Pelargonium graveolens* against standard bacterial strains. *P. aeruginosa, E. coli, S. aureus* and *B. subtilis*. Yeast and fungi. *C. albicans* and *A. niger*. The results showed that the essential oil (extracred in Clevenger-type apparatus), methanol and water extracts (extracred in a Soxhlet extractor) exhibit bactericide and fungicide effects, and in most cases, the concentrations required to kill the bacteria and fungi are two times higher than the concentration required to inhibit the bacteria and fungi as represented by the respective MBC and MIC values. Overall, the essential oil was more potent than the methanol and water extracts. Which MICs & MBCs of *Pelargonium graveolens* EO on standard bacterial strains (µg/ml) for *S. aureus, P. aeruginosa* & *E. coli was* MIC. 1.76-3.52, MBC. 3.52-7.04 µg/ml for all. While MICs & MBCs of *Pelargonium graveolens* leafe aquatic extract on standard bacterial strains (µg/ml) for both of *S. aureus* & *P. aeruginosa* was MIC. 5-10, MBC. 10-20 µg/ml, while (MIC. 10–20, MBC. 20–40 µg/ml against *E. coli.* While MICs & MBCs of *Pelargonium graveolens* leafe methanolic extract on standard bacterial strains (µg/ml) for both of *S. aureus* & *E. coli* was MIC. 5-10, MBC. 10-20 µg/ml, while MIC. 2.5-5, MBC. 5-10 µg/ml against *P. aeruginosa*. While MICs & MBCs of *Pelargonium graveolens* leafe aquatic extract on standard bacterial strains (µg/ml) for both of *S. aureus* & *P. aeruginosa* was MIC. 5-10, MBC. 10-20 µg/ml, while MIC. 10-20, MBC. 20-40 µg/ml against *E. coli*.
- In 2012, **Ghannadi *et al*** evaluated the antibacterial activity and composition of essential oils from *pelargonium graveolens* L'Her and *Vitex agnus-castus* L. The chemical compositions of essential oils were characterized by GC-MS. Disc diffusion method was used to study antimicrobial activity. The antibacterial activity of essential oils was investigated against six bacterial species included *L.monocytogenes, Salmonella enteritidis, P. aeruginosa, E. coli, S. aureus and B. subtilis*. The results revealed that, Inhibition zones

showed that the essential oils (obtained after hydro-distillation of the aerial parts of *P. graveolens* and the seeds of *V. agnus-castus*) of the two plants were active against all of the studied bacteria (except *Listeria monocytogenes*). The susceptibility of the strains changed with the dilution of essential oils in DMSO. The pure essential oils showed the most extensive inhibition zones and they were very effective antimicrobial compounds compared to chloramphenicol and amoxicillin. But at concentration of 200 mg/ml (1/4) the inhibition zones of *Pelargonium graveolens* essential oil was 14.3 ± 1.5, 7.0 ± 0.0, 15.3 ± 1.1 & 11.0 ± 0.0 mm/15µl/disk against *S. aureus*, *E. coli*, *P. aeruginosa* & *B. subtilis* respectively.

- In 2012, **Hsouna & Hamdi** investigated the Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from *pelargonium graveolens* growing in Tunisia. The GC-MS analysis of the essential oil revealed 42 compounds. Linallol L, Citronellol, Geraniol, 6-Octen-1-ol, 3,7-dimethyl, formate and Selinene were identified as the major components. The antimicrobial activities of *P. graveolens* essential oil and extracts (methanolic & ethylacetate) against the tested microorganisms were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, MIC and MBC values. The tested oil and organic extracts (methanolic & ethylacetate) exhibited a promising antimicrobial inhibitory activity against a panel of microorganisms (including within of it *S. aureus* & *K. pneumoniae*) with diameter inhibition zones ranging from 12 to 34 mm, where the antibacterial activity of *P. graveolens* essential oil & methanolic against *S. aureus & K. pneumoniae* was (24 & 13) & (13 & 15) mm /50 µl of 50 mg/ml/well respectively. Hexanic and Wateric exracts remained inactive in the range of the used concentration (4 mg/wells). MICs values ranging from 0.039 to 10 mg/ml, wher the MIC/MBC of *P. graveolens* essential oil & methanolic against *S. aureus & K. pneumoniae* was (0.312/0.625 & 25/10) & (10/10 & 0.312/10) mg/ml respectively. The investigation of the phenolic content showed that EtOAc, MeOH and water extracts had the highest phenolic contents.
- In 2007, **Rosato *et al*** evaluated the Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. As a first step growth

inhibition by some types of essential oils was assessed in five microbial species. The antimicrobial effects of *P. graveolens* oil, as well as those of its components, were evaluated by means of the agar dilution method (ADM) against *S. aureus, E. coli, B. cereus and B. subtilis*.The results revealed that, the *P. graveolens* & *Rosmarinus officinalis* oil had Antibacterial effect against all bacteria under study with MIC values of 0.72 & 5.60, 5.60 & 11.20, 0.36 & 1.40, 0.72 & 5.60 mg/ml respectively. While The results obtained highlighted the occurrence of a pronounced synergism between *P. graveolens* essential oil and Norfloxacin against three of the five bacterial species under study. Such antibacterial effects were also shown to increase, although to a lesser extent, when Norfloxacin was given with the main components of *P. graveolens* essential oil.

- In 2000**, Dorman & Deans** evaluated the Antimicrobial agents from plants. antibacterial activity of plant volatile oils. The volatile oils o*f black pepper , clove, Pelargonium graveolens, nutmeg, oregano, and thyme* were assessed for antibacterial activity against 25 different genera of bacteria, which included within of it *S. aureus, E. coli, K. pneumoniae & P. aeruginosa*. The results indicated that the *Pelargonium graveolens* volatile oil had antibacterial activity against *S. aureus, K. pneumoniae & P. aeruginosa* with inhibition zone of 13.6 ± 0.3, 13.8 ± 0.2 & 19.4 ± 0.1 mm/15µl/well, while *E. coli* showed no sensitivity against the *Pelargonium graveolens* volatile oil.
- In 1999, **Hammer *et al*** evaluated the Antimicrobial activity of essential oils and other plant extracts. In the present study, 52 plant EOs (including within of it *Pelargonium graveolens & Rosmainus officinalis)* and extracts were investigated for activity against 11 strains of bacteria (including within of it *E. coli, K. pneumoniae, P.* aeruginosa & *S. aureus*), using an agar dilution method. The results indicate that there are antibacterial activity against the tested bacteria, with MIC values against *E. coli, K. pneumoniae, P.* aeruginosa & *S. aureus* of 1.0, 2.0, > 2.0 & 1.0 % v/v respectively for *Rosmainus officinalis* herb EO, and MIC was 0.25, > 2.0, > 2.0 & 0.25 % v/v respectively for *Pelargonium graveolens* herb EO.

2.3.3. *Allium sativum*:

- In 2015, **Gupta *et al*** evaluated the antimicrobial activity of aqueous extract o f *Allium sativum* (AGE) against multidrug resistant clinical isolates of pathogenic bacteria found in human urine in cases of urinary tract infection (UTI). the results revealed that Among the 76 clinical pathogens *S. aureus* and *E. coli* were most susceptible with inhibition zone diameter of 26 & 28 mm / 100µl /well of 70mg/ml respectively followed by *Enterobacter* sp. and *Klebsiella* sp with inhibition zone diameter of 25 & 24 mm respectively, while *P. aeruginosa* had moderate susceptibility with inhibition zone diameter of 22 mm / 0.7mg /well. MIC of fresh aqueous garlic extract was found to be 35mg/ml where fresh AGE resulted in effective inhibition of bacterial growth.
- In 2014, **Al-Mariri, & Safi** evaluated the In Vitro Antibacterial Activity of Several Plant Extracts and Oils against Some Gram-Negative Bacteria. Experimental, in vitro,evaluation of the activities of 28 plant extracts and oils as well as some antibiotics against *E. coli* O157.H7, *Yersinia enterocolitica* O9, *Proteus* spp., and *K. pneumoniae* was performed. The activity against 15 isolates of each bacterium was determined by disc diffusion method at a concentration of 5%. Microdilution susceptibility assay was used in order to determine the minimal inhibitory concentrations (MICs) of the plant extracts, oils, and antibiotics. Among the evaluated herbs, *Allium sativum L* EO had antibacterial activity against all bacteria under study, with MIC 12.50, 6.25, 3.125 & 6.25 µl/ml against *E. coli, Yersinia enterocolitica*, *Proteus* spp and *K. pneumoniae* respectively.
- In 2014, **Viswanathan *et al*** evaluated the Antimycobacterial and Antibacterial Activity of *Allium sativum* Bulbs. The Antibacterial activity of *Garlic oil obtained by steam distillation* was evaluated against *S. aureus, E. coli, B. subtilis and MRSA* by zone of inhibition method. The results revealed that the *Allium sativum* EO had antibacterial activity against *S. aureus, E. coli, B. subtilis and MRSA* at concentration of 50 mg/ml with inhibition zones of 22.00, 18.67, 18.00 & 16.67 mm respectively.
- In 2014, **Gaherwal *et al*** observed the Anti-Bacterial Activities of *Allium sativum* against *E. coli, Salmonella Ser. Typhi and S. aureus*. The crude extract and methanolic extract of garlic were tested against *E. coli, S. aureus*

and Salmonella ser. Typhi. In result we found that in the well diffusion method, The crude extract of garlic was most efficient against the *S. typhi* followed by *S. aureus* and then *E. coli*, the inhibition zones were 10, 15 and 22 mm against *E. coli, S. aureus* and *S.ser. Typhi* respectively. The methanolic extract of garlic bulb was also effective against all the three bacteria under study, which had maximum inhibition zone against *S. aureus* followed by *E. coli* and then *S. ser. Typhi.* The inhibition zones were 10, 12 and 9 mm against *E. coli, S. aureus* and *S. ser. Typhi* respectively. Overall result shows that the crude extract was most powerful against the all pathogenic bacteria in comparison to methanolic extract, though both were effective.

- In 2013, **Akintobi *et al*** investigated the antimicrobial activity of garlic (*Allium sativum*) extract against Some Selected Pathogenic Bacteria using the agar well diffusion method. These bacteria include; *P. aeruginosa, S. aureus, Proteus mirabilis, E. coli, B. subtilis and S. typhi.* Four different extracts were obtained from the bulbs of garlic (water-soluble and ethanol-soluble extracts). There were zones of inhibitions around the wells, which indicate that the organisms were sensitive to both water and ethanol extract of garlic. The result showed that the isolates behaved differently in their sensitivity to the different extracts added to their growth medium. Aquatic extract of the garlic was effective against *Proteus mirabilis, S. typhi* and *S. aureus* with inhibition zones of 7, 6 & 5 mm respectively. *E. coli, P. aeruginosa* and *B. subtilis* were resistant to the aquatic extract. Ethanolic extract of the garlic was absolutely effective against *P. aeruginosa, S. aureus, Proteus mirabilis* & *S.typhi* pathogenic bacteria with inhibition zones of 7, 9, 6 & 19 mm respectively. *E. coli and B. subtilis* were resistant to the ethanolic extract. The quantitative and qualitative phytochemical analysis indicates that the extract of *Allium sativum* (garlic) constitutes antimicrobial activity. This investigation indicates that though plant had antimicrobial and greater inhibitory effect thus confirming its use in folk medicine.
- In 2013, **Sharma *et al*** investigated the antibacterial mechanism of action of *Allium sativum* rhizome essential oil (ASEO) against foodborne pathogens. The ASEO was obtained by hydrodistillation of *A. sativum* rhizome using a microwave-assisted extraction technique. The ASEO (1,000mg/disk) showed

potential antibacterial effect as diameters of inhibition zones against the tested foodborne pathogens American Type Culture Collection (ATCC) strains including within of it *S. aureus, E. coli, L. monocytogenes, S. Typhimurium and B. cereus*, which were found to be 14.00, 12.00, 21.00, 11.00 & 24.00 mm/ 10µL/1,000µg/disk respectively. The MIC/MBC values of ASEO against *S. aureus, E. coli, L. monocytogenes, S. Typhimurium and B. cereus* were found to be 125/250, 250/500, 125/250, 250/500 & 62.5/250 mg/mL respectively. At the MIC concentration, ASEO had potential inhibitory effect on the cell viability of the tested bacteria. In addition, the scanning electron microscopy analysis showed the inhibitory effect of ASEO as confirmed by the considerable morphological alterations on the cell walls of *B. cereus* and *E. coli*. Moreover, the ASEO revealed its mode of action on membrane integrity as confirmed by the release of extracellular adenosine 5′-triphosphate, 260-nm absorbing materials and potassium ions efflux against tested pathogens. These findings suggest that ASEO showed its effect on membrane permeability and surface characteristics.

- In 2013, **Arekemase *et al*** investigated the *in-vitro* sensitivity of selected enteric bacteria to extracts of *Allium sativum* L. The antimicrobial effects of *Allium sativum* (garlic) against some bacterial isolates were investigated using the agar diffusion well method. The results show that the Photochemical analyses of the ethanolic extracts showed the presence of many secondary metabolites such as saponins, tannins, alkaloid steroids and glycosides. Either the results show that the aquatic & ethanolic extracts revealed different degree of the antibacterial activity, which the aquatic extract was found to exhibit differential activity against *E. coli, P. aeruginosa and K. pueumoniae* with inhibition zone of 9.50, 6.00 & 7.00 mm/ 30 mg/ml/well respectively. Two bacteria, *S. aureus and B. subtilis* however showed resistance to the aqueous extract. Ethanolic extract had the most activity against *E. coli* (12.50 mm/ 30 mg/ml/well) while *S. aureus and B. subtilis* showed least sensitivity (4.50 & 5.00 mm/ 30 mg/ml/well), While *P. aeruginosa and K. pueumoniae* showed moderate sensitivity (11.00 & 9.50 mm/ 30 mg/ml/well). The MIC & MBC of the (garlic) was determined for both the aqueous and ethanolic extract. The ethanolic extract was more effective than the aqueous extract, inhibiting all the test organisms. While the aqueous extracts was effective against *E .coli, P.*

aeruginosa and K. pneumoniae. The MIC values for the aqueous extract at concentration of 30 mg/ml was detected against *E. coli, P. aeruginosa and K. pueumoniae*, but MIC not detected against *S. aureus and B. subtilis* at concentration of 30 mg/ml. The MBC values for the aqueous extract at concentration of 30 mg/ml were not detected against all tested bacteria. The MIC values for the ethanolic extract was detected against *E. coli, P. aeruginosa and K. pueumoniae, S. aureus and B. subtilis* at concentration of 30 mg/ml, while The MBC values for the ethanolic extract at concentration of 30 mg/ml was detected against *E. coli & P. aeruginosa*, and not detected against the rest of tested bacteria.

- In 2013, **Casella *et al*** investigated the role of diallyl sulfides and dipropyl sulfides in the *in-vitro* antimicrobial activity of the essential oil of garlic, *Allium sativum* L., and *leek, Allium porrum* L. The results revealed that *A. sativum* (garlic) EO showed a good antimicrobial activity against *S. aureus* (inhibition zone 14.8 mm), *P. aeruginosa* (inhibition zone 21.1 mm), and *E. coli* (inhibition zone 11.0 mm), whereas the EO of *A. porrum* (leek) had no antimicrobial activity. The main constituents of the garlic EO were diallyl monosulfide, diallyl disulfide (DADS), diallyl trisulfide, and diallyl tetrasulfide. The EO of *A. porrum* was characterized by the presence of dipropyl disulfide (DPDS), dipropyl trisulfide, and dipropyl tetrasulfide. The antimicrobial activities of the DADS and DPDS were also studied. The results obtained suggest that the presence of the allyl group is fundamental for the antimicrobial activity of these sulfide derivatives when they are present in *Allium* or in other species (DADS inhibition zone on *S. aureus* 15.9 mm, *P. aeruginosa* 21.9 mm, *E. coli* 11.4 mm).
- In 2012, **Pokhrel *et al*** made comparison of antimicrobial activity of crude ethanolic extracts and essential oils of spices against five strains of diarrhoea causing *E. coli.* Crude ethanolic extracts and essential oils of 5 spices including *coriander, Ginger, Turmeric, Cloves and garlic*, were examined for their antibacterial activity against 5 strains of diarrhoea causing *E. coli* (including within of it Enteropathogenic *E. coli* "EPEC") using disk diffusion methods. The results revealed that the *Allium sativum* EO had antibacterial activity against Enteropathogenic *E. coli* with inhibition zone of 10.00

mm/15µl/disk. The crude ethanolic extracts showed narrower antibacterial activity (20µl/disk) only cloves showed the highest inhibitory effect in the both case while coriander, ginger and turmeric showed no inhibitory effect in the case of crude ethanolic extracts.

- In 2012, **Karuppiah & Rajaram** evaluated the antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens (submitted by patients having suspected urinary tract and pus infections with multi-drug resistant *S. aureus*, *E. coli*, *Klebsiella sp* & *P. aeruginosa*). The results show that anti-bacterial potentials of the extracts of two crude garlic cloves and ginger rhizomes were tested against five gram negative and two gram positive multi-drug resistant bacteria isolates. All the bacterial isolates were susceptible to crude ethanolic extracts of both plants extracts. Except *Enterobacter sp.* and *Klebsiella sp* at concentration of 0.2 mg/ml with inhibition zone of 13.55, 15.50 & 14.50 mm/10µl/disk respectively, all other isolates were susceptible when subjected to ethanolic extracts of garlic and ginger. The highest inhibition zone was observed with garlic (19.45 mm) *against P. aeruginosa*. The minimal inhibitory concentration was 0.0789, 0.0655, 1.6020 & 0.0585 mg/ml against *S. aureus*, *E. coli*, *K. pneumoniae* & *P. aeruginosa*.
- In 2012, **Meriga *et al*** evaluate the insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum* (*A. sativum*). Dried bulbs of *Allium sativum* were extracted with different solvents and evaluated for insecticidal, antimicrobial and antioxidant activities. Aqueous and methanol extracts showed highest insecticidal activity (mortality rate of 81% and 64% respectively) against the larvae of *Spodoptera litura* (*S. litura*) at a concentration of 1000 ppm. With regard to antimicrobial activity, aqueous extract exhibited antibacterial activity against gram positive (*B. subtilis*, *S. aureus*) and gram negative (*E. coli* and *K. pneumoniae*) strains and antifungal activity against *C. albicans*. While methanol extract showed antimicrobial activity against all the tested microorganisms except two (*S. aureus* and *C. albicans*), the extracts of hexane, chloroform and ethyl acetate did not show any anti microbial activity. Minimum inhibitory concentration of aqueous and methanol extracts against tested bacterial and fungal strains was 100–150

μg/mL. Antioxidant activity of the bulb extracts was evaluated in terms of inhibition of free radicals by 2,2′-diphenly-1-picrylhydrazyl. Aqueous and methanol extracts exhibited strong antioxidant activity (80%–90% of the standard).

- In 2012, **Gull *et al*** investigated the inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. three types of extracts of each *Allium sativum* and *Zingiber officinale* including aqueous, methanol & ethanol extracts had been assayed separately against drug resistant bacteria including within of it *E. coli*, *P. aeruginosa*, *K. pneumoniae* & *S. aureus*. The antibacterial activity was determined by disc diffusion method. the results revaled that all tested strains were susceptible to garlic aqueous, methanol and ethanol extract but most effective was garlic aqueous extract, and showed poor susceptibility to the ginger aqueous extract. The MIC of different bacterial species varied from 0.05 mg/ml to 1.0 mg/ml. Which MIC for aquatic extract against E. coli, *P. aeruginosa*, *K. pneumoniae* & *S. aureus* was 0.1mg/ml, 0.09mg/ml, 0.2 mg/ml & 0.2 mg/ml respectively. While MIC for ethanolic extract against E. coli, *P. aeruginosa*, *K. pneumoniae* & *S. aureus* was 0.3mg/ml, 0.55mg/ml, 0.25 mg/ml & 0.83 mg/ml respectively. While MIC for methanolic extract against *E. coli*, *P. aeruginosa*, *K .pneumoniae* & *S. aureus* was 0.2mg/ml, 0.57mg/ml, 0.82 mg/ml & 1.00 mg/ml respectively.
- In 2010, Palaksha ***et al*** investigate the Antibacterial activity of garlic extract on streptomycin-resistant *S. aureus* and *E. colis* olely and in synergism with streptomycin. Gram-positive *S. aureus* and gram-negative *E. coli* were made resistant to standard antibiotic streptomycin used as a control in the experiment. Zones of inhibition of different treatment groups were measured by agar-well-diffusion assay and compared with control. The findings of this study reveal the distinct antibacterial profile of *Allium sativum* Linn. solely and in streptomycin synergism against streptomycin-resistant *S. aureus* and *E. coli* as witnessed from prominent zones of inhibition. Which the aquatic extract of garlic shown antibacterial activity against streptomycin-resistant *S. aureus* and *E. coli* with inhibition zone diameter of 14.00±0.58 & 14.50±0.29 mm (100μl/50%/well).

- In 2010, **Chandra *et al*** investigate the Antibacterial Activity of *Allium sativum* (L.) Against Bacteria Isolated from Upper Respiratory Tract. *In-vitro* antibacterial assay was investigated using aqueous extract of *Allium sativum* (L.) on certain bacterial species identified as *Staphylococcus* sp.(26.26%), *Micrococcus* sp. (25%), *Streptococcus* sp.(23%), *Klebsiella* sp.(18%) *Corynebacterium* sp. (15%) *Proteus* sp. (10%) and *E. coli* (8%) isolated from the samples collected from upper respiratory tract. The result of antimicrobial assay showed the zone of Inhibition (mm) at Concentrations of 100mg/ml of the aqueous extract of *A. sativum* was 37.6, 34.00 & 21.00 mm/100µl/well against *Staphylococcus* sp, *E. coli &* *Klebsiella* sp respectively. The Minimum Inhibitory Concentration (MIC) of the aqueous extract of *A. sativum* up to 12.5 mg/mL. However, the higher dose (100 mg/mL) was found potentially effective to control all the isolated bacterial population. The extent of affectivity was observed maximum on the *Micrococcus* sp. with a value 40.3 ± 0.3 mm and lowest on *Corynebacterium* sp. with a value 14.2 ± 1.2 mm.
- In 2009, **Abubakar** investigated the Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *E. coli*, *S. aureus*, *Streptococcus pneumoniea* and *P. aeruginosa*. The effects of water, ethanol and chloroform extracts of garlic against the nosocomial *S. aureus*, *E. coli*, *S. pneumoniea* and *P. aeruginosa* were investigated. At a concentration of 50 mg/ml, all the crude extracts inhibited the growth of the pathogenic bacteria, though with varying degrees of susceptibility. In this study, *S. aureus* (NSA1) was most susceptible to the active principles present in garlic, closely followed by *S. pneumoniae*. The results show that *S. aureus* had a zone of growth inhibition diameter of 28 mm in water, 24 mm in ethanol and 23 mm in chloroform extracts, while *S. pneumoniae* had a zone of growth of inhibition diameter of 23 mmin in water, 21 mm in ethanol and 19 mm in chloroform extracts. These bacteria may lack some alternative biochemical pathways, which cannot be affected by crude extract of the garlic. *E. coli* is less susceptible, with diameters of 20 mm in water, 18 mm in ethanol and 16 mm in chloroform extracts. *P. aeroginosa* was least susceptible of all the test bacteria used, with growth inhibition diameters of 10 mm in water, 8 mm in ethanol and 7 mm in chloroform extracts. The

MIC/MBC values of the aqueous extract against *S. aureus* was 50/75 mg/ml; *S. pneumoneae*, 75/100 mg/ml; *E. coli*, 100/125 mg/ml and *P. aeruginosa*, 125/150 mg/ml. The MIC/MBC values of the ethanolic extract against *S. aureus* was 75/100 mg/ml; *S. pneumoneae*, 100/125 mg/ml; *E. coli*, 125/150 mg/ml and *P. aeruginosa*, 150/175 mg/ml. The water extract was more potent than the organic extracts, and all were inferior in activity, when compared to the standard antibiotic, metronidazole. The gram positive *S. aureus* was more susceptible to the toxic effects of garlic than its gram-negative counterparts. The results obtained in this study indicate that water extracts of garlic can be used alongside conventional antibiotics to fight agents of nosocomial infections that are so prevalent in our hospitals.

2.3.4. *Ecballium elaterium*:

- In 2012, **Sasmakov *et al*** investigated the *In vitro* screening of the cytotoxic, antibacterial and antioxidant activities of some Uzbek plants used in folk medicine. The antimicrobial activity of the studied plant extracts was estimated for antibacterial activity against both Gram-positive bacteria *S. aureus* and *B. subtilis,* and Gram-negative bacteria *E. coli* & against *Candida maltosa*. The methanolic extracts of *Ecballium elaterium* showed moderate antibacterial activity against *S. aureus* with inhibition zone diameter of 8.00 mm/Forty microliters of test material (equivalent to 2 mg of the dried extract or 0.2 mg individual substances, dissolved in the same solvent used for extraction, was applied on sterile paper discs). While the *B. subtilis, E. coli* & *Candida maltosa* was not sensitive against methanolic extracts of *Ecballium elaterium*.
- In 2011, **Adwan *et al*** evaluated the antimicrobial effect of ethanolic extract of *Ecballium elaterium* fruits alone against *S. aureus* strains and *C. albicans* strains, or in combination with penicillin against *S. areus* strains. against *S. aureus* and *C. albicans*. Evaluation of the antimicrobial activity or synergy interaction was carried out using microdilution method. The results showed that ethanolic extract of *E. elaterium* fruits has antimicrobial activity against MRSA, MSSA and *C. albicans*. This extract showed a significant decrease in minimum inhibitory concentrations of penicillin against both MRSA and MSSA strains. MRSA, MSSA and *C. albicans* are susceptible organisms to

ethanolic extract of *E. elaterium* fruits. The MIC values of ethanolic extract of *E. elaterium* fruits alone against *S. aureus* were in the range of 0.195-1.563 mg/mL including *S. aureus* ATCC 25923 as a reference strain. The MIC values of penicillin alone against *S. aureus* were in the range of 0.195 to more than 100 U/mL. In case of combination between penicillin and *E. elaterium* ethanolic extract, 1/8-1/64 (0.024 mg/mL) of *E. elaterium* ethanolic extract MIC reversed the high level resistance of MRSA to penicillin.

- In 2010, **Koca *et al*** evaluated the Comparative in vitro activity of medicinal plants Arnebia densiflora and *Ecballium elaterium* aginst isolated strains of *K. pneumonia*. *In-vitro* activity of the extracts of medicinal plants *Ecballium elaterium* and *Arnebia densiflora*, which is endemic to Turkey, was screened against isolated strains of *K. pneumoniae* that were resistant in disc diffusion test (trimethoprime-sulfamethoxazol, sulbactam-ampicillin, clavulonat-amoxicillin, ceftriaxone, cefepime, imipenem, ceftazidime, tobramycin, gentamicin, ofloxacin, ciprofloxacin). Broth microdilution susceptibility testing was performed according to the Clinical and laboratory Standards Institute. The extracts of *A.densfilora* root, bark and E.*elaterium* leaf, stem, and fruits amples were analyzed against the strains of *K. pneumoniae* at the concentrations range from 128 to 0.0312 µg/ml.This is the first report shows that the extracts of *A.densiflora* and *E .elaterium* are effective as much as the antibiotics amoxicillin and ofloxacin against some of the isolated strains of *K. pneumoniae* (K2,K3,K5,K& and K10) at the concentration of 32 µg/mL. The extracts were active at the concentration of 64µg/mL onto rest of the strains, which are close to the effective dose of controls as well. However, the activities of all the extracts against standard control strain of *K. pneumoniae* (RSKK574) were comparatively better (8 µg/ml) than that of the tested isolated strains of *K. pneumoniae,* in which the activities varied between 32 to 64µg/ml.
- In 2009, **Oskay** *et al* evaluated the antibacterial activities of ethanolic extracts of 19 plant species were studied against multi-drug resistant clinical isolates using agar well diffusion method. Extracts of *Liquidambar orientalis, Vitis vinifera, Rosmarinus officinalis, Punica granatum, Cornus sanguinea, Euphorbia peplus, Ecballium elaterium, Inula viscosa and Liquidambar*

orientalis showed broad-spectrum antibacterial activity with inhibition zones ranging from 8 to 26 mm. The most resistant organisms were *E.* coli (*E. coli*), *Stenotrophomonas maltophilia* and *K. pneumoniae*, and the most susceptible species were *S. aureus* (Penicillin G- and oxacillin-resistant), *Streptococcus pyogenes* and *P. aeruginosa*, Ethanolic extract of *E. elaterium* fruits had antibacterial inhibitory activity against multi-drug resistant *S. aureus*, *E. coli*, *K. pneumoniae* & *P. aeruginosa* with inhibition zones of 14.00, 14.00, 8.00 & 20.00 mm/100µl/well of 4.0mg/ml stock solution respectively.

- In 2008, **Dogruoz *et al*** evaluated the antibacterial activity of some plant extracts**.** the tested plant extracts including within of it the aqueous extract of *Ecballium elaterium*, which estimated for their antibacterial activity by using agar well diffusion at concenration of 0.001mg/ml against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and other bactrial strains. The results revealed that, the aqueous extract of *Ecballium elaterium* was not active against all tested strains at this concenration.
- In 2007, **Oskay & Sarı** evaluated the Antimicrobial Screening of Some Turkish Medicinal Plants. Ethanol extracts of 19 Turkish medicinal plants, used in the traditional system of medicine, were investigated for their antimicrobial activity against 14 pathogenic bacterial species and a yeast, *Candida albicans*, using the agar well diffusion method. Anticandidal activity was detected in 10 plant extracts. Extracts including within of it *Rosmarinus officinalis* (leaves) & *Ecballium elaterium* (leaves, fruits; 2.1, v/v) which showed broad-spectrum antimicrobial activity with inhibition zones ranging from 4 to 34 mm/100µl/well of extract concentration (4mg/mL). Which the inhibition zones of *Rosmarinus officinalis* & *Ecballium elaterium* ethanolic extracts was of (20 & 10) & (10 & 20) mm against MRSA & *E. coli* respectiely. The minimum inhibitory concentration and minimal bactericidal concentration were determined for the seven highly active plants that showed antimicrobial activity against MRSA, *E. coli*, and *C. albicans*. The MICs of active extracts ranged from 8 to 14.2mg/mL while the MBCs was 14.2 to 24.4mg/mL**,** where the MIC/MBC of *Rosmarinus officinalis* ethanolic extracts was 9.4 / 18.2 mg/ml against MRSA, while MIC/MBC against *E. coli* was not determined at this concentration 4mg/ml. The MIC/MBC of *Ecballium*

elaterium ethanolic extracts was not not determined at this concentration (4mg/ml) against MRSA, while MIC/MBC against *E.coli* was 11.8 / 23.6 mg/ml.

2.3.5. *Ruta-graveolens*:

- In 2015, **Al-Shuneigat *et al*** investigated the The Chemical Composition and the Antibacterial Properties of *Ruta graveolens* L. Essential Oil grown in northern jordan. The chemical composition analysis was conducted using Gas Chromatography/Mass Spectrometry (GC-MS). The essential oil was isolated using hydrodistillation from the aerial parts of *Ruta graveolens* L. Six clinical isolates of bacteria were used in the present study; three strains of Gram-positive bacteria (*Methicillin-resistant S. aureus, S. epidermidis, and B. subtilis)* and three strains of Gram-negative bacteria (*E. coli, Enterobacter aerogenes, and P. aeruginosa*). The antibacterial activity was evaluated using a disc diffusion method (Sterile paper discs of 6 mm diameter were impregnated with 10 µL of essential oil and deposited on the agar surface). Thirty components, accounting for 98.1% of the oil, were identified. Ketones (43.02%), aldehydes (37.12%), esters (9.33%) and sesquiterpene hydrocarbons (5.22%), were the major constituents. The major compounds identified were 2-nonanone (37.13%), undecanal (34.69%), 2-acetoxydodecane (5.0%), and 2-decanone (3.31%). The results showed that the essential oil has a very potent activity against *MRSA, E. coli, P. aeruginosa, S. epidermidis, B. subtilis* & *E. aerogenes* with inhibition zone of 20, 14, 10, 22, 28 & 26 mm respectively. Overall, the essential oil of *Ruta graveolens* was more active against Gram-positive than Gram-negative bacteria.
- In 2015, **Orlanda & Nascimento** investigated the Chemical composition and antibacterial activity o*f Ruta graveolens* L. (Rutaceae) volatile oils (from frish leaves), from São Luís, Maranhão, Brazil. The EO was estimated for its antibacterial activity against of 8 different types of bacteria including within of i*t S. aureus, E. coli & P. aeruginosa* bacteria. The results revealed that, th*e Ruta graveolens* EO at concentration 75 µg/ml (10 µl/disk) had the highest antibacterial activity agains*t S. aureus*, with 22.00 ± 0.06 mm zone of inhibition & MIC of 1.00 ± 0.04 µg/ml, and the lowest antibacterial activity was observed agains*t P. aeruginosa* with 8.00 ± 0.05mm zone of inhibition &

MIC of 75.00 ± 0.09 µg/ml, and the moderate antibacterial activity was observed against *E. coli* with 17.70 ± 0.04 mm zone of inhibition & MIC of 1.00 ± 0.04 µg/ml

- In 2013, **Haddouchi *et al*** evaluated the Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria. These species including *Ruta angustifolia, Ruta chalepensis, Ruta graveolens and Ruta tuberculata*, against 12 bacteria including *S. aureus*, *E. coli, P. aeruginosa* & *K. pneumoniea*. The results obtained indicated that the essential oils of the *Ruta graveolens* when applied at 10 µl/disc had low *in-vitro* potential of antibacterial activity against all 12 bacteria tested with inhibition zone of 12, 7, 6 & 6 mm in diameter against *S. aureus*, *E. coli*, *K. pneumoniea* & *P. aeruginosa* respectively.
- In 2012, **jalali Moghadam *et al*** investigated the antimicrobial effects of hydro and hydroalcoholic (ethanol 70%) extracts of *Ruta graveolens* on 10 pathogenic bacteria. Standard strains of *Enterococcus faecalis, S. aureus, S. epidermidis, Strptococcus pneumoniae, Streptococcus pyogenesis, E. coli, K. pneumonia, S. typhi, seratia marcescens,* and *P. aerogenesis,* are used in this study. Effect of hydro and hydroalcholic extracts of this plant on growth of mentioned bacteria is determined by using disc diffusion method and by serial macrodilution for measuring MIC in comparison to effects of 11 common antibiotics on the same bacteria. In this study, Hydro and hydroalcoholic extracts of *Ruta graveolens* did not show inhibitory effect on growth of studied bacteria up to concentration 5mg/ml. It seems that lacking antibacterial effect of the herb extracts on the studied bacteria is due to resistant character of the bacteria and lacking of antibacterial continents in the extracts of the plant.
- In 2005, **Ivanova *et al*** evaluated the Antimicrobial and cytotoxic activity of *Ruta graveolens* at concentration of 0.5 mg/disk against 8 bacteria and fungi including within of it Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. The results obtained indicated that the *E. coli* was not sensetive to the *R. graveolens* methanolic extract at this concentration (diameter of the inhibitory zone less than 10 mm means absence of activity), while *S. aureus* was inhibited with inhibition zone 23.3 mm at concentration of 0.5 mg/disk.

- In 2000, **Ojala *et al*** evaluated the Antimicrobial activity of some coumarin containing herbal plants growing in Finland, against selected Gram-positive (*S. aureus*) and Gram-negative (*E. coli & P. aeruginosa*) bacteria, yeasts, mold, as well as plant pathogenic fungi, with emphasis on method optimization was carried out on methanol extracts prepared from seven plants including *Ruta graveolens* . The results obtained indicated that the Sensitivity to the extracts was found to vary considerably among the microorganisms, in which the antimicrobial activity of the leaf extracts when added as crude extracts of 0.126 mg/ml revealed no antimicrobial activity against *E. coli* (inhibition zone of sample < inhibition zone of methanol +1 mm), and revealed moderate antimicrobial activity against *S. aureus & P. aeruginosa* (I.Z. of sample 1–4 mm > I.Z. of methanol). *P. crispum* and *R. graveolens* affected the widest spectrum of microbes.
- In 1997, **Valsaraj *et al*** evaluated the Antimicrobial screening of selected medicinal plants from India. Which the antibacterial and antifungal screening of 83 extracts from different organs of the 78 investigated plants (including within of it *R. graveolens*) are screening for there antimicrobial against foure types of bacteria including within of it Gram-positive (*S.aureus*) and Gram-negative (*E. coli & P. aeruginosa*) bacteria, by using by agar diffusion method. The results obtained indicated that the *E. coli* was not sensetive to the *R. graveolens* ethanolic extract, while *S. aureus* & *P. aeruginosa* was inhibited at concentration of 12.5 & 25 mg/ml respectively.

2.3.6. *Phagnalon rupestre*:

- In 1998, **Ali-Shtayeh *et al*** investigated the antimicrobial activities of 20 Palestinian plants against five bacterial species (*S. aureus, E. coli, K. pneumoniae, Proteus vulgaris and P. aeruginosa*) and one yeast (*C. albicans*). The results showed that the most antimicrobial active plants were *Ph. rupestre* and *Micromeria nervosa*. Where the aquatic extracts of *Ph. rupestre* showed agood antibacterial activity against *S. aureus, E. coli, K. pneumoniae, P. aeruginosa* & *Proteus vulgaris*, while ethanolic extracts of *Ph. rupestre* showed agood antibacterial activity against *S. aureus, K. pneumoniae* &

P.vulgaris, while *E. coli* & *P. aeruginosa* was resistant to the *Phagnalon rupestre* ethanolic extract at concentration 200 mg/ml.

2.3.7. Antibacterial and synergistic effect:

- In 2011, **Elbashiti *et al*** evaluated the antibacterial and synergistic effect of some Palestinian plant extracts against *E. coli* and *S. aureus***.** Seven crude extracts from five plants obtained through four different extraction methods (water reflux, ethanol, methanol and ethanol reflux) were screened and tested against *E. coli* and *S. aureus*. Extracts from *Cakile maritima* (roots and shoots), *Cakile maritima* (seeds), *Mesembryanthemum crystallinum* (whole plant), *Atriplex halimus* (leaves), *Withania somnifera* (leaves), *Marrubium vulgare* (stem and leaves) were tested. There was no antibacterial activity in any plant extracts against *E. coli* except for *C. maritima* (seeds) when extracted by ethanol with an inhibition zone = 13 mm. However, antibacterial potentials were observed against *S. aureus* when treated with extracts of *W. somnifera* (leaves) with an inhibition zone = 25 mm, *M. vulgare* (stems) with an inhibition zone = 15 mm and *M. vulgare* (leaves) with an inhibition zone = 13 mm, all of which were extracted by ethanol. The synergistic effect of plant extracts and antibiotics showed promising results against antibiotic-resistant bacteria. The results obtained with *E. coli* were particularly interesting since it was inhibited by antibiotics combined with *C. maritima* (roots, shoots and seeds), *M. crystallinum* (whole plant), *M. vulgare* (stem and leaves) extracts at least in one extraction method (ethanol for 8 h). This inhibition was not observed with the individual plant extracts alone but when they were used with the ineffective antibiotics. Some of the extracts showed a synergistic activity when tested against *S. aureus*. However, when *A. halimus* (leaves) were extracted by water reflux and *C. maritima* (seeds), *W. somnifera* (leaves) and *M. vulgare* (stem) were extracted by methanol for 5 days, they showed no synergistic effect. Overall, the highest synergistic effect was observed when the plant extracts were treated with tetracycline and minocycline against both *E. coli* and *S. aureus*.
- In 2008, **Karmegam *et al*** evaluated the Antibacterial potency and synergistic effect of certain plant extracts against food-borne diarrheagenic bacteria**.** In this study**,** aqueous and ethanolic extracts of leaves of six commonly

available medicinal plants, *Balanites aegyptiaca (L.) Del. (Balanitaceae), Hyptis sauveolens Poit. (Lamiaceae), Lawsonia inermis L. (Lathyraceae),* Leucas aspera L. (Lamiaceae), Lobelia nicotianaefolia Roth. ex. Roem. & Schult. *(Lobeliaceae) and Phyllanthus madraspatana L. (Euphorbiaceae),* individually and in combinations were tested in crude form for their antibacterial activity against five different diarrheagenic bacteria, *Bacillus cereus, S. aureus, E. coli* O157.H7 (*enterohemorrhagic E. coli, EHEC*), *Salmonella enteritidis and Listeria monocytogenes*. *Cifroflaxacine* (20µg) was used as antimicrobial standard. The highest antimicrobial activity was recorded in both crude aqueous leaf extract (CALE) and crude ethanolic leaf extract (CELE) of L. nicotianaefolia when all the extracts were tested individually. *CALE and CELE* of *H. sauveolens* and *P. madraspatana* when tested individually showed least zone of inhibition (IZ) against test organisms (IZ, 0-1.2cm). Synergistic activity of CALE and CELE of selected plant leaves, in combination of two, three, four, five and six against test organisms ranged from 0-2.8 cm zome of inhibition**.** The IZ range of 2.6-2.8 was recorded for standard antibiotic, ciprofloxacin against test organisms.

Chapter 3

Materials and methods

3.1. Materials:

3.1.1. Chemicals and Culture Media:

Four types of media were used for carrying out this study Brain Heart Infusion Agar, Nutrient broth, Muellere Hinton Broth and Mueller-Hinton agar. Also ethanol and methanol used for extraction process. Ampicillin, Cefuroxime, Cefotaxime, Gentamicin, Erythromycin, Clindamycin, 0floxacin, Nalidixic acid, Norfloxacin, Ciprofloxacin , Amoxicillin-clavulanic acid, Ceflexin, Rifampicin & Amikacin used as reference antibiotics.

Table.3.1 list of antibiotic potency:

Antibiotics	**symbol**	**Antibiotics potency**	**Manufactured by**
Cefotaxime	CXM	30 mg	Himedia, Indian & Bioanalyse, Turkey
Cefuroxime	CTX	30 mg	Himedia, Indian & Bioanalyse, Turkey
Cefaclor	CEC, CF	30 mg	Himedia, Indian & Bioanalyse, Turkey
Cefalexin	CL,CN	30 mg	Himedia, Indian & Bioanalyse, Turkey
Ofloxacin	OFX	5 mg	Himedia, Indian & Bioanalyse, Turkey
Ciprofloxacin	CIP	5 mg	Himedia, Indian & Bioanalyse, Turkey
Norfloxacin	NOR	10 mg	Himedia, Indian & Bioanalyse, Turkey
Nalidixic acid	NA	30 mg	Himedia, Indian & Bioanalyse, Turkey
Amikacin	AK	30 µg	Himedia, Indian & Bioanalyse, Turkey
Gentamicin	GMN	10 mg	Himedia, Indian & Bioanalyse, Turkey
Ampicillin	AM	10 mg	Himedia, Indian & Bioanalyse, Turkey
Oxacilin	OX	1 mg	Himedia, Indian & Bioanalyse, Turkey
Amoxyclav	AMC	30 mg	Himedia, Indian & Bioanalyse, Turkey
Rifampicin	RIF	5 mg	Himedia, Indian & Bioanalyse, Turkey
Penicillin G	P	10 mg	Himedia, Indian & Bioanalyse, Turkey
Tetracycline	TE	30 mg	Himedia, Indian & Bioanalyse, Turkey

p- Iodonitrotetrazolium chloride (INT) were used as microbial growth indicator. Dimethyl sulfoxide (DMSO). These media, solvent, antibiotics and microbial growth indicator were purchased from high grade manfacture companies.

3.1.2. Plant Sample Collection:

The plant materials used in this study consisted of ***Allium sativum*** (bulbes)**,** ***Ecballium elaterium*** (fruite)**,** ***Pelargonium graveolens*** (shoots)**,** ***Rosmarinus officinalis*** (shoots)**,** ***Phagnalon rupestre*** (shoots) ***& Ruta graveolens*** (leaf) which are growing in Palestine were collected from khan yunes- Absan alkaberah in October 2014.

Table 3.2. List of medicinal plants used in the antimicrobial & synergistic assay:

Botanical Name	Family	Local Arabic Name	Part Used
Allium sativum	*Alliaceae*	ثومة	**Bulbes**
Ecballium elaterium	*Cucurbitaceae*	الضحاك (فقوس الحمار)	**Fruites**
Pelargonium graveolen	*Geraniaceae*	العطرة	**Shootes**
Rosmarinus officinalis	*Lamiaceae*	أكليل الجبل	**Shootes**
Phagnalon rupestre	*Asteraceae*	عشبة قديح	**Shootes**
Ruta-graveolens	*Rutaceae*	السذاب العطري	**Leafes**

3.1.3. Microorganisms:

The multi-drug resistant strains of *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* were obtained from microbiology department at Al-Shifa hospital, and were maintained on Brain Heart Infusion agar medium slant at 4 ºC until testing.

3.2. Methods:

3.2.1. Preparation of plant extract:

3.2.1.1. Preparation of aquatic extract:

A modification of previously described procedures (Boukhris *et al*., 2013) was followed to prepare the aquatic extracts of the plants as follows. The dried materials (20 g) were subjected to 150 ml solvent extraction water for 8 h in a Soxhlet extractor. The extracts were concentrated under reduced pressure (rotary evaporator) then preserved at 4 ºC in glass in the dark until its use.

3.2.1.2. Preparation of Methanolic Extract:

Extraction of plant secondary metabolite of selected plant parts was done by Soxhlet extraction method. Thirty gram of finely ground plant part powder was placed in porous bag made of muslin cloth, which was loaded into the main chamber of the Soxhlet extractor. The extraction was carried out with methanol as extraction solvent in 1.10 powder to solvent ratio at temperature 65 °C for 8 h, and then the extract was filtered and allowed to evaporate in open air. The dried extract is dissolved in 10% Dimethyl sulfoxide (DMSO) and stored in refrigerator until used (Chaudharia & Mahajanb., 2015; Albayrak, *et al.*, 2010).

3.2.1.3. Preparation of Ethanolic extract:

A modification of previously described procedures (Jameela *et al.*, 2011) was followed to prepare the ethanolic extracts of the plants as follows. 20 gram of each plant parts were extracted separately with 150 ml of ethanol solvent for 8 h by using soxhlet equipment. The solvent was made to evaporate in oven at 37ºC for three dayes. It was then collected and stored in airtight bottles for further microbiological assays**.**

3.2.1.4. Extraction of the Essential oils:

Essential oil was extracted from 500 g of fresh and cleaned parts of the secreened plants, by steam distillation method. In which, water was heated to produce steam that pass on the herb material to carry the most volatile chemicals. Following by cooling and condensation of the vapor mixture and the resulting distillate was collected and separated from the layer of water by extraction (The aqueous phase was extracted with chlorophorm (20 / 100 ml) and dried with anhydrous sodium sulphate), and stored in sealed glass vials at 4 - 5 ºC prior to testing (Pokhrel *et al*., 2012; Hsouna & Hamdi., 2012; Abu-Al-Basal., 2010 & Tsao & Yin., 2001).

3.2.2. Preparation of stock and test solutions:

A modification of previously described procedures (Mabrouk., 2012) was followed to prepare the stock and test solutions of the plant extracts. By using digital electronic balance, one gram of each aqueous, ethanolic & methanolic extracts & 1ml of essential oils was carefully taken in a standard measuring cylinder and 3 mL of Dimethyl sulfoxide (DMSO) was added to dissolve the aqueous, ethanolic, methanolic & essential oils extracts. Then each extract was made up to 5 mL by adding Dimethyl sulfoxide (DMSO) and stored under refrigerated (4°C) condition till use. This formed the stock solution of 200 mg/ml**.**

3.2.3. Standardization of inoculums:

Each culture was activated by transferring a loopful from Brain Heart Infusion (BHI) slants into sterile nutrient broth (10 ml) followed by incubation at 37 °C for 24 h. The optical density of each active culture was adjusted to 0.1 at 625 nm using fresh broth to give a standard inoculums of 10^6 colony forming units (cfu) per ml (Alzoreky & Nakahara., 2003).

3.2.4. The antibiotic sensitivity assay:

The antibiotic sensitivity of the isolates was determined using the disc diffusion method. Standardized inoculum (100µl) of the overnight grown nutrient broth cultures were spread on Mueller-Hinton agar plates using sterile swabs. The plates were dried at room temperature for 10 min, before placing the antibiotic discs at equidistance. The plates were incubated for 24 h at 37°C and the diameter of zone of inhibition was measured (Mabrouk, 2012; Kirbag *et al*, 2009; Sockett, 2006). Organisms were

classified as sensitive, intermediate or resistant, based on the NCCLS standards. A total of 15 antibiotics were used in this study as shown in Table 3.1.

3.2.5. Antimicrobial Activity Assays:

3.2.5.1. Evaluation of antibacterial activity of plant extracts by disc diffusion method:

A modification of previously described procedures (Gupta., *et al*, 2014; Casella *et al.*, 2013) was followed to evaluate of antibacterial activity of plant extracts. Standardized inoculums of each bacterium, i.e., 10^6 CFU (Colony Forming Units)/ml to 0.1 at 625 nm was introduced onto the surface of sterile Muller-Hinton (MH) agar plates and a sterile cotton swab was used for even distribution of inoculums. After a few minutes, sterile filter paper discs of 6 mm diameter were placed on the surface of inoculated and labeled MH agar plates and impregnated with 50 µL of known concentration of extracts (200 mg /ml) for aquatic, ethanolic, methanolic extracts & essential oils). Sterile paper discs containing Dimethyl sulfoxide alone was served as negative control. The plates were placed at 4ºC for 2 h. and then subsequently incubated at 37º C for 24 H. After incubation, the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. For each test solution, three replicates were maintained**.**

3.2.5.2. Determination of MIC and MBC of plant extracts by Microdilution Method:

A modification of previously described procedures (Cheraif *et al*, 2007) was followed to determinate of MIC and MBC of plant extracts. A broth microdilution method was used to determine the MIC and MBC. All tests were performed in Mueller Hinton Broth (MHB). The plant extracts were dissolved in dimethylsulphoxide (DMSO). Two fold dilution series was prepared to achieve a decreasing concentration ranging from 200 to 0.390 mg/ml of each extract, which was prepared in a 96-well microtiter plate. Overnight broth cultures of each strain were prepared and the plates were incubated at 37 ºC for 24 h. The MIC was defined as the lowest concentration of the plant extract at which the bacteria does not show visible growth. To determine MBC, broth was taken from each well and inoculated in Mueller Hinton agar for 24 h at 37 ºC. The MBC is defined as the lowest concentration of the plant extract at which

inoculated bacteria was totally killed. Amikacin and 10% DMSO solution served as positive and negative controls, respectively.

3.2.6. Evaluation of the synergistic effect:

A modification of previously described procedures (Elbashiti *et al*, 2011) was followed to evaluate of the synergistic effect. The bacterial cultures were grown in sterile nutrient broth medium at 37º C. After 4 h of growth, standardized inoculums of each bacterium, i.e., 10^6 CFU /ml to 0.1 at 625 nm was introduced onto the surface of sterile Muller-Hinton agar (MHA) plates and a sterile cotton swab was used for even distribution of inoculums. After a few minutes, the antibiotic filter paper disk of 6 mm in diameter were placed on the surface of inoculated and labeled MH agar plates and impregnated with 50 µL of known concentration of extracts (200 mg /ml for aquatic, ethanolic, methanolic extracts & 200 µl/ml for essential oils). The plates were incubated at 37º C for 24 h. The diameters of cleared zones were measured and compared with that of the antibiotic alone.

3.3. Statistical Analysis:

All data were expressed as the mean ± standard deviation (SD) by measuring three independent replicates. One-way analysis of variance (SAS, 1990; ANOVA procedure) followed by Duncan's test was performed to compare means and to test the significance of differences between means obtained among the treatments at $p < 0.05$ level of significance using SPSS 18 software.

Chapter 4

Results

4.1. Evaluation of antibiotics activity:

The results of antibacterial susceptibility testing represented in table 4.1 showed that all the bacterial pathogens, *S. aureus* (Figure 4.1.a&b), *Escherichia coli* (Figure 4.2.a&b), *K. pneumoniae* (Figure 4.3.a&b), *P. aeruginosa* (Figure 4.4.a&b) were highly resistant to many antibiotics including. Ciprofloxacin (CIP), Ampicillin (AM), Cefotaxime (CTX), Nalidixic acid (NA), Norofloxacin (NOR), Cefuroxime (CXM), Cefaclor (CF or CEC), Ofloxacin (OFX), Cefalexin (CL or CN), Tetracycline (TE), Rifampicin (RIF), Amoxyclav (AMC), Gentamycin (GMN), Penicillin (P) and Oxacillin (OX), while Amikacin (AK) was used as positive control agent (Table 4.1).

(Table 4.1). Effect of antibiotic reference standard on pathogenic bacteria (inhibition zone expressed by mm):

Antibiotic / Bacteria	CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN	P	OX	AK
E. coli	0	0	0	0	0	0	0	0	0	9	8	0	11	*	*	20
K.pneumonia	0	0	0	0	0	0	0	0	0	10	8	0	*	*	*	19
P.aeruginosa	0	0	0	0	0	0	0	0	0	8	8	7	*	*	*	21
S. aureus	0	0	0	*	0	0	0	0	0	*	*	0	*	7	0	22

*. not tested, mm. millimeter.

Antibiotic susceptibility against tested bacteria.

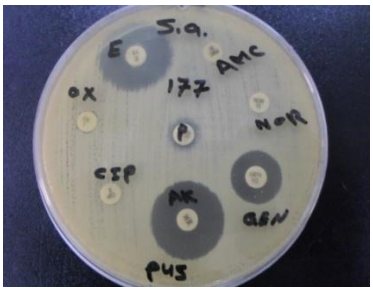


Figure 4.1.a Against *S. aureus*

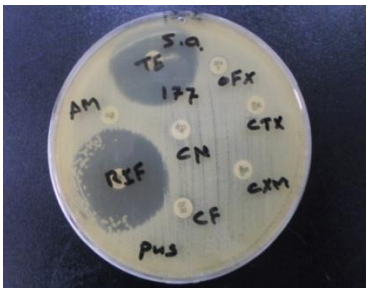


Figure 4.1.b Against *S.* *aureus*

Figure 4.2.a. Against *E. coli*

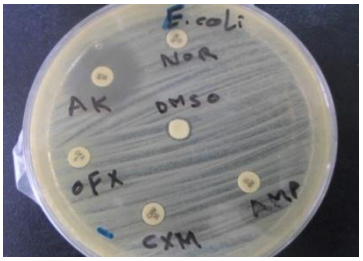


Figure 4.2.b. Against *E. coli*

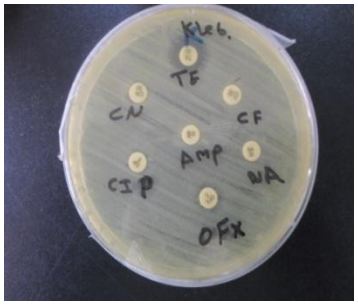


Figure 4.3.a. Against *K. pneumoniae*

Figure 4.3.b. Against *K. pneamoniae*

Figure 4.4.a. Against *P. aeruginosa*

Figure 4.4.b. Against *P. aeruginosa*

4.2. Evaluation of antibacterial activity of plant extracts:

The results of antibacterial activity of Aquatic extracts, Ethanolic extracts, Methanolic extracts & Essential oils of all the six plants when tested individually for their antibacterial activity against the four isolated bacterial species, which are known to cause infection in humans, are shown in Table 4. 2 - 4.5

4.2.1. Antibacterial activity of plant extracts against *Staphylococcus aureus*:

The disc diameters of zone of inhibition of plants extracts against *S. aureus* are shown in **table 4.2**.

4.2.1.1. The aquatic extracts:

The Aquatic extracts of all the plants secreened showed various inhibitory effects ranged between 8.33- 12.66 mm in diameter against *S. aureus*. The largest zone of inhibition were observed from the *Rosmarinus officinalis* with inhibition zone diameter of 12.66 mm (Figure 4.8), followed by *Pelargonium graveolen* (Figure 4.7), *Ecballium elaterium* (Figure 4.6), *Allium sativum* (Figure 4.5) & *Ruta graveolens* (Figure 4.10) with inhibition zone diameter of 10.66, 10.00, 10.00 &9.66 mm respectively. The lowest activity was the *Phagnalon rupestre* with inhibition zone diameter of 8.33mm (Figure 4.9).

4.2.1.2. The ethanolic extracts:

The ethanolic extract of *Rosmarinus officinalis* were the most effective extract against *S. aureus* which it is showing the highest antibacterial activity against this bacteria with inhibition zone diameter of 13.33mm (Figure 4.8), followed strictly were extracts of *Pelargonium graveolen* (Figure 4.7), *Ruta graveolens* (Figure 4.10), *Ecballium elaterium* (Figure 4.6) & *Allium sativum* (Figure 4.5) with inhibition zone diameter of 13.00, 11.66, 11.33 &11.66 mm respectively. The least effective were ethanolic extract of *Phagnalon rupestre* with inhibition zone diameter of 10.66 mm (Figure 4.9).

4.2.1.3. The methanolic extracts:

The methanolic extract of the screened plants that extracted with a soxhlet extractor for 8hr. showed various degree of antibacterial activity against *S. aureus* ranged between 8.33 - 14.66 mm. The highest antibacterial activity were observed for *Rosmarinus officinalis* with inhibition zone diameter of 14.66 mm (Figure 4.8) followed by *Allium sativum* (Figure 4.5), *Pelargonium graveolen* (Figure 4.7),

Ecballium elaterium (Figure 4.6) & *Phagnalon rupestre* (Figure 4.9) with inhibition zone diameter of 12.66, 11.00, 10.66 &9.66 mm respectively. The lowest antibacterial effect was for *Ruta graveolens* with inhibition zone diameter of 8.33 mm (Figure 4.10).

Table 4.2. Antibacterial Effect of different plant Extracts Against *S. aureus* in mm:

		Group	No.	Inhibition Zones	± Standard Deviation	Factor	Sig.
Staphylococcus aureus	**Aquatic**	**A**	3	10.00	1.00	**2.549**	**0.086**
		B	3	10.00	1.00		
		C	3	10.66	1.15		
		D	3	12.66	2.08		
		E	3	8.33	0.57		
		F	3	9.66	2.52		
	Ethanolic	**A**	3	11.66	0.57	**1.196**	**0.368**
		B	3	11.33	1.53		
		C	3	13.00	1.73		
		D	3	13.33	2.31		
		E	3	10.66	0.57		
		F	3	11.66	2.08		
	Methanolic	**A**	3	12.66	0.57	**2.941**	**0.058**
		B	3	10.66	1.15		
		C	3	11.00	1.73		
		D	3	14.66	4.62		
		E	3	9.66	1.53		
		F	3	8.33	1.53		
	Essential oil	**A**	3	12.66	0.57	**3.116**	**0.050**
		B	3	9.00	1.73		
		C	3	12.33	2.52		
		D	3	11.33	1.53		
		E	3	10.00	2.00		
		F	3	8.33	1.53		

(**A.** ***Allium sativum,*** **B.** ***Ecballium elaterium,*** **C.** ***Pelargonium graveolen,*** **D.** ***Rosmarinus officinalis,*** **E:*Phagnalon rupestre* &** **F.** ***Ruta-graveolens*****).**

Antibacterial activity of plant extracts against *S. aureus*

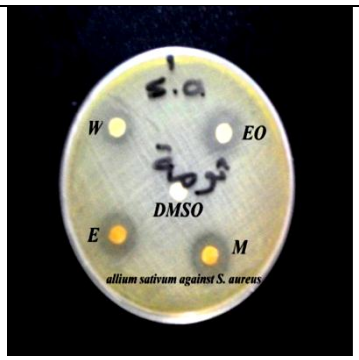


Figure 4.5. ***Allium sativum extracts against S. aureus***

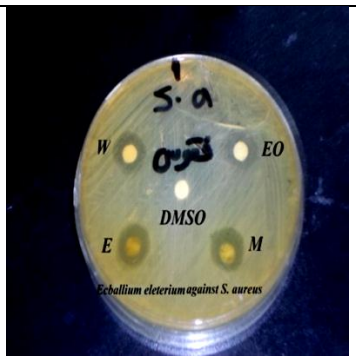


Figure 4.6. ***Ecballium elaterium extracts against S. aureus***

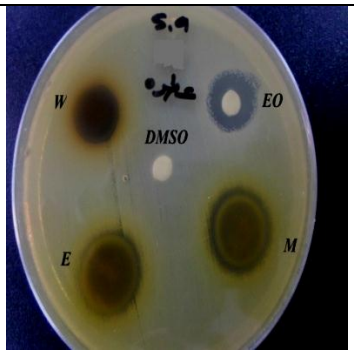


Figure 4.7. ***Pelargonium graveolen extracts against S. aureus***

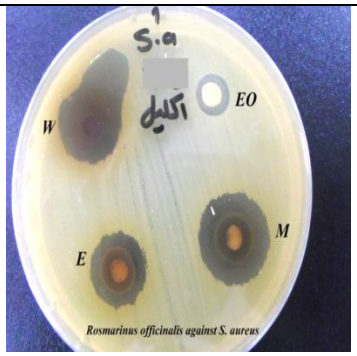


Figure 4.8. ***Rosmarinus officinalis extracts against S. aureus***

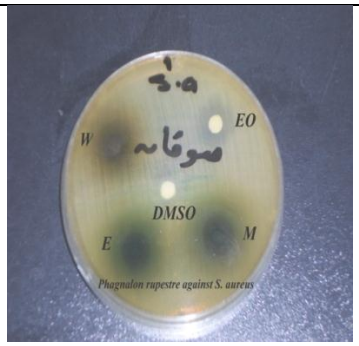


Figure 4.9. ***Phagnalon rupestre extracts against S. aureus***

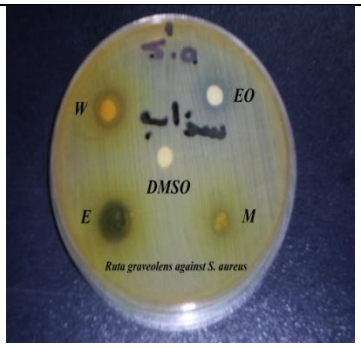


Figure 4.10. ***Ruta graveolen extracts against S. aureus***

W: Aquatic, E: Ethanolic, M: Methanolic & EO: Essential oil

4.2.1.4. The essential oils:

The EOs of the tested plants gives various degree of antibacterial activity (8.33- 12.66 mm/50µl EO) against *S. aureus*. The extract of *Allium sativum* presented the highest antibacterial activity with inhibition zone diameter of 12.66 mm (Figure 4.5), followed by *Pelargonium graveolen* (Figure 4.7), *Rosmarinus officinalis* (Figure 4.8), *Phagnalon rupestre* (Figure 4.9) & *Ecballium elaterium* (Figure 4.6) which have an

intermediate activity against *S. aureus* with inhibition zone diameter of 12.33, 11.33, 10.00& 9.00 mm. The lowest activity was measured in *Ruta graveolens* with inhibition zone diameter of 8.33mm (Figure 4.10).

4.2.2. Antibacterial activity of plant extracts against *Escherichia coli*:

The antibacterial activities of the extracts obtained from the plants under study by the diffusion method against *Escherichia coli* are summarized in Table 4.3.

4.2.2.1. The aquatic extracts:

The results obtained from the disk diffusion method, indicated that the aquatic extracts of the tested plants exhibited antibacterial activity against *E. coli* at concentration 50µl/disk of 200 mg/ml. As can be seen from Table 4.3 the aquatic extract of *Pelargonium graveolens* was the most effective against *E. coli* with inhibition zone of 14.00 mm (Figure 4.13). In contrast, the *Rosmarinus officinalis*, *Ruta-graveolens*, *Phagnalon rupestre* & *Ecballium elaterium* have moderate antibacterial activity with inhibition zone diameter of 9.33, 9.00, 8.50 &8.00 mm respectively (Table 4.3 & Figures 4.14,13,15 & 12). The lowest antibacterial effect was with *Allium sativum* with inhibition zone diameter of 7.00 mm (Figure 4.11).

4.2.2.2. The ethanolic extracts:

The ethanolic extracts of the tested plants (shoot part of *Pelargonium graveolens*, *Rosmarinus officinalis* & *Phagnalon rupestre* except the bulbs of *Allium sativum*, the fruits of *Ecballium elaterium* & the leave of *Ruta-graveolens*) that extracted by using 80% ethanol in soxhlet extractor for 8 h give various degree of antibacterial activity against *E. coli* ranged between 9.33 - 16.66 mm, where the highest activity was for *Pelargonium graveolen* with inhibition zone diameter of 16.66 mm (Table 4.3 & Figure 4.13), followed by *Phagnalon rupestre*, *Ecballium elaterium*, *Allium sativum* & *Rosmarinus officinalis* with inhibition zone diameter of 13.66, 10.66, 10.66 &9.66 mm respectively (Table 4.3 & Figures 4.15, 12, 11& 14), and the lowest antibacterial effect against *E .coli* was for *Ruta graveolens* with inhibition zone diameter of 9.33 mm (Table 4.3 & Figure 4.16).

4.2.2.3. The methanolic extracts:

The methanolic extracts of different plants that were screened for their antibacterial activity against *E. coli* were showed different antibacterial activity on the growth of

the tested bacteria. *Pelargonium graveolen* exhibited the largest zone of inhibition diameter of 16.00 mm (Table 4.3 & Figure 4.13).

Table 4.3. Antibacterial effect of different plant extracts against *E. coli* in mm:

		Group	No.	Inhibition Zones	± Standard Deviation	Factor	Sig
Escherichia coli	**Aquatic**	**A**	3	7.00	0.00	**26.273**	**0.000**
		B	3	8.00	0.00		
		C	3	14.00	1.00		
		D	3	9.33	0.57		
		E	3	8.50	1.32		
		F	3	9.00	1.00		
	Ethanolic	**A**	3	10.66	0.57	**6.921**	**0.003**
		B	3	10.66	1.53		
		C	3	16.66	0.57		
		D	3	9.66	2.52		
		E	3	13.66	3.21		
		F	3	9.33	1.15		
	Methanolic	**A**	3	10.00	1.73	**4.084**	**0.021**
		B	3	8.66	0.57		
		C	3	16.00	1.73		
		D	3	10.33	2.08		
		E	3	12.66	4.04		
		F	3	11.33	1.53		
	Essential oil	**A**	3	12.00	0.00	**5.548**	**0.007**
		B	3	8.33	0.57		
		C	3	10.00	1.00		
		D	3	10.66	2.08		
		E	3	10.00	1.73		
		F	3	7.33	0.57		

(A: Allium sativum, B: Ecballium elaterium, C: Pelargonium graveolens, D: Rosmarinus officinalis, E: Phagnalon rupestre & F: Ruta-graveolens).

The methanolic extracts of *Ecballium elaterium* showed the lowest activity with inhibition zone of 8.66 mm in diameter (Table 4.3 & Figure 4.12). The data also showed the inhibition zone of 12.66 mm were observed for methanolic extracts of the *Phagnalon rupestre* (Table 4.3 & Figure 4.15). The other tested methanolic extracts showed a zone of 11.33, 10.33 & 10.00 mm for *Ruta-graveolens, Rosmarinus officinalis* & *Allium sativum* respectively (Table 4.3 & Figures 4.16, 14 & 11).

Antibacterial activity of plant extracts against *E. coli*:

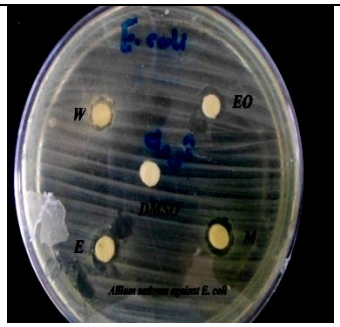


Figure 4.11. ***Allium sativum extracts against E. coli***

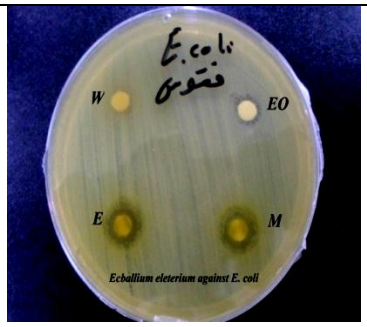


Figure 4.12. ***Ecballium elaterium extracts against E. coli***

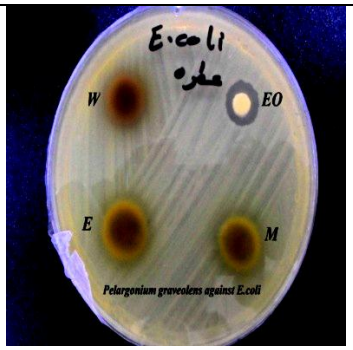


Figure 4.13. ***Pelargonium graveolen extracts against E. coli***

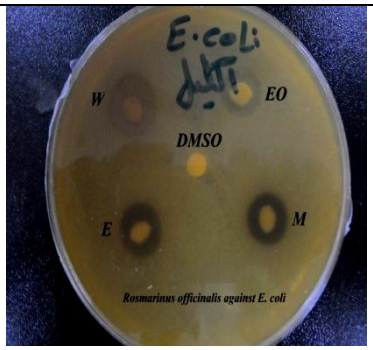


Figure 4.14. ***Rosmarinus officinalis extracts against E. coli***

Figure 4.15. ***Phagnalon rupestre extracts against E. coli***

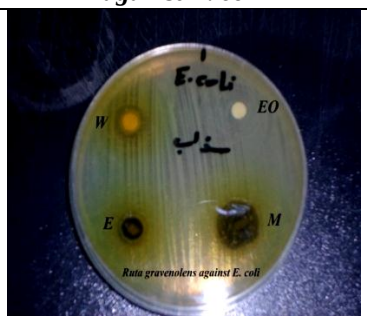


Figure 4.16. ***Ruta graveolen extracts against E. coli***

W: Aquatic, E: Ethanolic, M: methanolic, EO: Essential oil

4.2.2.4. The essential oils:

The EOs of the screened plants extracted with steam distillation for 4 hr showed various degree of antibacterial activity against *E. coli* ranged between 7.33 - 12 mm, where the highest antibacterial activity were observed for *Allium sativum* with inhibition zone diameter of 12.00 mm (Table 4.3 & Figure 4.11), followed by *Rosmarinus officinalis*, *Phagnalon rupestre*, *Pelargonium graveolen* & *Ecballium elaterium* with inhibition zone diameter of 10.66, 10.00, 10.00 & 8.33 mm respectively (Table 4.3 & Figures 4.12 - 15), and the lowest effect were for *Ruta graveolens* with inhibition zone diameter of 7.33 mm (Table 4.3 & Figure 4.16).

4.2.3. Antibacterial activity of plant extracts against *Klebsilla pneumoniae*:

The susceptibility pattern of the herbal extracts against the *K. pneumoniae* bacteria are showed in Table 4.4.

4.2.3.1. The aquatic extracts:

The Aquatic extract of *Pelargonium graveolens* were the most effective extract showing the highest antibacterial activity against *K. pneumoniae* bacteria with inhibition zone diameter of 13.00 mm (Table 4.4 & Figure 4.19), followed strictly were extracts of *Rosmarinus officinalis*, *Ruta-graveolens*, *Allium sativum* and *phagnalon rupestre* with inhibition zone diameter of 8.66, 8.00, 8.00 &7.66 mm respectively (Table 4.4 & Figures 4. 17- 22). The least effective were the aquatic extract of Ecballium *elaterium* with inhibition zone diameter of 7.50 mm (Table 4.4 & Figure 4.18).

4.2.3.2. The ethanolic extracts:

The Ethanolic extracts of the tested plants give various degree of activity (7.33- 17.00 mm inhibition zone / 50 µl) against *K. pneumoniae*. the extract of *Pelargonium graveolens* presented the highest antibacterial activity with inhibition zone diameter of 16.00 mm (Table 4.4 & Figure 4.19), followed by *Ecballium elaterium*, *Allium sativum*, *Rosmarinus officinalis* & *Ruta graveolens* which have an intermediate activity against *K. pneumoniae* with inhibition zone diameter of 10.00, 10.00, 9.66 &9.00 mm respectively (Table 4.4 &Figure 4.18, 17, 20 & 22). The lowest activity was the *Phagnalon rupestre* with inhibition zone diameter of 7.33 mm (Table 4.4 & Figure 4.21).

Table 4.4. Antibacterial effect of different plant extracts against *K. pneumoniae*: in mm

		Group	No.	Inhibition Zones	± Standard Deviation	Factor	sig
Klebsilla pneumoniae	**Aquatic**	**A**	3	8.00	0.00	**2.140**	**0.000**
		B	3	7.50	0.50		
		C	3	13.00	1.00		
		D	3	8.66	1.15		
		E	3	7.66	0.57		
		F	3	8.00	1.00		
	Ethanolic	**A**	3	10.00	1.00	**16.221**	**0.000**
		B	3	10.00	2.00		
		C	3	16.00	1.00		
		D	3	9.66	1.53		
		E	3	7.33	0.57		
		F	3	9.00	1.00		
	Methanolic	**A**	3	10.00	1.00	**17.391**	**0.000**
		B	3	9.66	1.53		
		C	3	17.00	0.00		
		D	3	10.66	1.53		
		E	3	11.00	1.00		
		F	3	11.00	1.00		
	Essential oil	**A**	3	10.00	1.00	**2.057**	**0.142**
		B	3	8.00	1.00		
		C	3	11.33	1.53		
		D	3	11.66	2.88		
		E	3	9.66	1.15		
		F	3	9.66	1.15		

(A: Allium sativum, B: Ecballium elaterium, C: Pelargonium graveolens, D: Rosmarinus officinalis, E: Phagnalon rupestre & F: Ruta-graveolens).

Antibacterial activity of plant extracts against *Klebsilla pneumoniae*:

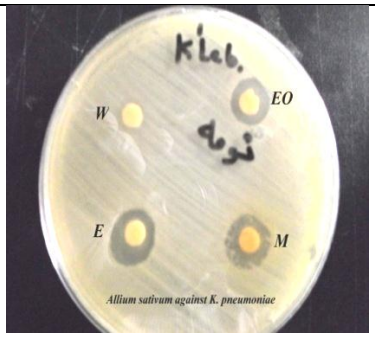


Figure 4.17. *Allium sativum* extracts against *K. pneumoniae*

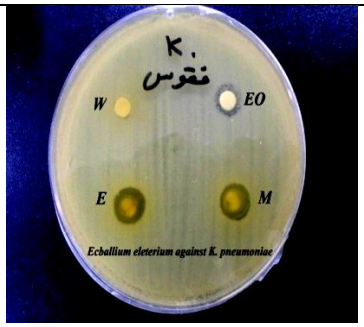


Figure 4.18. *Ecballium elaterium* extracts against *K. pneumoniae*

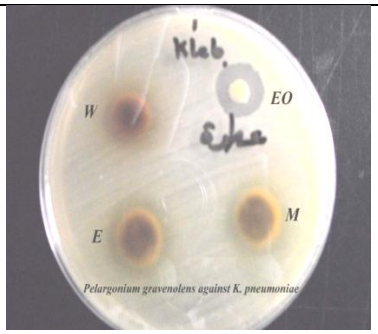


Figure 4.19. *Pelargonium graveolen* extracts against *K. pneumoniae*

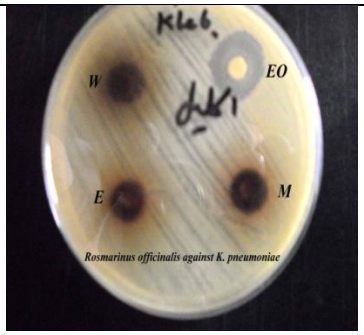


Figure 4.20. *Rosmarinus officinalis* extracts against *K. pneumoniae*

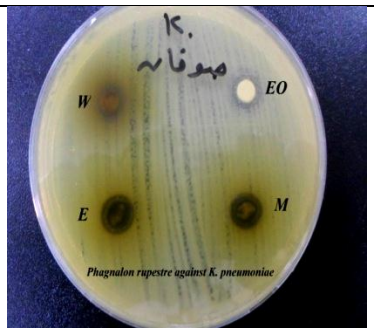


Figure 4.21. *Phagnalon rupestre* extracts against *K. pneumoniae*

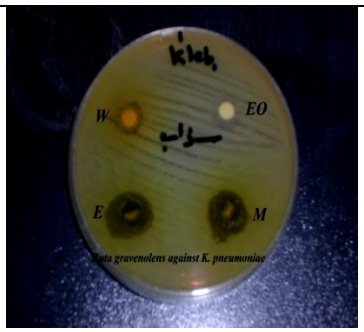


Figure 4.22. *Ruta graveolen* extracts against *K. pneumoniae*

W: Aquatic, E: Ethanolic, M: methanolic, EO: Essential oil

4.2.3.3 The methanolic extracts:

The Methanolic extract of *Pelargonium graveolens* were have the highest antibacterial activity against *K. pneumoniae* bacteria with inhibition zone diameter of 17.00 mm (Table 4.4 & Figure 4.19), followed strictly were extracts of *Phagnalon rupestre*, *Ruta-graveolens*, *Rosmarinus officinalis & Allium sativum* with inhibition

zone diameter of 11.00, 11.00, 10.66 &10.00 mm respectively (Table 4.4 & Figure 4. 20 - 22 &4 -17). The least antibacterial activity against *K. pneumoniae* bacteria were the methanolic extract of *Ecballium elaterium* with inhibition zone diameter of 9.66 mm (Table 4.4 & Figure 4.18).

4.2.3.4. The essential oils:

The EOs of different plants that were screened for their antibacterial activity against *K. pneumonia*e was showed different antibacterial activity on the growth of the tested bacteria. The EO of *Rosmarinus officinalis* exhibited the largest zone of inhibition of 11.66 mm in diameter (Table 4.4 & Figure 4.20). The EO for *Ecballium elaterium* showed lower activity with inhibition zone of 8.00 mm in diameter (Figure 4.18). The data also showed that the inhibition zone of 11.33 mm was observed for the EO of *Pelargonium graveolens* (Table 4.4 & Figure 4.19). The other tested EOs showed a zone of 10.00, 9.66 & 9.66 mm for *Allium sativum*, *Phagnalon rupestre* & *Ruta graveolens* respectively (Table 4.4 & Figure 4.17, 21 & 22).

4.2.4. Antibacterial activity of plant extracts against *Pseudomonas aeruginosa*:

The *in-vitro* antibacterial activity of sex plants extracts estimated by the diameter of inhibition where are varied according to plant type & bacterial strain (Table 4.5).

4.2.4.1. The aquatic extracts:

In the aquatic extracts the extract of *Pelargonium graveolens* has the highest activity were observed against *P. aeruginosa* with the strongest inhibition zone of 11.66 ± 3.51 mm in diameter (Table 4.5 & Figures 4. 25), followed by *Ruta-graveolens*, *Rosmarinus officinalis*, *Ecballium elaterium*, *Allium sativum* with inhibition zone of 11.00, 10.00, 7.83 &7.00 mm respectively (Table 4.5 & Figures 4. 28, 26, 25 & 23). The least activity was the *Phagnalon rupestre* with inhibition zone of 7.00 mm (Table 4.5 & Figure 4. 27).

4.2.4.2. The ethanolic extracts:

The Ethanolic extracts of the different plants presented different degree of antibacterial activity against the tested microorganism. The extract of *Pelargonium graveolens* presented the highest activity with inhibition zone diameter of 15.33 mm (Table 4.5 & Figures 4. 25), followed by *Allium sativum*, *Rosmarinus officinalis*, *Ecballium elaterium* &*Phagnalon rupestre* which have an intermediate activity

against *P. aeruginosa* with inhibition zone diameter of 12.66, 9.33, 9.33 &8.66 mm respectively (Table 4.5 & Figures 4. 23, 26, 24 & 27). The least activity was the *Ruta graveolens* with inhibition zone diameter of 8.33 mm (Table 4.5 & Figure 4. 28).

Table 4.5. Antibacterial effect of different plant extracts against *P. aeruginosa* in mm:

		Group	No.	Inhibition zones	± Standard Deviation	Factor	sig
Pseudomonas aeruginosa	**Aquatic**	**A**	3	7.00	1.00	**4.189**	**0.020**
		B	3	7.83	0.28		
		C	3	11.66	3.51		
		D	3	10.00	2.00		
		E	3	7.00	0.00		
		F	3	11.00	1.00		
	Ethanolic	**A**	3	12.66	0.57	**13.953**	**0.000**
		B	3	9.33	1.15		
		C	3	15.33	1.53		
		D	3	9.33	2.08		
		E	3	8.66	1.15		
		F	3	8.33	0.57		
	Methanolic	**A**	3	9.66	0.57	**29.108**	**0.000**
		B	3	11.00	1.00		
		C	3	15.00	1.00		
		D	3	7.66	0.57		
		E	3	8.66	1.15		
		F	3	8.66	0.57		
	Essential oil	**A**	3	11.33	0.57	**5.762**	**0.006**
		B	3	9.33	1.53		
		C	3	12.66	0.57		
		D	3	12.00	2.00		
		E	3	8.66	1.15		
		F	3	12.33	0.57		

(**A.:** ***Allium sativum,*** **B:** ***Ecballium elaterium,*** **C:** ***Pelargonium graveolen,*** **D:** ***Rosmarinus officinalis,*** **E:** ***Phagnalon rupestre*** **&** **F:** ***Ruta-graveolens*****).**

4.2.4.3 .The methanolic extracts:

The Methanolic extracts of all plants screened showed various inhibitory effects (8.66 - 15 mm/ 50µl inhibition zone) against *Pseudomonas aeruginosa.* The largest zone of inhibition were observed from *Pelargonium graveolens* with inhibition zone diameter of 15.00 mm (Table 4.5 & Figure 4. 25), followed by *Ecballium elaterium, Allium sativum, Phagnalon rupestre & Ruta graveolens* with inhibition zone diameter of 11.00, 9.66, 8.66 & 8.66 mm respectively (Table 4.5 & Figures 4. 24, 23, 27 & 28). The lowest activity was observed from the *Rosmarinus officinalis* with inhibition zone diameter of 7.66 mm (Table 4.5 & Figure 4. 26).

4.2.4.4. The essential oils:

The EOs of all plants inhibited the growth of *Pseudomonas aeruginosa* producing different clear zone of growth inhibition. *Pelargonium graveolens* produced an largest average zone of inhibition of 12.66 mm (Table 4.5 & Figure 4. 25), while *Phagnalon rupestre* produced an lowerest average zone of inhibition of 8.66 mm (Table 4.5 & Figure 4. 27), while *Ruta-graveolens, Rosmarinus officinalis*, *Allium sativum* & *Ecballium elaterium* produced an average zone of inhibition of 12.33, 12.00, 11.33 &9.33 mm respectively (Table 4.5 & Figures 4. 28, 26, 23 & 24), from triplicate assays.

The acceptable standard diameter zone of inhibition for sensitive organism for the standard antibiotic Amikacin is ≥ 17mm (NCCLS 1993).

However, for the plant extracts, the average zone of inhibition observed against the tested clinical pathogenic bacteria ranged from 7.00 - 14 mm in aquatic extracts & from 7.33 - 16.66 mm in ethanolic extracts & from 7.66 - 17.00 mm in methanolic extracts & from 7.33 - 12.66 mm in EOs. These values *full* within the range of resistant & /or intermediate sensitive when compared with control antibiotic. At the same time, several workers have reported bioactivity of crude extracts of medicinal plants within such range of diameter zone of inhibition (Karmegam, *et al*., 2008).

Antibacterial activity of plant extracts against *Pseudomonas aeruginosa*

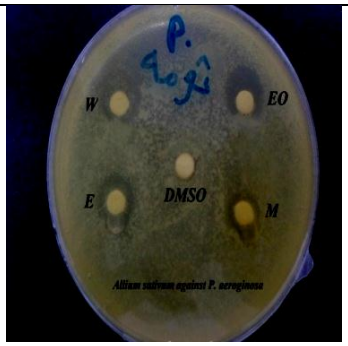


Figure 4.23. *Allium sativum* extracts against *P. aeruginosa*

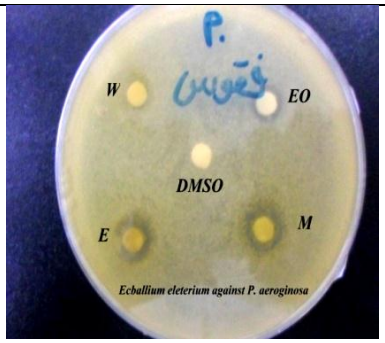


Figure 4.24. *Ecballium elaterium* extracts against *P. aeruginosa*

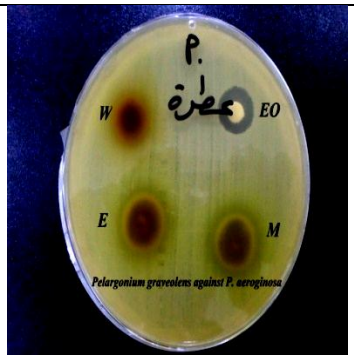


Figure 4.25. *Pelargonium graveolen* extracts against *P. aeruginosa*

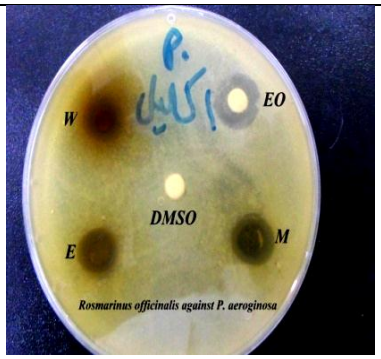


Figure 4.26. *Rosmarinus officinalis* extracts against *P. aeruginosa*

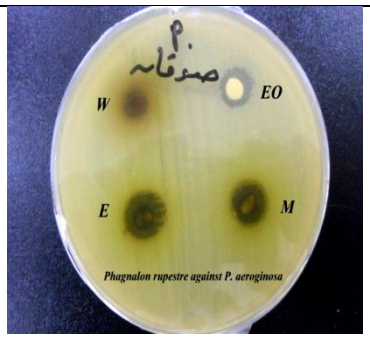


Figure 4.27. *Phagnalon rupestre* extracts against *P. aeruginosa*

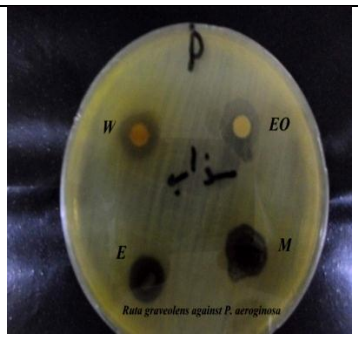


Figure 4.28. *Ruta graveolen* extracts against *P. aeruginosa*

W: Aquatic, E: Ethanolic, M: Methanolic, EO: Essential oil

4.3. Synergistic antibacterial activity:

To evaluate the possible synergistic effects of the extracts with antibiotics, six plants (A. *Allium sativum,* B. *Ecballium elaterium,* C. *Pelargonium graveolen* , D. *Rosmarinus officinalis,* E. *Phagnalon rupestre* & F. *Ruta-graveolens)* were tested. The diameter of zone of inhibition of different combinations of plant extracts is represented in Tables (4. 6 to 21). Combinations of the spices in several cases

demonstrated synergistic, additive or antagonistic effects on microorganisms. Enlargement of inhibition zones indicates a positive interaction (synergism) (Ahmad, & Aqil, 2007).

4.3.1. Synergistic effect against *Staphylococcus aureus*:

The plant extracts differed significantly in their synergistic ability to inhibit the growth of *S. aureus*, which was extracted using socxhlet apparatus for 8 hours.

4.3.1.1. The aquatic extracts:

Table 4.6 & Figures 4.29. A - L summarizes the synergistic effect of plant extracts against *S. aureus*, which was extracted using distilled water for 8 hours.

Among the six extracts (i.e. A. *Allium sativum* (bulbes), B. *Ecballium elaterium* (fruite), C. *Pelargonium graveolen* (shoots), D. *Rosmarinus officinalis* (shoots), E. *Phagnalon rupestre* (shoots) & F. *Ruta-graveolens* (leaf) were adds as crude extracts of 50µl/disc of 200 mg/ml), *Rosmarinus officinalis* (shoots) was the only one that had the most synergistic inhibitory effect against *S. aureus*.

Table 4.6. Synergistic activity of different plant extracts with different antibiotics against *S. aureus*:

			CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Aquatic	Antibiotic alone		0	0	0	0	0	0	0	0	0	7	0
	Extract alone												
	A	10.00	**13**	0	0	0	0	0	0	0	0	10	0
	B	10.00	0	0	0	0	0	0	0	0	0	0	7
	C	10.66	10	8	9	8	9	9	10	8	8	**12**	0
	D	**12.66**	**28**	**24**	**24**	**25**	**26**	**28**	**22**	**24**	**23**	**21**	**22**
	E	8.33	0	0	0	0	0	0	0	0	0	8	0
	F	9.66	9	8	0	0	0	0	0	0	0	0	0

(**A.:** ***Allium sativum*****, B:** ***Ecballium elaterium*****, C:** ***Pelargonium graveolen*****, D:** ***Rosmarinus officinalis*****, E:*****Phagnalon rupestre* & F:** ***Ruta-graveolens*****).**

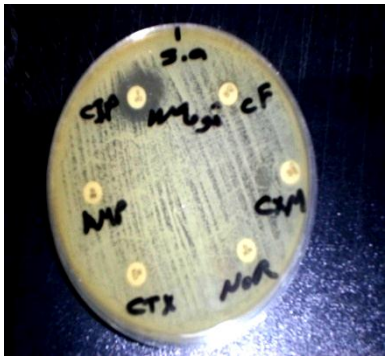


Figure 4.29. A. combination of *Allium sativum* aquatic extract with antibiotics against *S. aureus*

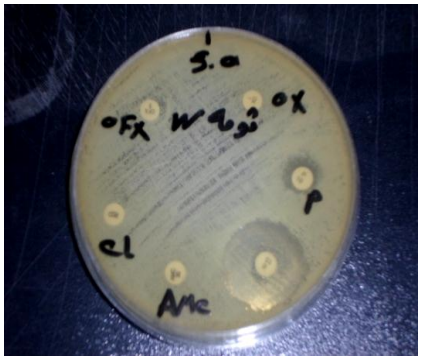


Figure 4.29. B. combination of *Allium sativum aquatic* extract with antibiotics against *S. aureus*

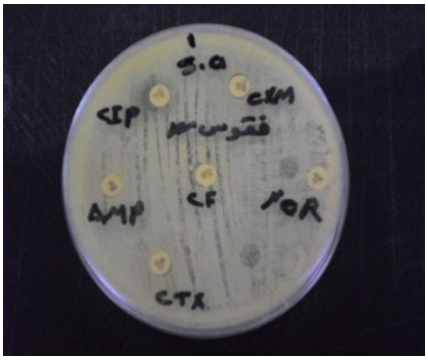


Figure 4.29. C. combination of *Ecballium elaterium aquatic* extract with antibiotics against *S. aureus*

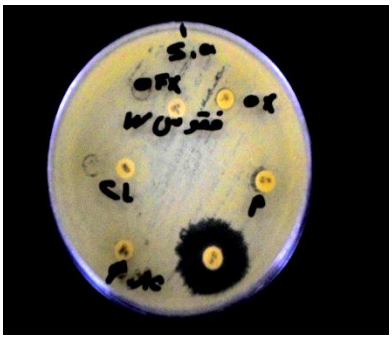


Figure 4.29.D. combination of *Ecballium elaterium* aquatic extract with antibiotics against *S. aureus*

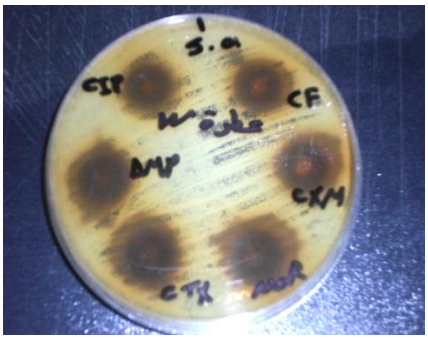


Figure 4.29. E. combination of. *Pelargon-ium graveolen* aquatic extract with antibiotics against *S. aureus*

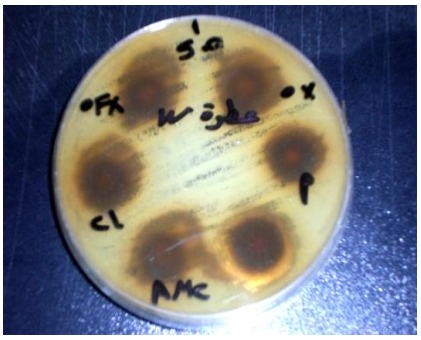


Figure 4.29. F. combination of. *Pelargon-ium graveolen* aquatic with antibiotics extract against *S. aureus*

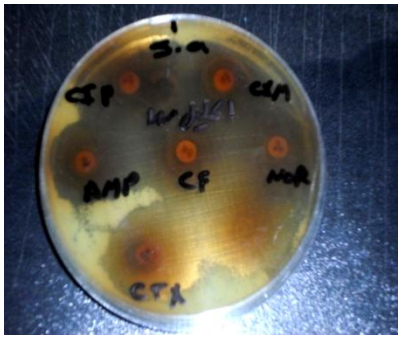


Figure 4.29. G. combination of *Rosmar-inus officinalis* aquatic extract with antibiotics against *S. aureus*

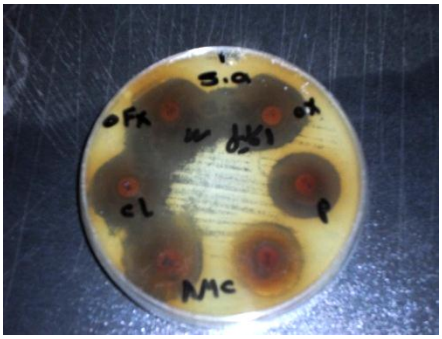


Figure 4.29. H. combination of *Rosmarinus officinalis* aquatic extract with antibiotics against *S. aureus*

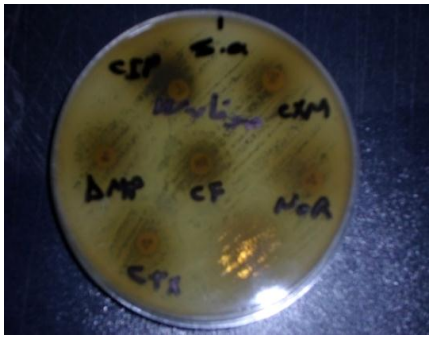


Figure 4.29.I. combination of *Phagnalon rupestre* aquatic extract with antibiotics against *S. aureus*

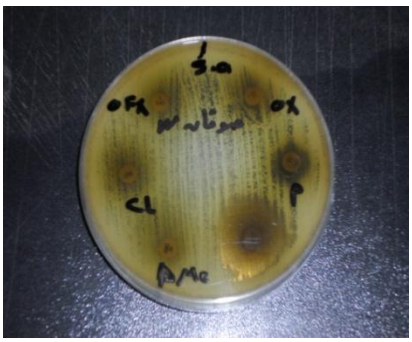


Figure 4.29. J. combination of *Phagnalon rupestre* aquatic extract with antibiotics against *S. aureus*

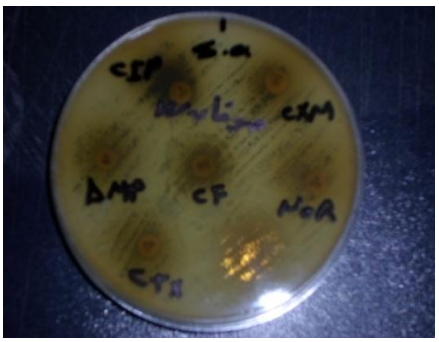


Figure 4.29. K. combination of *Ruta graveolen* aquatic extract with antibiotics against *S. aureus*

Figure 4.29. L. combination of *Ruta graveolen* aquatic extract with antibiotics against *S. aureus*

The results showed that *Rosmarinus officinalis* (shoots) had a synergistic effect with all antibiotics & was able to suppress the *S. aureus* growth and their extracts alone were effective. While the results showed that the *Allium sativum* (bulbes) had additive effect with all antibiotics except with CIP where its effect was synergistic effect and the *Ecballium elaterium* (fruite) had additive effect with all antibiotics, except with P & OX where had antagonistic effect. The *Pelargonium graveolen* (shoots) had antagonistic effect with most antibiotics except with p were had synergistic effect on *S. aureus*. The *Phagnalon rupestre* (shoots) & *Ruta graveolens* (leaf) both of it had additive effect with most tested antibiotics on *S. aureus*. Overall, the best synergism between the aquatic extracts and antibiotics against *S. aureus* was presented in Table 4.7.

Table 4.7. The best synergism with aquatic extracts against *S. aureus*:

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Aquatic	**Group**	D	D	D	D	D	D	D	D	D	D	**D**
	Means	28.00	24.00	24.00	25.00	26.00	28.00	22.00	24.00	23.00	21.00	**22.00**
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**1.00**
	F	240.60	524.80	855.90	913.50	995.10	1146.30	744.00	844.80	779.10	283.95	**1131.36**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**0.001**

D: *Rosmarinus officinalis*

4.3.1.2. The ethanolic extracts:

Table 4.8 & Figures 4.30. A - L summarizes the synergistic effect of plant extracts against *S. aureus*, which was extracted using 80% ethanol for 8 hours.

Among the six extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf), the *Pelargonium graveolen* (shoots), *Rosmarinus officinalis* (shoots) & *Ruta graveolens* (leaf) had the most synergistic inhibitory effect against *S. aureus* but the best synergistic effect was with *Rosmarinus officinalis* (shoots) which had inhibition zone ranged from 12 - 23 mm in diameter.

The results in table 4.8 also showed that, *Allium sativum* (bulbes) & *Ecballium elaterium* (fruite) had antagonistic effect with most antibiotics except with CIP where had synergistic effect against *Staphylococcus aureus*, while *Phagnalon rupestre* (shoots) had antagonistic effect with all antibiotics except with AM, CTX, NOR & OX which had additive effect against *S. aureus*.

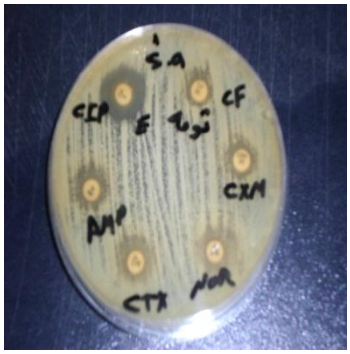


Figure 4.30. A. combination of *Allium sativum* ethanolic extract with antibiotics against *S. aureus*

Figure 4.30. B. combination of *Allium sativum* ethanolic extract with antibiotics against *S. aureus*

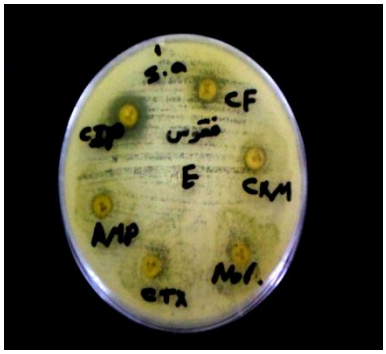


Figure 4.30. C. combination of *Ecballium elaterium* ethanolic extract with antibiotics against *S. aureus*

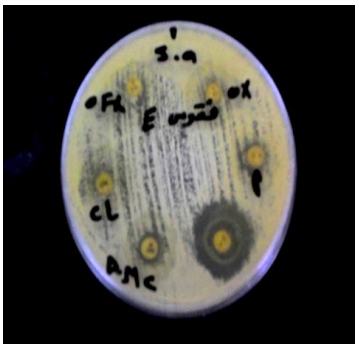


Figure 4.30. D. combination of *Ecballium elaterium* ethanolic extract with antibiotics against *S. aureus*

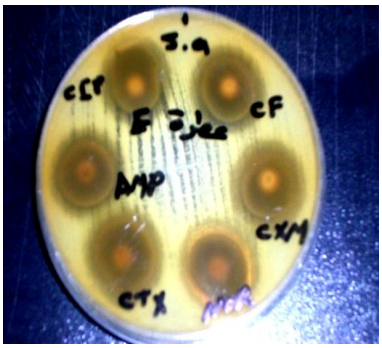


Figure 4.30. E. combination of. *Pelargonium graveolen* ethanolic extract with antibiotics against *S. aureus*

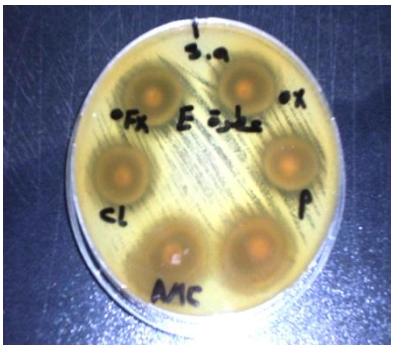


Figure 4.30. F. combination of. *Pelargonium graveolen* ethanolic extract with antibiotics against *S. aureus*

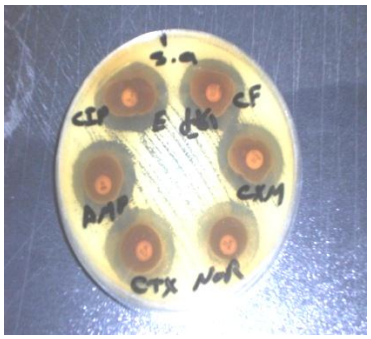


Figure 4.30. G. combination of *Rosmarinus officinalis* ethanolic extract with antibiotics against *S. aureus*

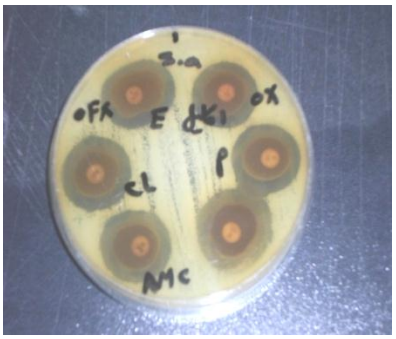


Figure 4.30. H. combination of *Rosmarinus officinalis* ethanolic extract with antibiotics against *S. aureus*

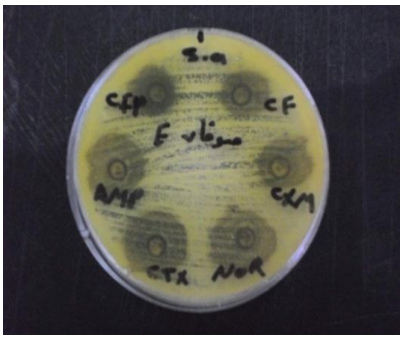


Figure 4.30. I. combination of *Phagnalon rupestre* ethanolic extract with antibiotics against *S. aureus*

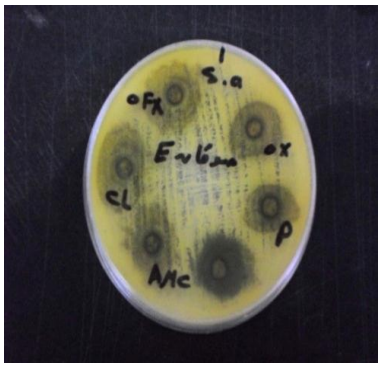


Figure 4.30. J. combination of *Phagnalon rupestre* ethanolic extract with antibiotics against *S. aureus*

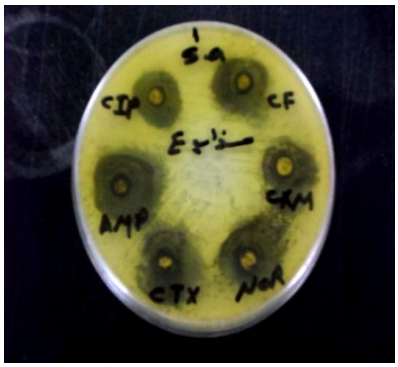


Figure 4.30. K. combination of *Ruta graveolen* ethanolic extract with antibiotics against *S. aureus*

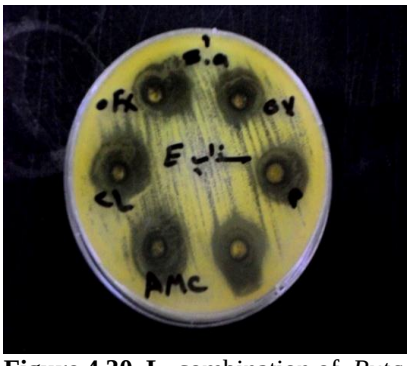


Figure 4.30. L. combination of *Ruta graveolen* ethanolic extract with antibiotics against *S. aureus*

Table 4.8. Synergistic activity of different plant extracts with different antibiotics against *S .aureus*:

			CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Ethanolic	Antibiotic alone		0	0	0	0	0	0	0	0	0	7	0
	extract												
	A	11.66	17	11	10	8	9	9	0	0	8	11	8
	B	11.33	12	0	0	0	8	8	0	0	9	10	0
	C	13.00	16	18	17	17	18	19	18	18	18	17	17
	D	13.33	22	23	23	22	22	23	21	22	21	22	22
	E	10.66	10	0	0	0	8	8	8	8	7	0	0
	F	11.66	16	19	16	17	12	12	13	16	14	15	14

(**A:** ***Allium sativum,*** **B:** ***Ecballium elaterium,*** **C:** ***Pelargonium graveolen,*** **D:** ***Rosmarinus officinalis,*** **E:** ***Phagnalon rupestre*** **& F:** ***Ruta-graveolens*****).**

Overall, the best synergism between the ethanolic extracts and antibiotics against *S. aureu*s was presented in Table 4.9**.**

Table 4.9. The best synergism with ethanolic extracts against *S. aureu*s:

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Ethanolic	**Group**	D	D	D	D	D	D	D	D	D	D	**D**
	Means	22.00	23.00	23.00	22.00	22.00	23.00	21.00	21.66	21.00	22.00	**22.00**
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.57	1.00	1.00	**1.00**
	F	52.50	445.35	403.20	399.00	103.70	121.70	358.20	472.90	114.40	202.68	**371.55**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**0.001**

D. *Rosmarinus officinalis*

4.3.1.3. The methanolic extracts:

Table 4.10 & Figures 4.31. A - L summarize**s** the synergistic effect of plant extracts against *S. aureu*s, which was extracted by methanol for 8 hours by using soxhlet extractor. Among the six extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf) added as crude extract of 50 µl/ disc of 200 mg/ml, *Pelargonium graveolen* (shoots) & *Rosmarinus officinalis* (shoots) had the most synergistic inhibitory effect against *S. aureu*s, but the best synergistic effect was for *Rosmarinus officinalis* (shoots) followed by *Pelargonium graveolen* (shoots) with all antibiotics & was able to suppress the growth of *S. aureu*s, which give inhibition zone ranged from 24-28 mm & 14-17 mm in diameter respectively. The results showed that *Allium sativum* (bulbes) had

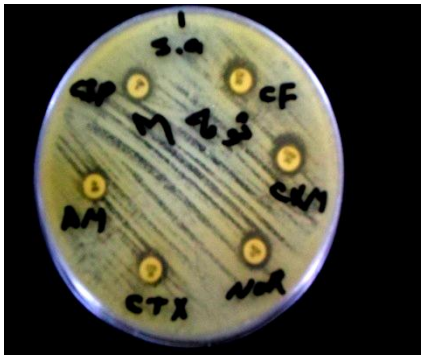


Figure 4.31. A. combination of *Allium sativum* methanolic extract with antibiotics against *S.aureus*

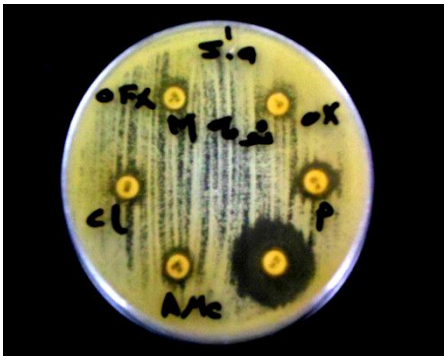


Figure 4.31. B. combination of *Allium sativum* methanolic extract with antibiotics against *S.aureus*

Figure 4.31. C.combination of *Ecballium elaterium* methanolic extract with antibiotics against *S.aureus*

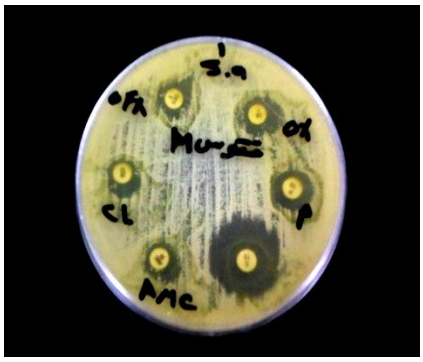


Figure 4.31. D combination of *Ecballium elaterium* methanolic extract with antibiotics against *S.aureus*

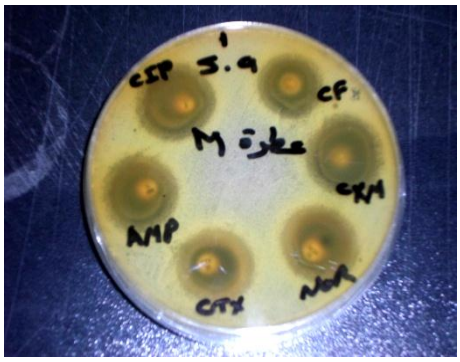


Figure 4.31. E.combination of. *Pelargonium graveolen* methanolic extract with antibiotics against *S.aureus*

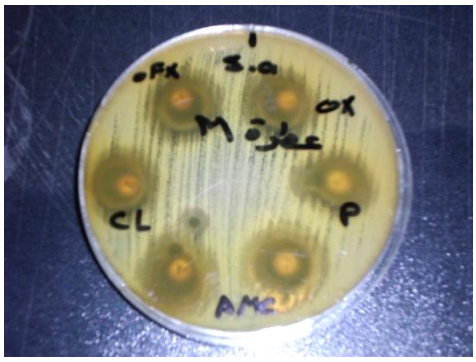


Figure 4.31. F.combination of. *Pelargonium graveolen* methanolic extract with antibiotics against *S.aureus*

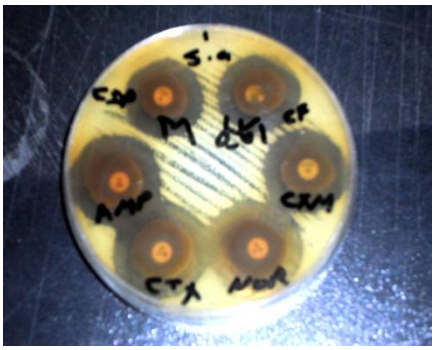


Figure 4.31. G.combination of *Rosmarinus officinalis* methanolic extract with antibiotics against *S.aureus*

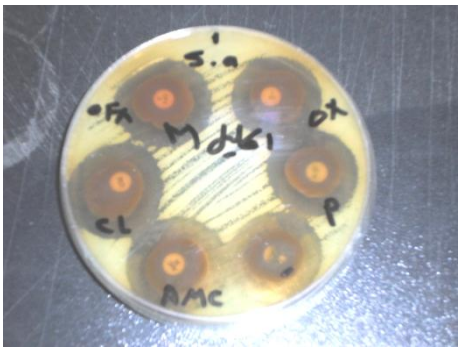


Figure 4.31. H. combination of *Rosmarinus officinalis* methanolic extract with antibiotics against *S.aureus*

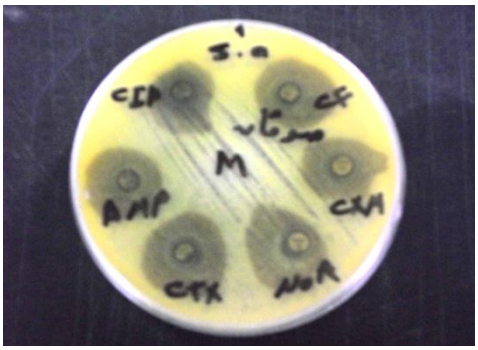


Figure 4.31. I. combination of *Phagnalon rupestre* methanolic extract with antibiotics against *S.aureus*

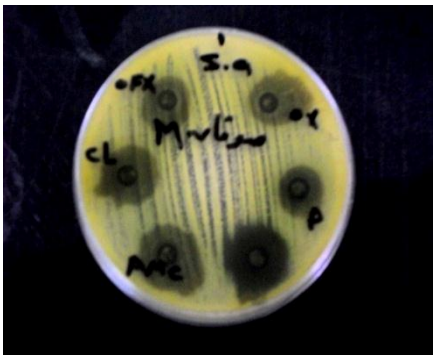


Figure 4.31. J. combination of *Phagnalon rupestre* methanolic extract with antibiotics against *S.aureus*

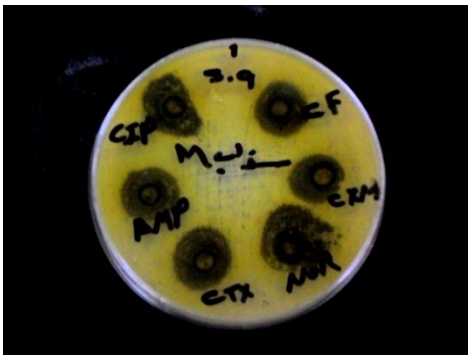


Figure 4.31. K. combination of *Ruta graveolen* methanolic extract with antibiotics against *S.aureus*

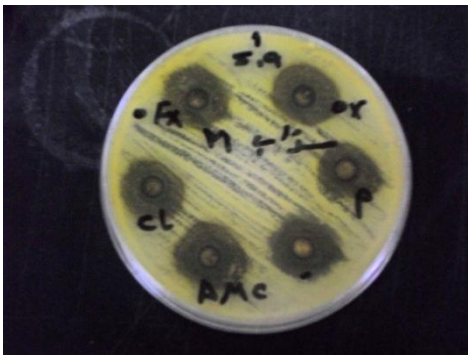


Figure 4.31. L. combination of *Ruta graveolen* methanolic extract with antibiotics against *S.aureus*

antagonistic effect with all antibiotics on *S. aureus*, while *Ecballium elaterium* (fruite) had antagonistic effect with all antibiotics except with CIP, P & OX which had synergistic effect, furthermore the combination of *Phagnalon rupestre* (shoots) methanolic extract with the antibiotics give antagonistic effect with all antibiotics except with OFX &CL which had additive effect & with CIP which give synergistic effect against *Staphylococcus aureus*. On the other hand the combination of *Ruta graveolens* (leaf) methanolic extract with the antibiotics give a synergistic effect with CIP, CTX, CF, AMP, P & OX while give antagonistic effect with the rest of antibiotics against *S.aureus* bacteria.

Table 4.10. Synergistic activity of different plant extracts with different antibiotics against *S.aureus*:

			CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Methanolic	Antibiotic alone		0	0	0	0	0	0	0	0	0	7	0
	extract												
	A	12.66	9	8	9	9	10	9	8	9	9	11	8
	B	10.66	13	10	0	0	11	11	10	11	10	12	12
	C	11.00	17	15	14	15	16	14	15	15	17	14	15
	D	14.66	25	28	28	27	25	25	27	24	26	25	25
	E	9.66	12	8	9	8	8	9	0	0	8	9	8
	F	8.33	9	8	8.5	8	8	9	8	8	9	10	11

(***A: Allium sativum, B: Ecballium elaterium, C: Pelargonium graveolen, D: Rosmarinus officinalis, E: Phagnalon rupestre & F: Ruta-graveolens*).**

Overall, the best synergism between the methanolic extracts and antibiotics against *S. aureus* was presented in Table 4.11.

Table 4.11. The best synergism with methanolic extracts against *S. aureus*:

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Methanolic	**Group**	D	D	D	D	D	D	D	D	D	D	**D**
	Means	25.00	28.00	28.00	27.00	25.00	25.00	27.00	24.00	26.00	25.00	**25.00**
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**1.00**
	F	110.90	187.70	311.19	298.68	129.60	118.10	296.16	229.56	150.50	104.10	**121.70**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**0.001**

D: *Rosmarinus officinalis*

.

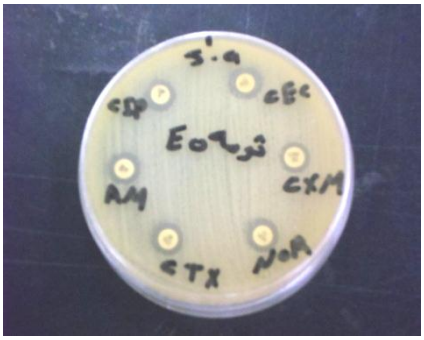


Figure 4.32. A. combination of *Allium sativum* EO with antibiotics against *S. aureus*

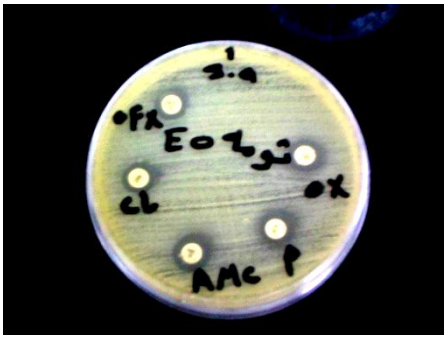


Figure 4.32. B. combination of *Allium sativum* EO with antibiotics against *S. aureus*

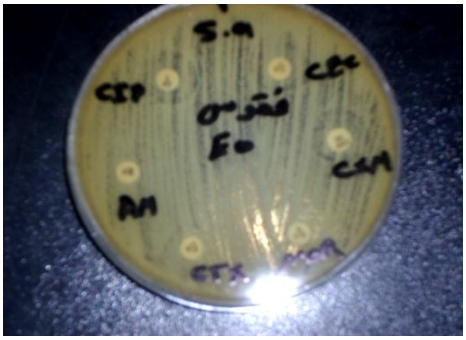


Figure 4.32. C. combination of *Ecballium elaterium* EO with antibiotics against *S. aureus*

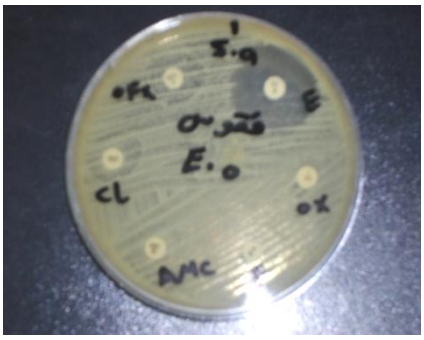


Figure 4.32. D. combination of *Ecballium elaterium* EO with antibiotics against *S. aureus*

Figure 4.32. E.combination of. *Pelargonium graveolen* EO with antibiotics against *S. aureus*

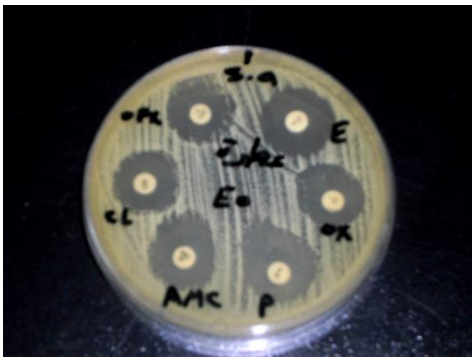


Figure 4.32. F.combination of. *Pelargonium graveolen* EO with antibiotics against *S. aureus*

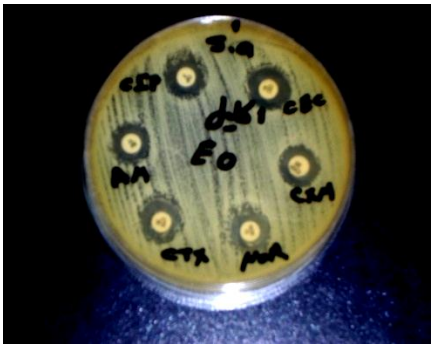


Figure 4.32. G. combination of *Rosmarinus officinalis* EO with antibiotics against *S. aureus*

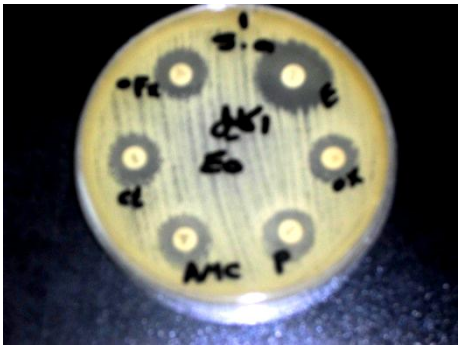


Figure 4.32. H. combination of *Rosmarinus officinalis* EO with antibiotics against *S. aureus*

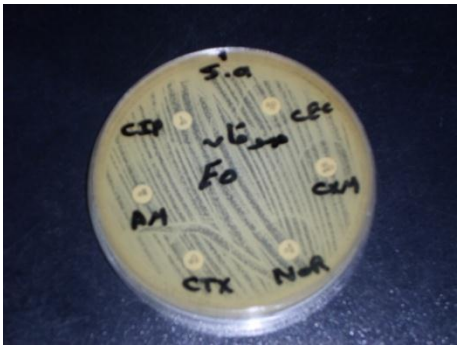


Figure 4.32. I. combination of *Phagnalon rupestre* EO with antibiotics against *S. aureus*

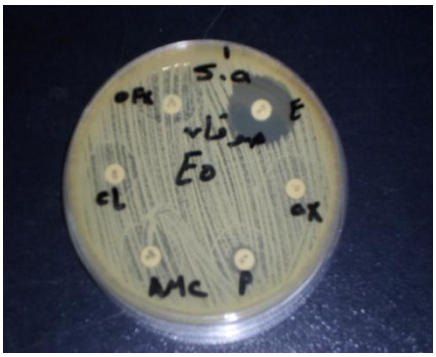


Figure 4.32. J. combination of *Phagnalon rupestre* EO with antibiotics against *S. aureus*

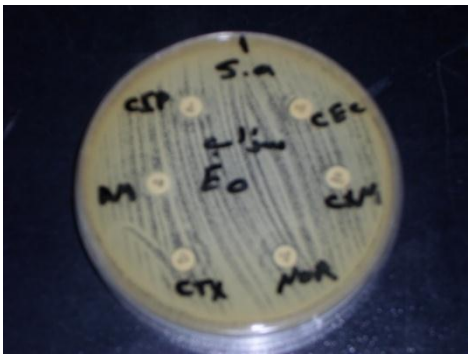


Figure 4.32. K. combination of *Ruta graveolen* EO with antibiotics against *S. aureus*

Figure 4.32. L. combination of *Ruta graveolen* EO with antibiotics against *S. aureus*

Table 4.12. Synergistic activity of different plant extracts with different antibiotics against *S.aureus*:

Essential Oils		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
	Antibiotic alone / extract	0	0	0	0	0	0	0	0	0	7	0
	A 12.66	11	10.5	10	11	10	11	11	10	11.5	11	11
	B 9.00	0	0	0	0	0	0	0	0	0	0	0
	C 12.33	19	18	17.5	18.5	17	16	18	20	19.5	18	18
	D 11.33	14	12.5	13.5	12	13	15	15	14.5	15	14	14
	E 10.00	0	0	0	0	0	0	0	0	0	10	0
	F 8.33	0	0	0	7	0	0	8	8	10	7	7

(**A:** ***Allium sativum,*** **B:** ***Ecballium elaterium,*** **C:** ***Pelargonium graveolen,*** **D:** ***Rosmarinus officinalis,*** **E:** ***Phagnalon rupestre*** **&** **F:** ***Ruta-graveolens*****).**

4.3.1.4. The essential oils:

Table 4.12 & Figures 4.32. A- L summarizes the synergistic effect of Essential oils against *S. aureu*s, which was extracted by using steam distillation apparatus for 4 h. Among the six screened EOs (i.e. A. *Allium sativum* (bulbes) EO, B. *Ecballium elaterium* (fruite) EO, C. *Pelargonium graveolens* (shoots) EO, D. *Rosmarinus officinalis* (shoots) EO, E. *Phagnalon rupestre* (shoots) EO & F. *Ruta-graveolens*(leaf) EO), the *Allium sativum* (bulbes) EO had antagonistic effect with all tested antibiotics on *S. aureu*s, *and Ecballium elaterium* (fruite) & *Phagnalon rupestre* (shoots) had additive effect with all tested antibiotics except the *Ecballium elaterium* (fruite) EO with P which had antagonistic effect ,and *Ruta graveolens* (leaf) EO had synergistic inhibitory effect with AMC only on *S. aureu*s and had antagonistic effect with NOR, OFX, CL, P & OX antibiotics and had additive effect with CIP, AM, CTX, CXM &CF antibiotics. The results in Table 4.12 also show that the best synergistic effect was for *Pelargonium graveolen* (shoots) EO followed by *Rosmarinus officinalis* (shoots) EO with inhibition zone diameter ranged from 16-20 mm &12-15 mm respectively. Overall, the best synergism between the EOs and antibiotics against *S. aureu*s was presented in Table 4.13.

Table 4.13. The best synergism with methanolic extracts against *S. aureus*:

		CIP	AM	CTX	NOR	CXM	CF	OFX	CL	AMC	P	OX
Essential oil	**Group**	C	C	C	C	C	C	C	C	C	C	**C**
	Means	19.00	18.00	17.50	18.50	17.00	16.00	18.00	20.00	19.50	18.00	**18.00**
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**1.00**
	F	426.40	372.40	370.00	292.66	349.60	369.60	255.00	283.38	283.35	160.94	**302.77**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**0.001**

C: ***Pelargonium graveolen***

4.3.2. Synergistic effect against *Esherichia coli*:

Although the antibacterial activities of *Allium sativum* (bulbes), *Ecballium elaterium* (fruites), *Pelargonium graveolen* (shoots), *Rosmarinus officinalis* (shoots), *Phagnalon rupestre* (shoots) & *Ruta graveolens* (leaves) extracts have been accepted, synergism assay were carried out for them & synergism rate differed significantly in their synergistic ability to inhibit the growth of *E. coli* depending on the solvent of extraction & the plant extracts.

4.3.2.1: The aquatic extracts:

Table 4.14 & Figures 4.33. A - L summarizes the synergistic effect of plant extracts against *E. coli*, which was extracted using distilled water for 8 hours. The extracts were added as 50 µl/disc of 200 mg/ml concentration.

Among the six extracts *Rosmarinus officinalis* (shoots) aquatic extract had synergistic inhibitory effect against *E. coli* with most antibiotics except with CIP, TE & RIF that had antagonistic effects & with AMC, which had additive effect on *E. coli*. Our result also showed that *Allium sativum* (bulbes) aquatic extract had synergistic inhibitory effect against *E. coli* with CIP & TE and was able to suppress the *E. coli* growth, and had antagonistic effect with GEN, and had additive effects with the rest of tested antibiotics.

Ecballium elaterium (fruites) extract, also had a synergistic inhibitory effect with CIP, AM, CXM, TE, RIF & AMC. The highest synergistic inhibitory effect was observed with TE & CXM with inhibition zone of 12 & 11 respectively. The results in the table showed either that the *Pelargonium graveolen* (shoots) extract had additive effects with CIP, AM, CTX, NA, NOR, CF, OFX, AMC & RIF while with CXM, CL & TE had antagonistic effect on *E. coli. Phagnalon rupestre* (shoots) aquatic extract had additive with all antibiotics except with TE, AMC & GEN that had antagonistic effects on *E. coli*. .

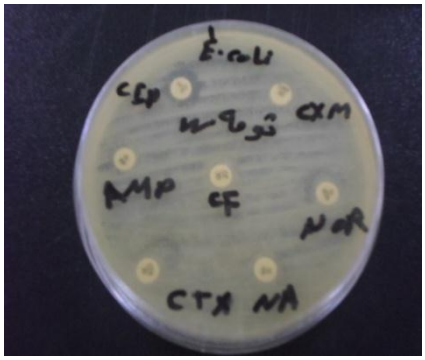


Figure 4.33. A. combination of *Allium sativum* aquatic extract with antibiotics against *E. coli*

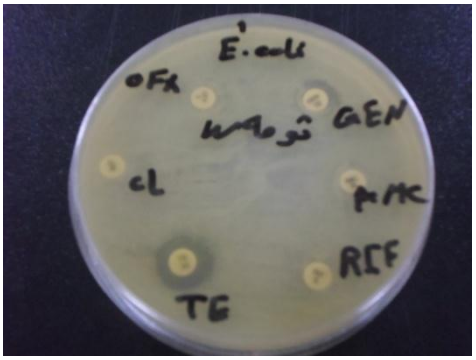


Figure 4.33. B. combination of *Allium sativum aquatic* extract with antibiotics against *E. coli*

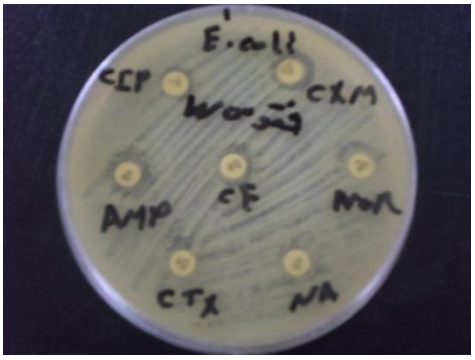


Figure 4.33. C. combination of *Ecballium elaterium aquatic* extract with antibiotics against *E. coli*

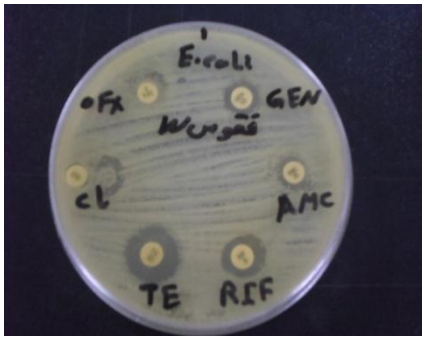


Figure 4.33. D. combination of *Ecballium elaterium* aquatic extract with antibiotics against *E. coli*

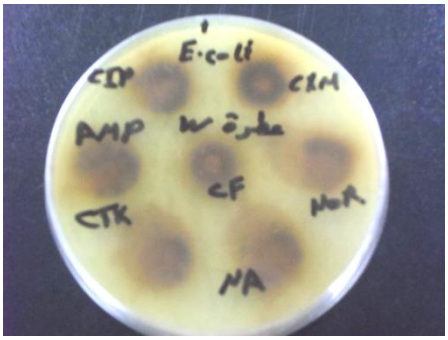


Figure 4.33. E. combination of. *Pelargonium graveolen* aquatic extract with antibiotics against *E. coli*

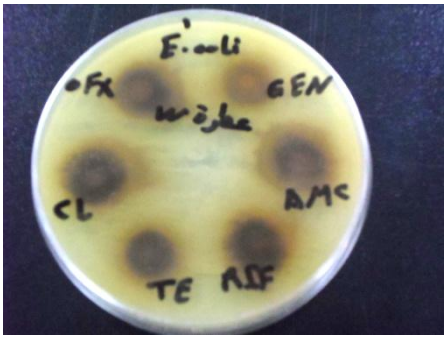


Figure 4.33. F. combination of. *Pelargonium graveolen* aquatic with antibiotics extract against *E. coli*

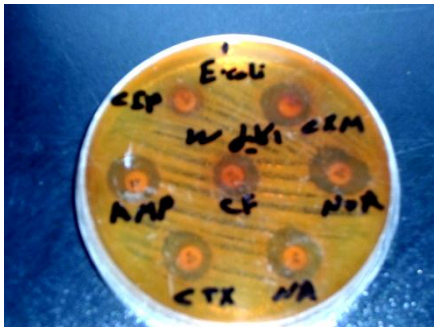


Figure 4.33. G. combination of *Rosmar-inus officinalis* aquatic extract with antibiotics against *E. coli*

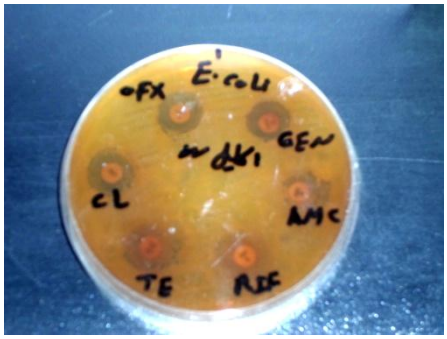


Figure 4.33. H. combination of *Rosmarinus officinalis* aquatic extract with antibiotics against *E. coli*

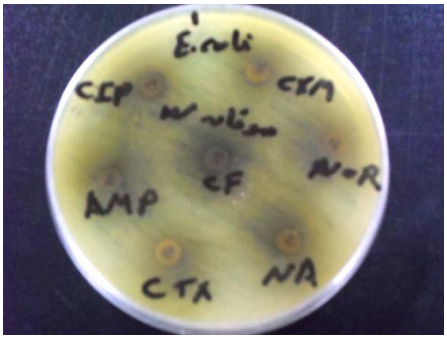


Figure 4.33. I. combination of *Phagnalon rupestre* aquatic extract with antibiotics against *E. coli*

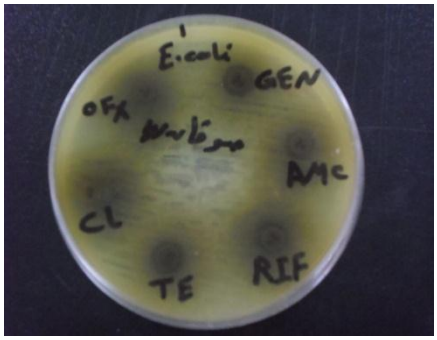


Figure 4.33. J. combination of *Phagnalon rupestre* aquatic extract with antibiotics against *E. coli*

Figure 4.33. K. combination of *Ruta graveolen* aquatic extract with antibiotics against *E. coli*

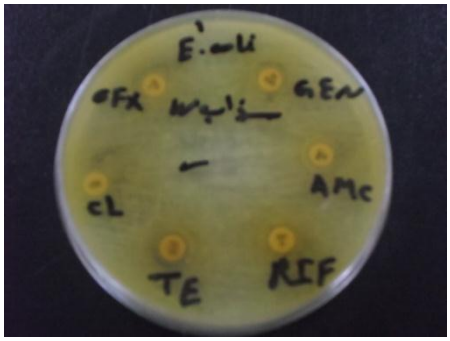


Figure 4.33. L. combination of *Ruta graveolen* aquatic extract with antibiotics against *E. coli*

The *Ruta graveolens* (leaves) aquatic extract when combined with the antibiotics found to be had additive effects with all the tested antibiotics except with TE, RIF & GEN that had antagonistic effects on *E. coli.*

Table 4.14. Synergistic activity of different plant extracts with different antibiotics against *E. coli*:

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN
Aquatic	Antibiotic alone	0	0	0	0	0	0	0	0	0	9	8	0	11
	extract													
	A 7.00	**11**	0	0	0	0	0	0	0	0	**13**	0	0	10
	B 8.00	**9**	**10**	0	0	0	**11**	8	0	0	**12**	**11**	**10**	10
	C 14.00	0	0	0	0	0	8	0	0	10	12	8	0	0
	D 9.33	9	**13**	**12**	**14**	**13**	**14**	**13**	**14**	**14**	0	0	0	**13**
	E 8.50	0	0	0	0	0	0	0	0	0	8	8	8	9
	F 9.00	0	0	0	0	0	0	0	0	0	0	0	0	0

(**A:** ***Allium sativum,*** **B:** ***Ecballium elaterium,*** **C:** ***Pelargonium graveolen,*** **D:** ***Rosmarinus officinalis,*** **E:** ***Phagnalon rupestre*** **&** **F:** ***Ruta-graveolens).***

Overall, the best synergism between the aquatic extracts and antibiotics against *E. coli* was presented in Table 4.15.

Table 4.15. The best synergism with aquatic extracts against *E. coli:*

	CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN
Group	A	D	D	D	D	D	D	D	D	A	B	B	D
Means	11.00	13.00	12.00	14.00	13.00	14.00	13.00	14.00	14.00	13.00	11.00	10.00	13.00
SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
F	171.40	325.50	432.00	588.00	507.00	239.40	287.10	588.00	360.00	165.15	153.00	198.00	140.00
Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

A: *Allium sativum***, B:** *Ecballium elaterium***, D:** *Rosmarinus officinalis***.**

4.3.2.2. The ethanolic extracts:

Table 4.16 & Figures 4.34. A - L summarizes the synergistic effect of all plant extracts against *E. coli*, which was extracted using 80% ethanol for 8 hours. The six plant extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf), had different degree of effect ranged between synergistic, antagonistic or additive effect on *E. coli* when added as crude extracts of 50µl /disc of 200 mg/ml. The results in Table 4.16 showed that *Allium*

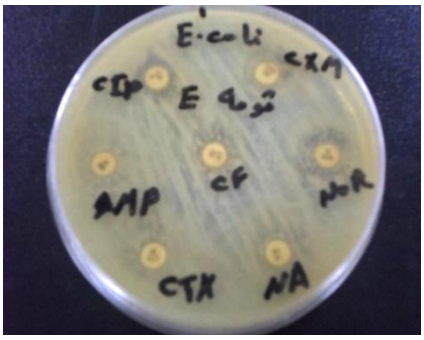


Figure 4.34. A. combination of *Allium sativum* ethanic extract with antibiotics against *E. coli*

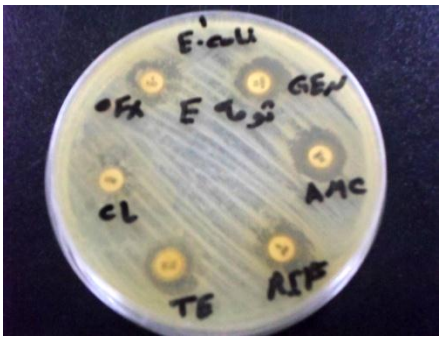


Figure 4.34. B. combination of *Allium sativum* ethanic extract with antibiotics against *E. coli*

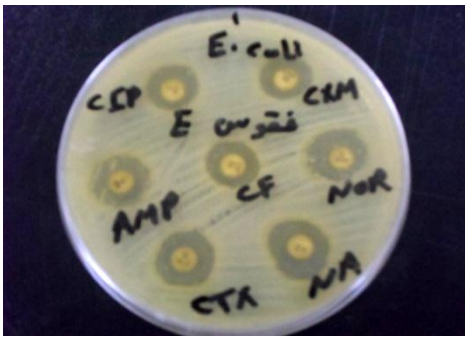


Figure 4.34. C. *combination of Ecballium elaterium* ethanic extract with antibiotics against *E. coli*

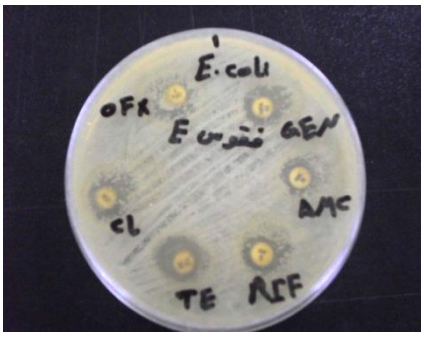


Figure 4.34. D. combination of *Ecballium elaterium* ethanic extract with antibiotics against *E. coli*

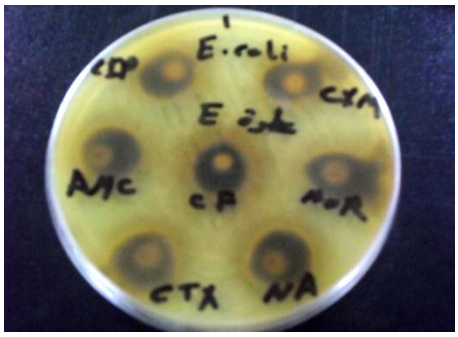


Figure 4.34. E. combination of. *Pelargonium graveolen* ethanic extract with antibiotics against *E. coli*

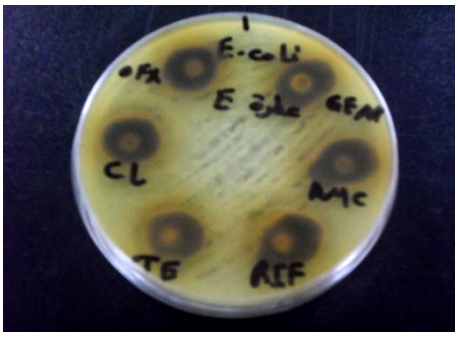


Figure 4.34. F. combination of. *Pelargonium graveolen* ethanic extract with antibiotics against *E. coli*

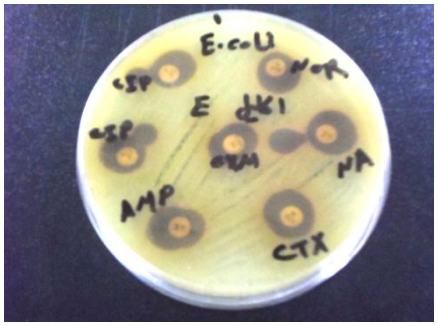


Figure 4.34. G. combination of *Rosmarinus officinalis* ethanic extract with antibiotics against *E. coli*

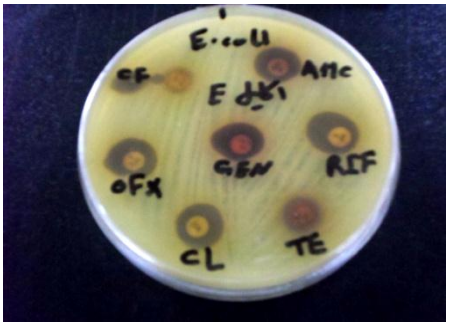


Figure 4.34. H. combination of *Rosmarinus officinalis* ethanic extract with antibiotics against *E. coli*

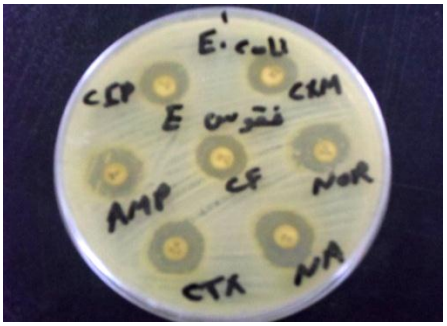


Figure 4.34. I. combination of *Phagnalon rupestre* ethanic extract with antibiotics against *E. coli*

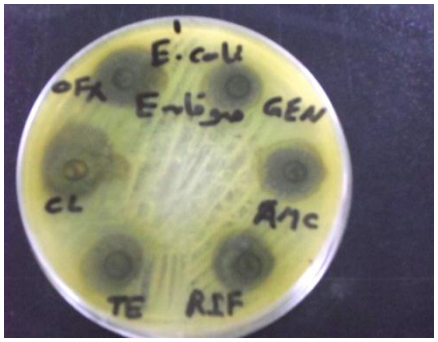


Figure 4.34. J. combination of *Phagnalon rupestre* ethanic extract with antibiotics against *E. coli*

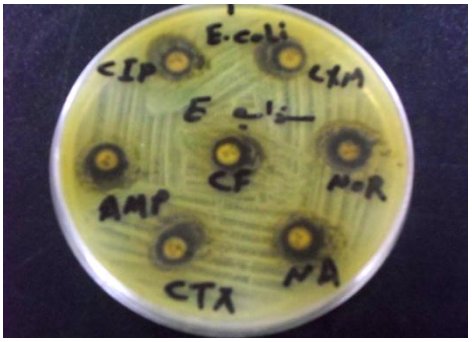


Figure 4.34. K. combination of *Ruta graveolen* ethanic extract with antibiotics against *E. coli*

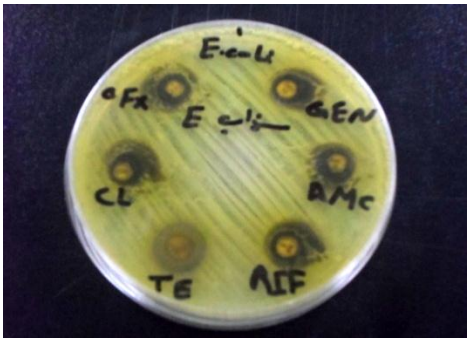


Figure 4.34. L. combination of *Ruta grave- olen* ethanic extract with antibiotics against *E. coli*

sativum (bulbes) ethanolic extract had a synergistic inhibitory effects with some of the tested antibiotics including NOR, CXM, OFX, AMC & GEN, and the best synergism was with CXM & AMC followed by NOR, OFX & GEN and was able to suppress the growth of *E. coli* bacteria and their extract alone was effective. The results showed also that *Allium sativum* (bulbes) ethanolic extract had antagonistic effects with CIP, CF, TE & RIF, and had additive effects with the rest of antibiotics.

The results also showed that *Ecballium elaterium* (fruites) had a synergistic inhibitory effects with AM, CTX, NA, CF &TE, and had antagonistic effects with CIP, NOR,CXM, CL, RIF, AMC &GEN, while had additive effects with OFX & GEN only.

Table 4.16. Synergistic activity of different plant extracts with different antibiotics against *E. coli*:

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN
Ethanolic	Antibiotic alone	0	0	0	0	0	0	0	0	0	9	8	0	11
	extract													
	A 10.66	10	0	0	0	**12**	**13**	9	**12**	0	10	10	**13**	**12**
	B 10.66	10	**14**	**14**	**14**	10	10	**12**	0	10	**12**	9	8	11
	C 16.66	13	14	12	12	13	12	12	14	12	14	10	12	13
	D 9.66	**10**	8	**10**	**12**	**10**	**12**	7	9	**10**	8	**10**	**10**	8
	E 13.66	9.5	8	10	0	12	12	0	**15**	0	8	12	12	12
	F 9.33	**10**	8	8	**10**	**14**	**11**	**11**	**11**	8	**14**	**10**	**10**	10

(A: *Allium sativum*, B: *Ecballium elaterium*, C: *Pelargonium graveolen*, D: *Rosmarinus officinalis*, E: *Phagnalon rupestre* & F: *Ruta-graveolens*).

The results in table 4.16 also showed that *Pelargonium graveolen* (shoots) ethanolic extract when combined with the tested antibiotics had antagonistic effects with all tested antibiotics. While *Rosmarinus officinalis* (shoots) ethanolic extract when combined with the tested antibiotics had a synergistic effects with CIP, CTX, NA, NOR, CXM, CL, RIF & AMC and was able to suppress the growth of *E. coli* (the best synergism was with NA & CXM with inhibition zone of 12 mm for both), and had antagonistic effect with the rest of the tested antibiotics.

The ethanolic extract of *Phagnalon rupestre* (shoots) when combined with the antibiotics had given different results varied from synergestic, antagonistic or additive effect on *E. coli*, which had a synergistic effect with OFX with inhibition zone of 15

mm & was able to suppress the *E. coli* growth, while it was had antagonistic effects with the rest of antibiotics except with NA, CF & CL where it had additive effects on *E. coli*. The *Ruta-graveolens* (leaves) was had a synergistic effects with most of the antibiotics including CIP, NA, NOR, CXM, CF, OFX, TE, RIF & AMC but the best synergism was with NOR & TE antibiotics with inhibition zone of 14 mm in diameter for both of it, and was able to suppress the *E. coli* growth, and had antagonistic effects with the rest of the tested antibiotics against *E. coli*. Overall, the best synergism between the ethanolic extracts and antibiotics against *E. coli* was presented in Table 4.17.

Table 4.17. The best synergism with ethanolic extracts against *E. coli:*

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN
Ethanolic	**Group**	F	B	B	B	F	A	B	E	D	F	F	A	A
	Means	10.00	14.00	14.00	14.00	14.00	13.00	12.00	15.00	10.00	14.00	12.00	13.00	12.00
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	F	5.63	96.00	84.96	187.95	7.70	3.2	89.36	105.72	155.00	22.80	2.90	10.10	9.60
	Sig	0.007	0.001	0.001	0.001	0.002	0.046	0.001	0.001	0.001	0.061	0.001	0.001	0.001

A: *Allium sativum*, **B:** *Ecballium elaterium*, **C:** *Pelargonium graveolen*, **E:** *Phagnalon rupestre*, **E:***Phagnalon rupestre* & F. *Ruta-graveolens*,

4.3.2.3. The methanolic extracts:

Table 4.18 & Figures 4.35. A - L summarizes the synergistic effect of all plant extracts against *E. coli*, which was extracted using HPLC methanol for 8 hours by using soxhlet apparatus.

Among the six plant extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf) which had different degree of effect ranged between synergistic, antagonistic or additive effect on *E. coli* when added as crude extracts of 50 µl /disc of 200 mg/ml), *Allium sativum* (bulbes) , *Ecballium elaterium* (fruites) & *Ruta graveolens* (leaves) had the most synergistic inhibitory effects with all the tested antibiotics against *E. coli*, but *Allium sativum* (bulbes) had the best synergistic effects with CTX, NA, NOR, CI, AMC &GEN with inhibition zone diameter of 14 mm for all that antibiotics, and *Ecballium elaterium* (fruites) had the best synergistic effects with CF, AMC & GEN with inhibition zone diameter of 12, 11.5 &13.5 mm respectively, and *Ruta-graveolens*(leaves) had the best synergistic effects with CIP, CTX, NA, CF, CL, AMC & GEN with inhibition zone diameter of 14, 15, 14, 14, 15, 14 & 14 mm

respectively. *Pelargonium graveolen* (shoots) methanolic extract when combined with the antibiotic-tics had additive effects with all antibiotics except with TE & AMC, which had antagonistic effects. *Rosmarinus officinalis* (shoots) extract also had a synergistic effects AM, CTX, NOR, CXM, OFX, CL, TE, AMC & GEN antibiotics, but the best synergism was observed with CTX & AMC with inhibition zone diameter of 14 & 13 mm respectively against *E. coli* and was able to suppress the growth of this bacteria, and had antagonistic effects with the rest of the tested antibiotics. *Phagnalon rupestre* (shoots) methanolic extract when combined with the antibiotics had antagonistic effects with all the tested antibiotics except with RIF & AMC which had a synergistic inhibitory effects against *E. coli* with inhibition zone diameter of 13 mm for both of it, and was able to inhibit the growth of this bacteria.

Table 4.18. Synergistic activity of different plant extracts with different antibiotics against *E. coli*:

			CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN
Methanolic	Antibiotic alone		0	0	0	0	0	0	0	0	0	9	8	0	11
	extract														
	A	10.00	**12.5**	**13**	**14**	**14**	**14**	**11**	**13**	**13**	**14**	**12**	**13**	**14**	**14**
	B	8.66	**10**	**11**	**9.5**	**10**	**11**	**10**	**12**	**10**	**10**	**10**	**10**	**11.5**	**13.5**
	C	16.00	0	0	0	0	0	0	0	0	0	14	0	14	11
	D	10.33	10	**12**	**14**	10	**12**	**12**	10	**12**	**12**	**12**	10	**13**	**12**
	E	12.66	9	12	10	9	12	12	10	10	10	11	**13**	**13**	12
	F	11.33	**14**	**12**	**15**	**14**	**13**	**13**	**14**	**13**	**15**	**13**	**12**	**14**	**14**

(A: *Allium sativum*, B: *Ecballium elaterium*, C: *Pelargonium graveolen*, D: *Rosmarinus officinalis*, E: *Phagnalon rupestre* & F: *Ruta-graveolens*).

Overall, the best synergism between the methanolic extracts and antibiotics against *E. coli* was presented in Table 4.19.

Table 4.19. The best synergism with methanolic extracts against *E. coli:*

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN
Mthanolic	**Group**	F	A	F	A,F	A	F	F	A, F	F	C	A,E	A, F	**A, F**
	Means	14.00	13.00	15.00	14.00	14.00	13.00	14.00	13.00	15.00	14.00	13.00	14.00	**14.00**
	SD	1.00	0.50	1.00	0.50	0.50	1.00	1.00	1.00	1.00	1.00	0.57	0.50	**0.50**
	F	101.54	103.34	132.32	111.38	112.94	84.48	92.76	87.36	122.68	6.00	87.36	3.34	**5.40**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.005	0.001	0.040	**0.008**

A: *Allium sativum*, **C:** *Pelargonium graveolen*, **E:** *Phagnalon rupestre*, & F: *Ruta-graveolens*,

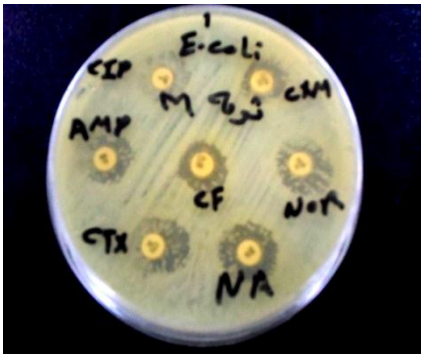


Figure 4.35. A. combination of *Allium sativum* methanolic extract with antibiotics against *E. coli*

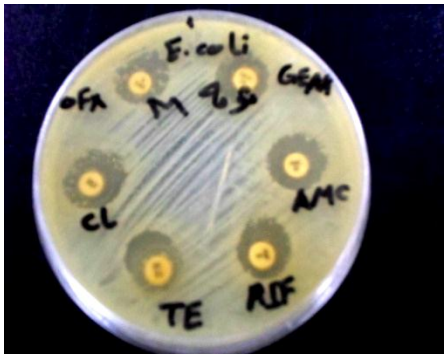


Figure 4.35. B. combination of *Allium sativum* methanolic **extract** with antibiotics against *E. coli*

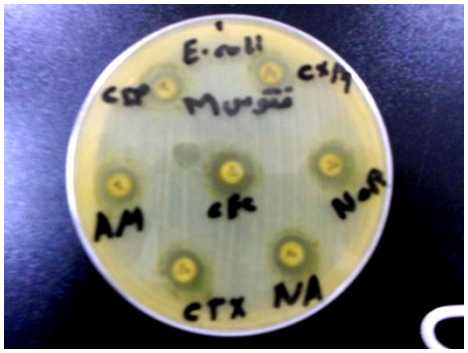


Figure 4.35. C. combination of *Ecballium elaterium* methanolic extract with antibiotics against *E. coli*

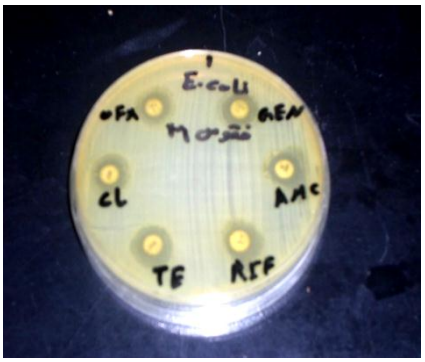


Figure 4.35. D. combination of *Ecballium elaterium* methanolic extract with antibiotics against *E. coli*

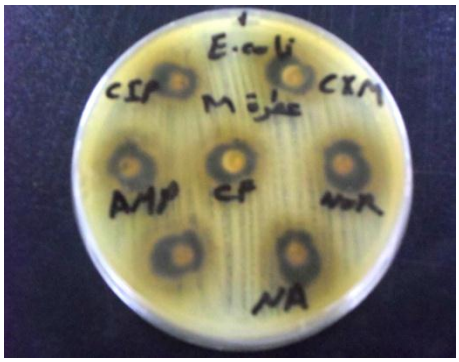


Figure 4.35. E. combination of. *Pelargonium graveolen* methanolic extract with antibiotics against *E. coli*

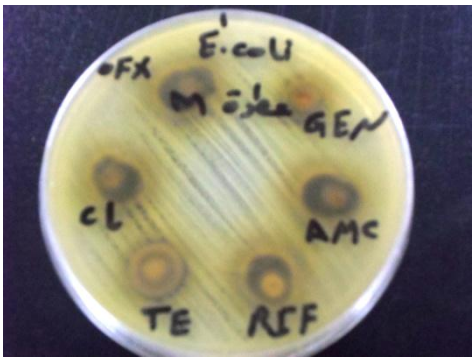


Figure 4.35. F. combination of. *Pelargonium graveolen* methanolic extract with antibiotics against *E. coli*

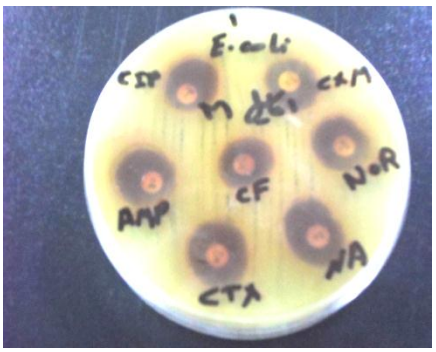


Figure 4.35. G. combination of *Rosmarinus officinalis* methanolic extract with antibiotics against *E. coli*

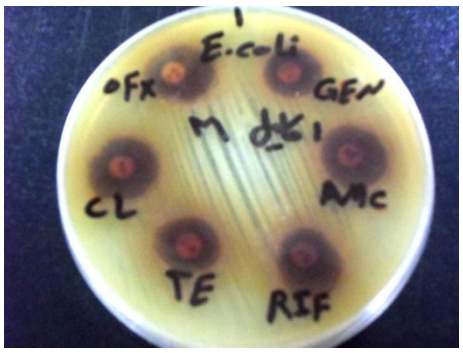


Figure 4.35. H. combination of *Rosmarinus officinalis* methanolic extract with antibiotics against *E. coli*

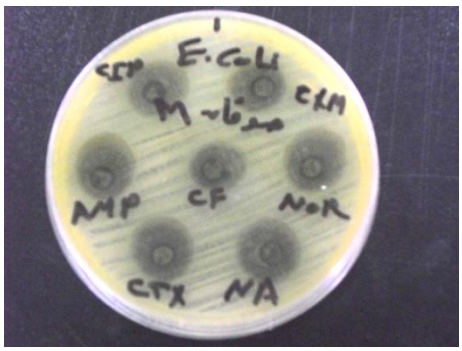


Figure 4.35. I. combination of *Phagnalon rupestre* methanolic extract with antibiotics against *E. coli*

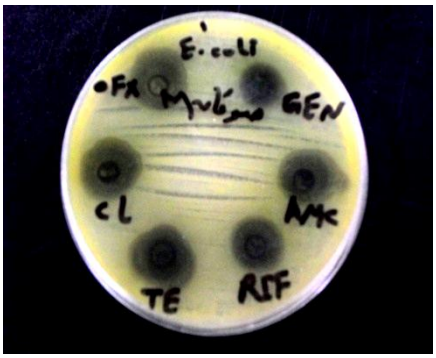


Figure 4.35. J. combination of *Phagnalon rupestre* methanolic extract with antibiotics against *E. coli*

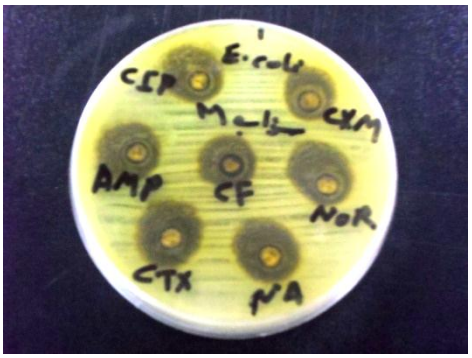


Figure 4.35. K. combination of *Ruta graveolen* methanolic extract with antibiotics against *E. coli*

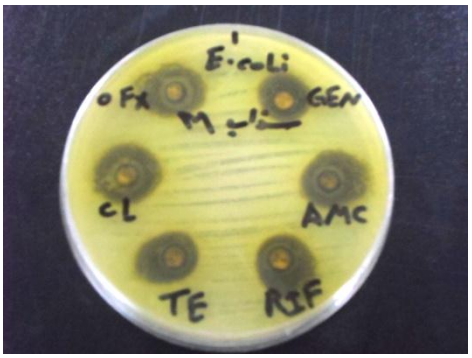


Figure 4.35. L. combination of *Ruta graveolen* methanolic extract with antibiotics against *E. coli*

4.3.2.4. The essential oils:

Table 4.20 & Figures 4.36. A - L summarizes the synergistic effect of Essential oils against *E. coli*, which was extracted by using steam distillation apparatus for 4 hours.

Table 4.20. Synergistic activity of different EOs with different antibiotics against *E. coli*:

Essential Oils		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN
	Antibiotic alone / extract	0	0	0	0	0	0	0	0	0	9	8	0	11
A	12.00	**17**	**16**	**16**	**15**	**16**	**14**	**15**	**17**	**16**	**14**	**14**	**14.5**	**14**
B	8.33	0	7	0	0	0	0	0	0	7	7	8	**10**	**12**
C	10.00	**18**	**16**	**17**	**17**	**16.5**	**16.5**	**17**	**16.5**	**15**	**15.5**	**14**	**15**	**13**
D	10.66	**16**	**14**	**14.5**	**14.5**	**15.5**	**13**	**15**	**17**	**14**	**15**	**15**	**14.5**	**14**
E	10.00	8	8.5	7.5	8	8	9	8	8	8	8	**11**	**12**	**13**
F	7.33	**8**	0	7	**8**	**8**	**8.5**	0	**10**	**9.5**	**10**	**10**	**10**	**12**

(**A:** ***Allium sativum,*** **B:** ***Ecballium elaterium,*** **C:** ***Pelargonium graveolen,*** **D:** ***Rosmarinus officinalis,*** **E:** ***Phagnalon rupestre*** **&** **F:** ***Ruta-graveolens*****).**

Among the six EOs (i.e. A. *Allium sativum* (bulbes) EO , B. *Ecballium elaterium* (fruites) EO ,C. *Pelargonium graveolen* (shoots) EO , D. *Rosmarinus officinalis* (shoots) EO, E. *Phagnalon rupestre* (shoots) EO &F. *Ruta graveolens* (leaves) EO which had different degree of effect ranged between synergistic, antagonistic or additive effect on *E. coli* when added as crude extracts of 50 µl /disc of 200 mg/ml), *Allium sativum* (bulbes) EO, *Pelargonium graveolen* (shoots) EO & *Rosmarinus officinalis* (shoots) EO had the most synergistic inhibitory effects with all the tested antibiotics against *E. coli*, but *Allium sativum* (bulbes) EO had the best synergistic effects with CIP, AM, CTX, NOR, OFX & CL with inhibition zone diameter of 17, 16, 16, 16, 17 & 16 mm respectively, and *Pelargonium graveolen* (shoots) EO had the best synergistic effects with CIP, CTX, NA & CF with inhibition zone diameter of 18, 17, 17 & 17 mm respectively against *E. coli*, and *Rosmarinus officinalis*(shoots) EO had the best synergistic effects with CIP, NOR & OFX with inhibition zone diameter of 16, 15.5 & 17 mm respectively. *Ecballium elaterium* (fruites) EO when combined with the antibiotics had synergistic effects with AMC & GEN against *E. coli* and was able to suppress the growth of this bacterium, and had antagonistic effects with AM, CL, TE & RIF, and had additive effects with the rest of antibiotics.

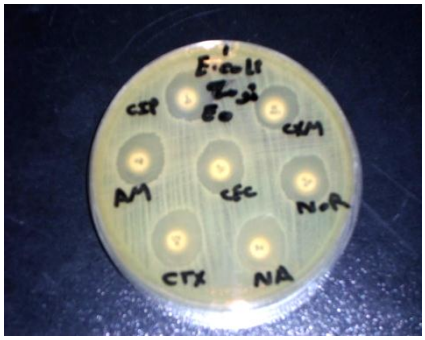


Figure 4.36. A. combination of *Allium sativum* EO with antibiotics against *E. coli*

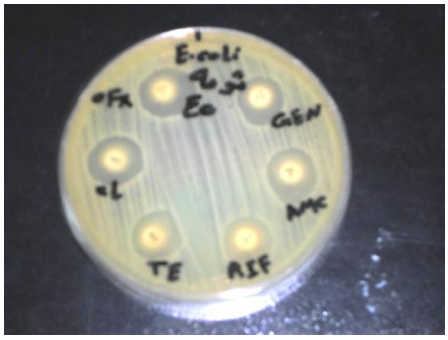


Figure 4.36. B. combination of *Allium sativum* EO with antibiotics against *E. coli*

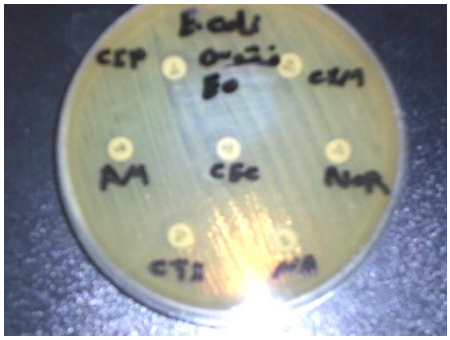


Figure 4.36. C. combination of *Ecballium e-laterium* EO with antibiotics against *E. coli*

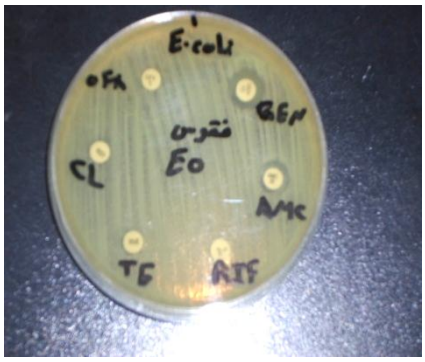


Figure 4.36. D. combination of *Ecballium elaterium* EO with antibiotics against *E. coli*

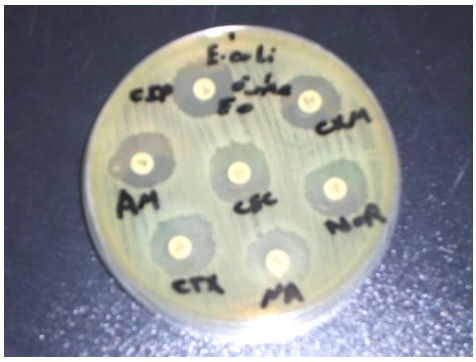


Figure 4.36. E. combination of. *Pelargonium graveolen* EO with antibiotics against *E. coli*

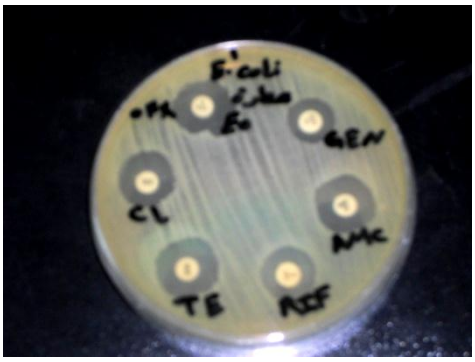


Figure 4.36. F. combination of. *Pelargonium graveolen* EO with antibiotics against *E. coli*

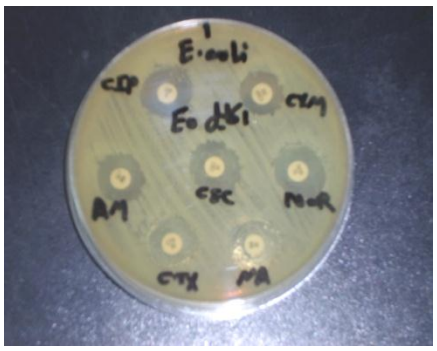


Figure 4.36. G. combination of *Rosmarinus officinalis* EO with antibiotics against *E. coli*

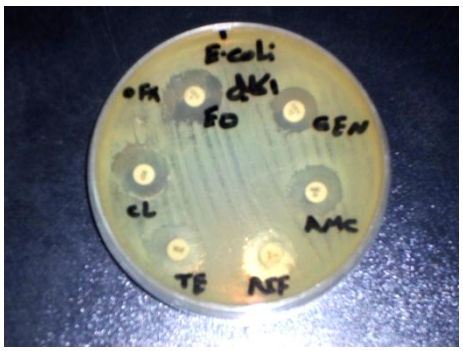


Figure 4.36. H. combination of *Rosmarinus officinalis* EO with antibiotics against *E. coli*

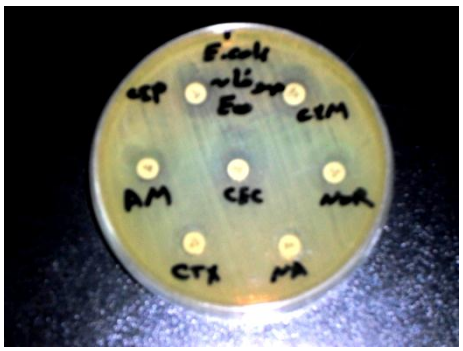


Figure 4.36. I. combination of *Phagnalon rupestre* EO with antibiotics against *E. coli*

Figure 4.36. J. combination of *Phagnalon rupestre* EO with antibiotics against *E. coli*

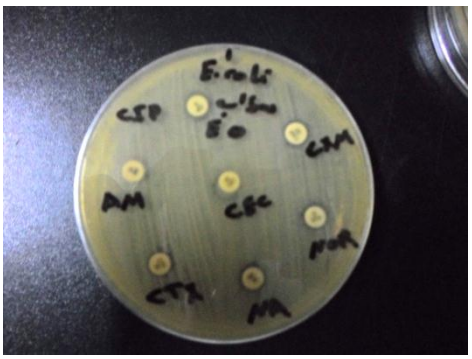


Figure 4.36. K. combination of *Ruta graveolen* EO with antibiotics against *E. coli*

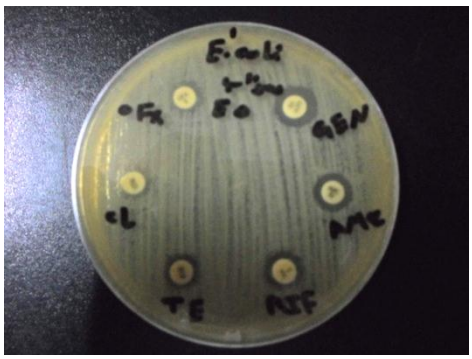


Figure 4.36. L. combination of *Ruta graveolen* EO with antibiotics against *E. coli*

Phagnalon rupestre (shoots) EO when combined with the tested antibiotics had a synergistic effect with RIF, AMC & GEN with inhibition zones of 11, 12 & 13 mm respectively, and had antagonistic effects with the rest of the tested antibiotics. *Ruta-graveolens*(leaves) EO had different degree of effect ranged between synergistic, antagonistic or additive effect on *E. coli* when added as crude extracts of 50 µl /disc, which had a synergistic inhibitory effects with CIP, NA, NOR, CXM, OFX, CL, TE, AMC & GEN, but had the best synergism with OFX, AMC & GEN with inhibition zones of 10, 10, 12 mm respectively and was able to suppress the *E. coli* growth, and had antagonistic effect with CTX, and had additive effects with AM & CF antibiotics. Overall, the best synergism between the EOs and antibiotics against *E. coli* was presented in Table 4.21.

Table 4.21. The best synergism with EOs against *E. coli:*

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC	GEN
Essential Oil	**Group**	C	C	C	C	C	C	C	A, D	A	C	D	C	A, D
	Means	18.00	16.33	17.00	17.00	16.50	16.50	17.00	17.00	16.00	15.50	15.00	15.00	14.00
	SD	0.50	0.57	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.50	0.50	0.50	0.50
	F	325.74	350.44	633.12	263.40	279.93	175.20	614.74	303.98	72.20	66.44	36.48	32.20	3.84
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.026

A: ***Allium sativum*****, C:** ***Pelargonium graveolen*** **& D:** ***Rosmarinus officinalis***

4.3.3. Synergistic effect against *Klebsilla pneumoniae*:

This experiment was carried out to screen the effect of *Allium sativum* (bulbes), *Ecballium elaterium* (fruites), *Pelargonium graveolen* (shoots), *Rosmarinus officinalis* (shoots), *Phagnalon rupestre* (shoots) *& Ruta-graveolens* (leaves) extracts in combination with selected antibiotics to identify systems, which might be used to improve the efficiency of these antibiotics against *K. pneumoniae.* The extracts were added as 50 µl /disc of 200 mg/ml concentration.

4.3.3.1. The aquatic extracts:

Table 4.22 & Figures 4.37. A - L summarizes the synergistic effect of plant extracts against *K. pneumoniae,* which was extracted using distilled water for 8 hours.

Among the six extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf) added as crude extract of

Figure 4.37. A. combination of *Allium sativum* aquatic extract with antibiotics against *K. pneumoniae*

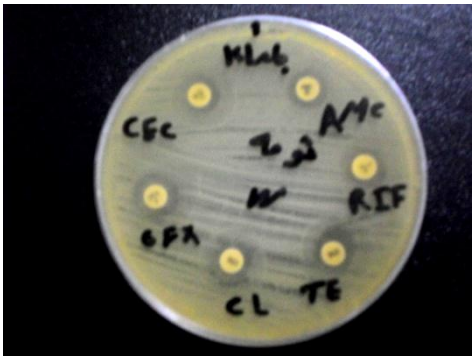


Figure 4.37. B. combination of *Allium sativum aquatic* extract with antibiotics against *K. pneumoniae*

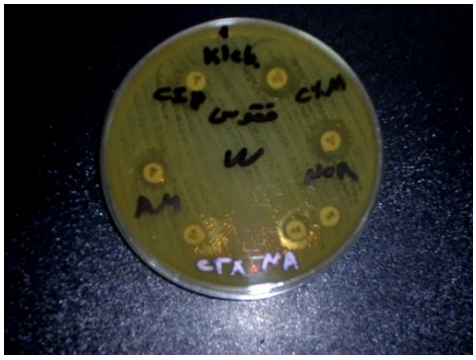


Figure 4.37. C. combination of *Ecballium elaterium aquatic* extract with antibiotics against *K. pneumoniae*

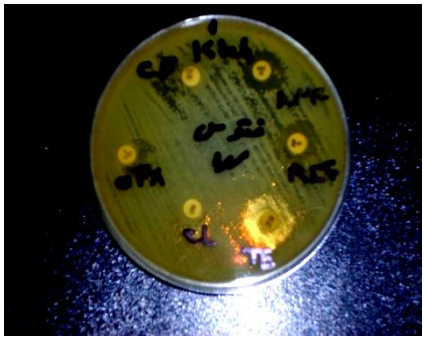


Figure 4.37. D. combination of *Ecballium elaterium* aquatic extract with antibiotics against *K. pneumoniae*

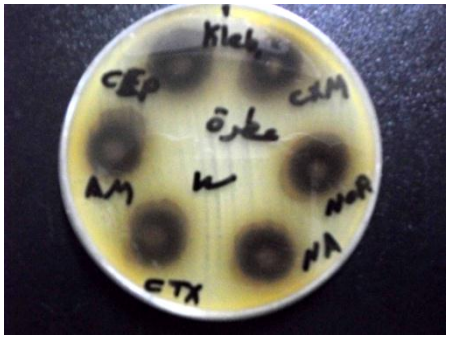


Figure 4.37. E. combination of. *Pelargonium graveolen* aquatic extract with antibiotics against *K. pneumoniae*

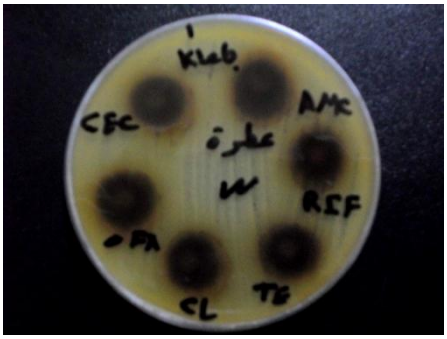


Figure 4.37. F. combination of. *Pelargonium graveolen* aquatic with antibiotics extract against *K. pneumoniae*

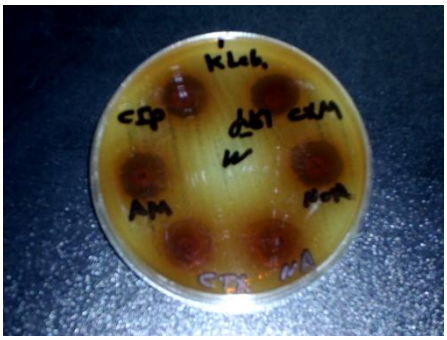


Figure 4.37. G. combination of *Rosmainus officinalis* aquatic extract with antibiotics against *K. pneumoniae*

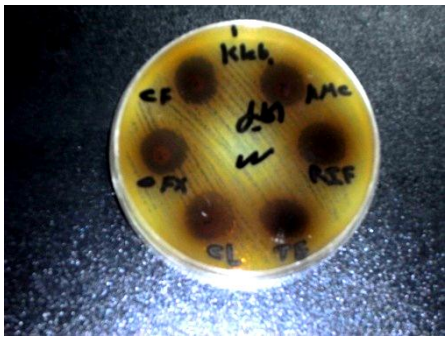


Figure 4.37. H. combination of *Rosmarinus officinalis* aquatic extract with antibiotics against *K. pneumoniae*

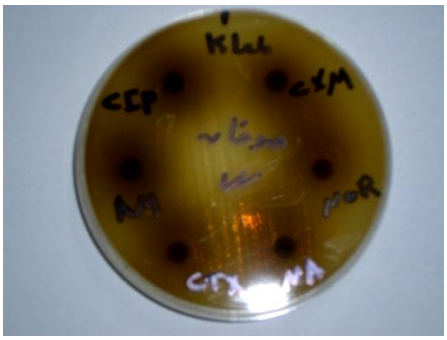


Figure 4.37. I. combination of *Phagnalon rupestre* aquatic extract with antibiotics against *K. pneumoniae*

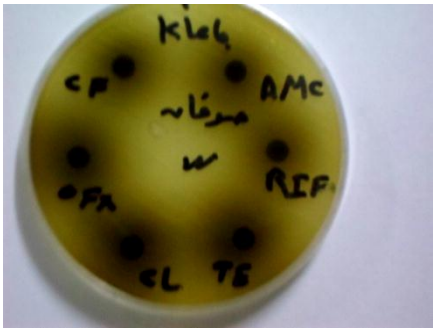


Figure 4.37. J. combination of *Phagnalon rupestre* aquatic extract with antibiotics against *K. pneumoniae*

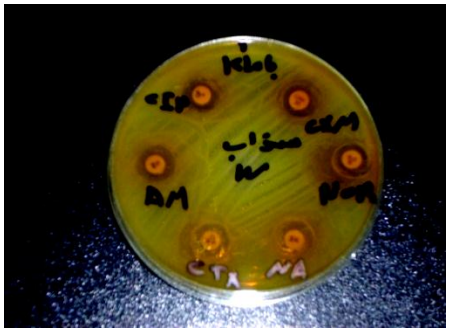


Figure 4.37. K. combination of *Ruta graveolen* aquatic extract with antibiotics against *K. pneumoniae*

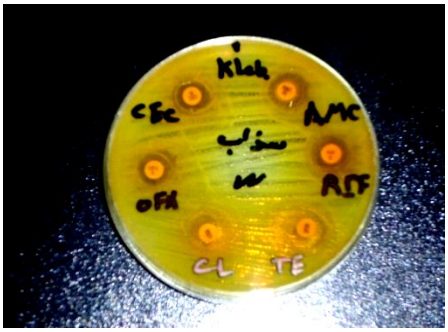


Figure 4.37. L. combination of *Ruta graveolen* aquatic extract with antibiotics against *K. pneumoniae*

50µl /disc of 200 mg/ml concentration, *Allium sativum* (bulbes) aquatic extract when combined with the selected antibiotics had a synergistic effects with all of these antibiotics and was able to suppress the *K. pneumoniae* growth except with TE which had antagonistic inhibitory effects, but the best synergism was observed with CTX with inhibition zone diameter of 14 mm. *Ecballium elaterium* (fruites) aquatic extract had a synergistic effect when combined with AM, NA, NOR, CXM, TE, RIF & AMC, but the best synergism was observed with TE & AMC with inhibition zone diameter of 16 & 14 mm, and had antagonistic effects with CF, and had additive effects with the rest of the selected antibiotics.

Table 4.22. Synergistic activity of different plant extracts with different antibiotics against *K. pneumoniae*:

			CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Aquatic	Antibiotic alone		0	0	0	0	0	0	0	0	0	10	8	0
	extract													
	A	8.00	**11**	**12**	**14**	**12**	**10**	**10**	**10**	**11**	**10**	9	**10**	**10**
	B	7.50	0	**9**	0	**12**	**13**	**12**	7	0	0	**16**	**11**	**14**
	C	13.00	**14**	12.5	13	**13.5**	**14**	12.5	**14**	**15**	12	13	12	**14**
	D	8.66	**14.5**	**15**	**16**	**14**	**14.5**	**13.5**	**13**	**15.5**	**16.5**	**12.5**	**14.5**	**14.5**
	E	7.66	0	0	0	0	0	0	0	**8**	**10**	0	0	**10**
	F	8.00	**11.5**	**12**	**11**	**13**	**11**	**12**	**11**	**10.5**	**12**	**11.5**	**13**	**12**

(A: *Allium sativum*, B: *Ecballium elaterium*, C: *Pelargonium graveolen*, D: *Rosmarinus officinalis*, E: *Phagnalon rupestre* & F: *Ruta-graveolens*).

Pelargonium graveolen (shoots) extract had a synergistic effect with most the tested antibiotics including CIP, NA, NOR, CXM, CF, OFX & AMC, but the best synergism was observed with OFX, CIP, NOR, CF & AMC with inhibition zone diameter of 15 mm for OFX and 14 mm for CIP, NOR, CF &AMC.

Rosmarinus officinalis (shoots) aquatic extract when added to the antibiotics it was observed to be had a synergistic effects with all of these antibiotics and was able to suppress the *K. pneumoniae* growth, but the best synergism was observed with AM, CTX, OFX & CL with inhibition zone diameter of 15, 16, 15.5 & 16.5 mm. *Phagnalon rupestre* (shoots) extract when combined with the selected antibiotics was observed to be had a synergistic effects with OFX, CL & AMC with inhibition zone diameter of 8, 10 & 10 mm, and had antagonistic effects with TE & RIF, and had additive effects with the rest of these selected antibiotics . *Ruta-graveolens* (leaves)

aquatic extract when added as crude extract of 50µl / disc of 200 mg/ml concentration to antibiotics it was had synergistic effects with all the tested antibiotics, but the best synergism was observed when the *Ruta graveolens* (leaves) aquatic extract was combined with AM, NA, CXM, CL & AMC with inhibition zone diameter of 12, 13, 12, 12 & 12 mm respectively, and was able to suppress the *K. pneumoniae* growth. Overall, the best synergism between the aquatic extracts and antibiotics against *E. coli* was presented in Table 4.23.

Table 4.23. The best synergism with aquatic extracts against *K. pneumoniae:*

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Aquatic	**Group**	D	D	D	D	D	D	C	D	**D**	B	D	**D**
	Means	14.50	15.00	16.00	14.00	14.50	13.50	14.00	15.50	16.50	16.00	14.66	**14.50**
	SD	1.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.28	**0.50**
	F	250.34	144.21	283.57	120.17	123.00	130.11	134.57	210.11	127.23	158.23	119.64	**20.36**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**0.001**

B: ***Ecballium elaterium***, C: ***Pelargonium graveolen*** & D: ***Rosmarinus officinalis***

4.3.3.2. The ethanolic extracts:

Table 4.24 & Figures 4.38. A - L summarizes the synergistic effect of all plant extracts against *K. pneumoniae*, which was extracted using 80% ethanol for 8 hours. The six plant extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf), had different degree of effect ranged between synergistic, antagonistic or additive effect on *E. coli* when added as crude extracts of 50µl / disc of 200 mg/ml concentration.

Table 4.24. Synergistic activity of different plant extracts with different antibiotics against *K. pneumoniae*:

			CIP	**AM**	**CTX**	**NA**	**NOR**	**CXM**	**CF**	**OFX**	**CL**	**TE**	**RIF**	**AMC**
Ethanolic	Antibiotic alone		0	0	0	0	0	0	0	0	0	10	8	0
	Extract													
	A	10.00	10	9	10	**12**	**11**	**13**	**11**	**12**	10	10	10	10
	B	10.00	**14**	**13**	**13**	**12**	**12.5**	**11**	**11**	**12.5**	10	**16.5**	**12**	**11.5**
	C	16.00	14	15	14.5	13	14.5	15	12.5	12	12.5	14	14	15
	D	9.66	**12**	**12.5**	**12.5**	**13.5**	**13**	8.5	**12**	**13**	**12.5**	**12.5**	8	**13**
	E	7.33	**10**	**10**	**9**	**8.5**	**9**	**9.5**	**11**	**9**	**10.5**	7	0	**9**
	F	9.00	**11**	**14**	**12**	**11.5**	**12**	**12.5**	**10.5**	**12**	**11**	**12.5**	**13**	**13.5**

(A: *Allium sativum*, B: *Ecballium elaterium*, C: *Pelargonium graveolen*, D: *Rosmarinus officinalis*, E: *Phagnalon rupestre* & F: *Ruta-graveolens*).

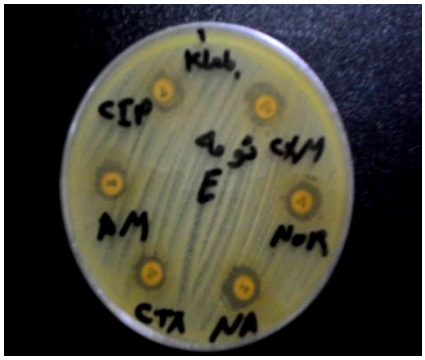


Figure 4.38. A. combination of *Allium sativum* ethanolic extract with antibiotics against *K. pneumoniae*

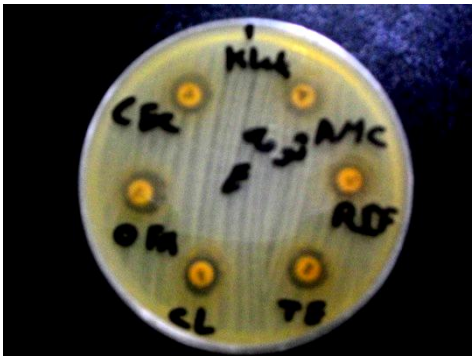


Figure 4.38. B. combination of *Allium sativum* ethanolic extract with antibiotics against *K. pneumoniae*

Figure 4.38. C. combination of *Ecballium elaterium* ethanolic extract with antibiotics against *K. pneumoniae*

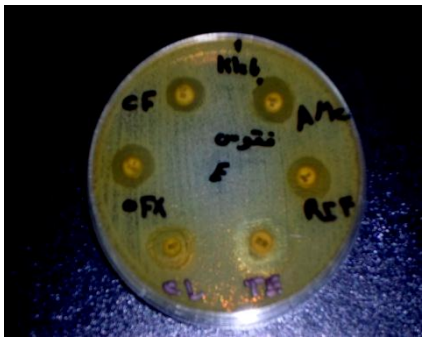


Figure 4.38. D. combination of *Ecballium elaterium* ethanolic extract with antibiotics against *K. pneumoniae*

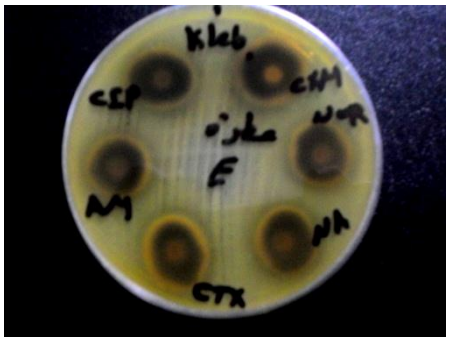


Figure 4.38. E. combination of. *Pelargonium graveolen* ethanolic extract with antibiotics against *K. pneumoniae*

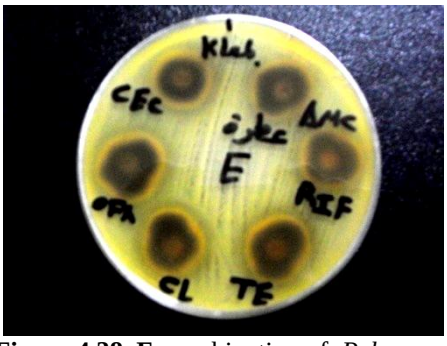


Figure 4.38. F. combination of. *Pelargonium graveolen* ethanolic with antibiotics extract against *K. pneumoniae*

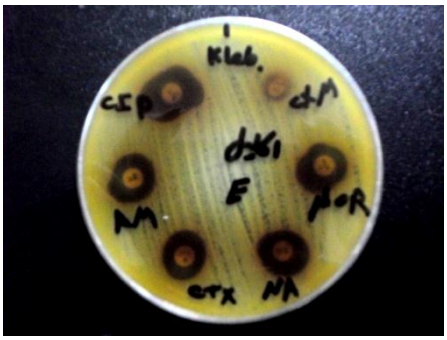


Figure 4.38. G. combination of *Rosmarinus officinalis* ethanolic extract with antibiotics against *K. pneumoniae*

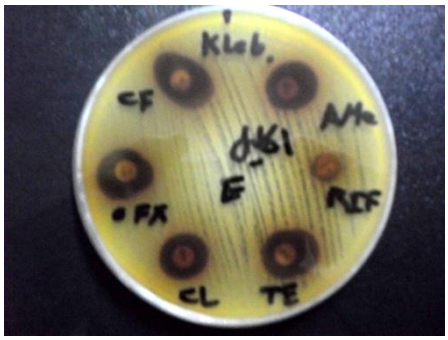


Figure 4.38. H. combination of *Rosmarinus officinalis* ethanolic extract with antibiotics against *K. pneumoniae*

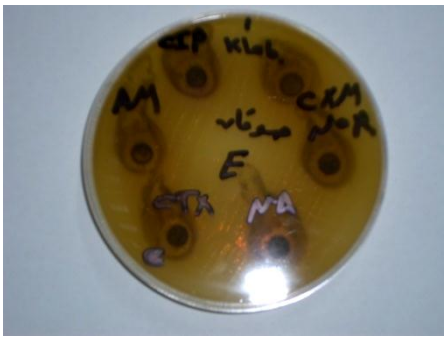


Figure 4.38. I. combination of *Phagnalon rupestre* ethanolic extract with antibiotics against *K. pneumoniae*

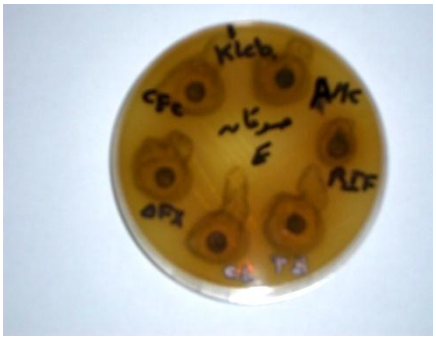


Figure 4.38. J. combination of *Phagnalon rupestre* ethanolic extract with antibiotics against *K. pneumoniae*

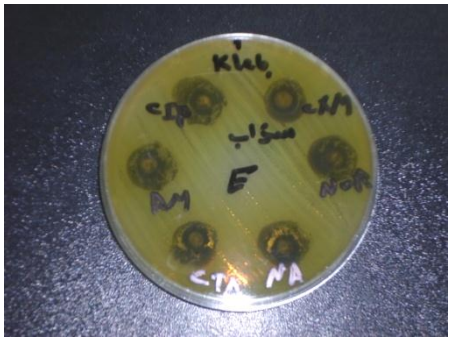


Figure 4.38. K. combination of *Ruta graveolen* ethanolic extract with antibiotics against *K. pneumoniae*

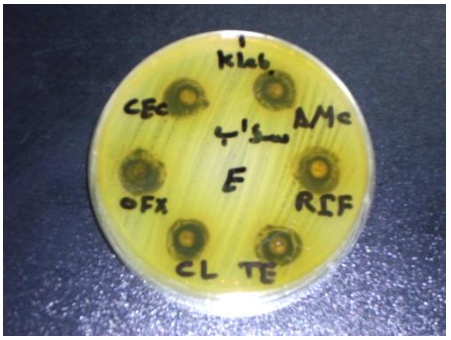


Figure 4.38. L. combination of *Ruta graveolen* ethanolic extract with antibiotics against *K. pneumoniae*

The results in table 4.24 showed that *Allium sativum* (bulbes) ethanolic extract when combined with the tested antibiotics had a synergistic effects with NA, NOR,, CXM, CF & OFX, but the best synergism was observed with NA, CF & OFX with inhibition zone diameter of 12, 13 & 12 mm respectively and was able to suppress the *K. pneumoniae* growth, and had additive effect with TE, and had antagonistic effect with the rest of antibiotics. *Ecballium elaterium* (fruites) ethanolic extract had a synergistic effect when combined with all the tested antibiotics except with CL, which had additive effect, but the best synergism was observed with CIP & TE with inhibition zone diameter of 14 & 16.5 mm respectively. *Pelargonium graveolen* (shoots) ethanolic extract had antagonistic effect with all the tested antibiotics. *Rosmarinus officinalis* (shoots) ethanolic extract when added to the antibiotics as crude extracts of 50µl /disc it was observed to be had a synergistic effects with all of these antibiotics except with CXM & RIF which had antagonistic & additive effects respectively, but the best synergism was observed with NA, NOR, OFX & AMC with inhibition zone diameter of 13.5, 13, 13 & 13 mm respectively and was able to suppress the *K. pneumoniae* growth. *Phagnalon rupestre* (shoots) extract when combined with the selected antibiotics it was observed to be had a synergistic effects with all of these antibiotics except with TE & RIF, which had antagonistic effects, but the best synergism was observed with CF & CL with inhibition zone diameter of 11 & 10.5 mm respectively and was able to suppress the *K. pneumoniae* growth. *Ruta-graveolens* (leaves) ethanolic extract when added as crude extract of 50 µl /disc to antibiotics it was had a synergistic effects with all the tested antibiotics, but the best synergism was observed when the *Ruta graveolens* (leaves) ethanolic extract was combined with AM, CXM & AMC with inhibition zone diameter of 14, 12.5 & 13.5 mm respectively. Overall, the best synergism between the ethanolic extracts and antibiotics against *K. pneumoniae* was presented in Table 4.25.

Table 4.25. The best synergism with ethanolic extracts against *K. pneumoniae:*

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Ethanolic	**Group**	C	F	B	D	D	C	D	D	D	B	C	F
	Means	14.00	15.00	13.00	13.50	14.50	13.00	12.00	13.00	12.50	16.50	13.00	13.50
	SD	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.00
	F	10.10	18.43	20.91	14.76	14.00	40.35	2.27	6.77	6.44	96.67	172.15	33.38
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.114	0.003	0.004	0.001	0.001	0.001

B: *Ecballium elaterium*, C: *Pelargonium graveolen* & D: *Rosmarinus officinalis* & *F: Ruta-graveolens*.

4.3.3.3. The methanolic extracts:

Table 4.26 & Figures 4.39. A - L summarizes the synergistic effect of all plant extracts against *K. pneumoniae*, which was extracted using methanol for 8 hours by using soxhlet apparatus.

The six plant extracts (i.e. A. *Allium sativum* (bulbes) , B. *Ecballium elaterium* (fruite) , C. *Pelargonium graveolens* (shoots) , D. *Rosmarinus officinalis* (shoots) , E. *Phagnalon rupestre* (shoots) & F. *Ruta graveolens* (leaf) had different degree of effects ranged between synergistic, antagonistic or additive effect on *K. pneumoniae* when added as crude extracts of 50 µl / disc of 200 mg/ml concentration. The results in table 4.26 showed that *Allium sativum* (bulbes) methanolic extract had a synergistic effects when combined with CIP, NOR, CF, OFX, CL & TE, but the best synergism was observed with NOR & OFX with inhibition zone diameter of 12.5 & 13 mm respectively and was able to suppress the *K. pneumoniae* growth, and had antagonistic effect with CXM and additive effects with the rest of antibiotics.

Ecballium elaterium (fruites) methanolic extract when added as crude extract of 50 µl /disc to the antibiotics it was had a synergistic effects with all the tested antibiotics, but the best synergism was observed when the *Ecballium elaterium* (fruites) methanolic extract was combined with CIP, AM & NOR with inhibition zone diameter of 15, 14.5 & 14.5 mm respectively.

Pelargonium graveolen (shoots) methanolic extract had antagonistic effect with all the tested antibiotics. *Rosmarinus officinalis* (shoots) methanolic extract when added to the antibiotics as crude extracts of 50µl /disc it was observed to be had a synergistic effects with all of these antibiotics, but the best synergism was observed when the *Rosmarinus officinalis* (shoots) methanolic extract added to the CF with inhibition zone diameter of 15 mm & OFX & AMC with inhibition zone diameter of 14.5 mm for both and was able to suppress the *K. pneumoniae* growth. *Phagnalon rupestre* (shoots) extract when combined with the selected antibiotics it was observed to be had a synergistic effects with CL & TE, and had additive effects with NOR & RIF, and had antagonistic effects with the rest of antibiotics. *Ruta graveolens* (leaves) methanolic extract when added as crude extract of 50 µl /disc to the antibiotics it was had a synergistic effects with all the tested antibiotics except with TE which had additive effects, but the best synergism was observed when the *Ruta graveolens*

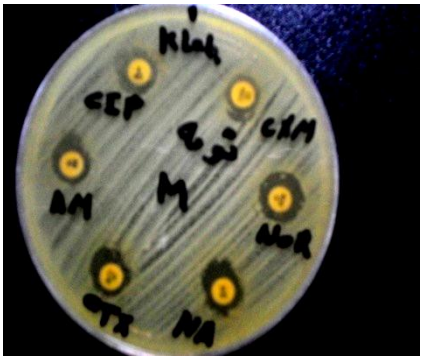


Figure 4.39. A. combination of *Allium sativum* methanolic extract with antibiotics against *K. pneumoniae*

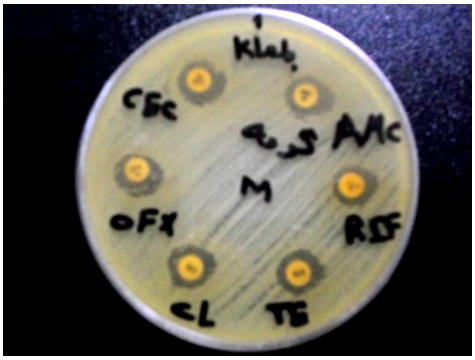


Figure 4.39. B. combination of *Allium sativum* methanolic extract with antibiotics against *K. pneumoniae*

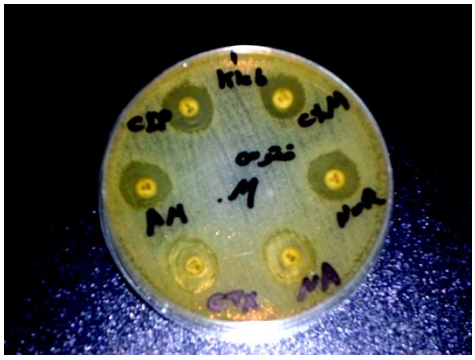


Figure 4.39. C. combination of *Ecballium elaterium* methanolic extract with antibiotics against *K. pneumoniae*

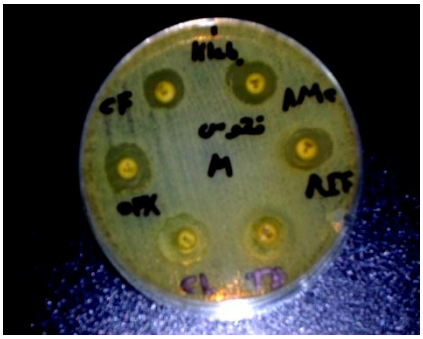


Figure 4.39. D. combination of *Ecballium elaterium* methanolic extract with antibiotics against *K. pneumoniae*

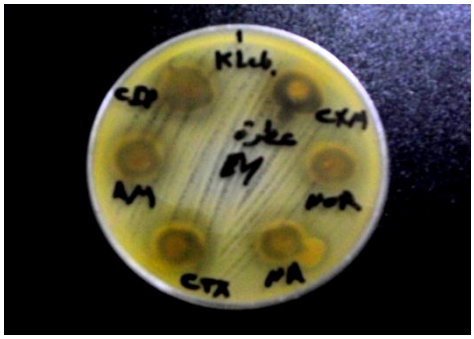


Figure 4.39. E. combination of *Pelargonium graveolen* methanolic extract with antibiotics against *K. pneumoniae*

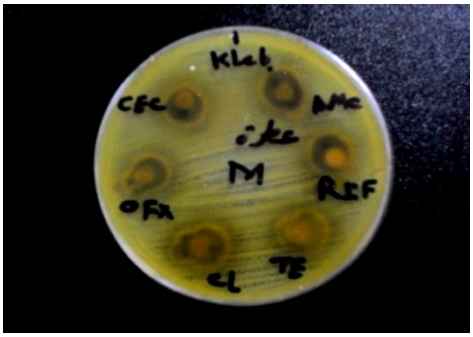


Figure 4.39. F. combination of *Pelargonium graveolen* methanolic extract with antibiotics extract against *K. pneumoniae*

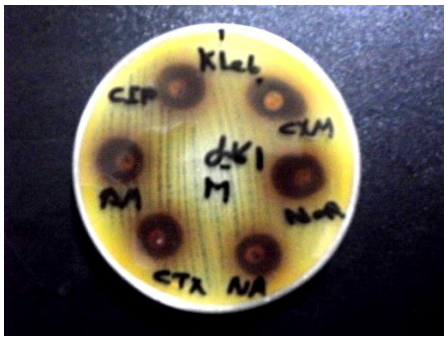


Figure 4.39. G. combination of *Rosmarinus officinalis* methanolic extract with antibiotics against *K. pneumoniae*

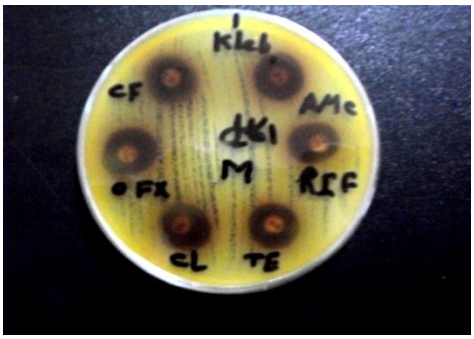


Figure 4.39. H. combination of *Rosmarinus officinalis* methanolic extract with antibiotics against *K. pneumoniae*

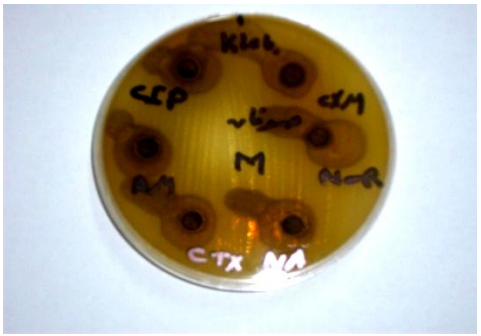


Figure 4.39. I. combination of *Phagnalon rupestre* methanolic extract with antibiotics against *K. pneumoniae*

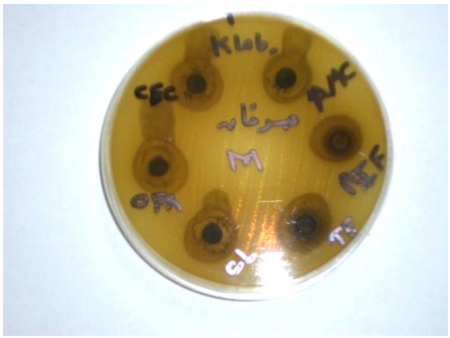


Figure 4.39. J. combination of *Phagnalon rupestre* methanolic extract with antibiotics against *K. pneumoniae*

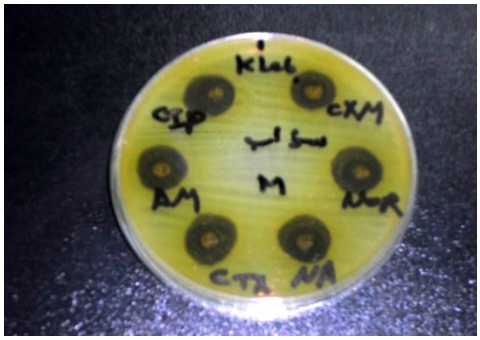


Figure 4.39. K. combination of *Ruta graveolen* methanolic extract with antibiotics against *K. pneumoniae*

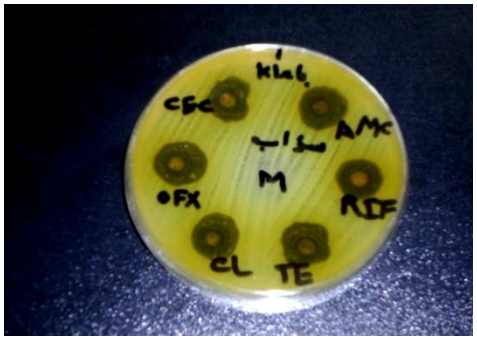


Figure 4.39. L. combination of *Ruta graveolen* methanolic extract with antibiotics against *K. pneumoniae*

(leaves) methanolic extract combined with AM, CTX & NOR with inhibition zone diameter of 15, 14 & 14.5 mm respectively.

Table 4.26. Synergistic activity of different plant extracts with different antibiotics against *K. pneumoniae*:

			CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Methanolic	Antibiotic alone		0	0	0	0	0	0	0	0	0	10	8	0
	Extract													
	A	10.00	**11**	10	10	10	**12.5**	9	**12**	**13**	**11**	**12**	10	10
	B	9.66	**15**	**14.5**	**14**	**14**	**14.5**	**13**	**12**	**13.5**	**13.5**	**13**	**11**	**14**
	C	17.00	11	10.5	12	11.5	12	11.5	12	12.5	11	11	11.5	12.5
	D	10.66	**14**	**13.5**	**13**	**12**	**14**	**13**	**15**	**14.5**	**14**	**14**	**12.5**	**14.5**
	E	11.00	8	7	8.5	8	0	9	10	8	**12**	**12**	11	10
	F	11.00	**13.5**	**15**	**14**	**12**	**14.5**	**13.5**	**12**	**11.5**	**12**	11	**13**	**13.5**

(**A:** ***Allium sativum,*** **B:** ***Ecballium elaterium,*** **C:** ***Pelargonium graveolen,*** **D:** ***Rosmarinus officinalis,*** **E:** ***Phagnalon rupestre*** **&** **F:** ***Ruta-graveolens*****).**

Overall, the best synergism between the methanolic extracts and antibiotics against *K. pneumoniae* was presented in Table 4.27.

Table 4.27. The best synergism with methanolic extracts against *K. pneumoniae:*

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Methanolic	**Group**	B	F	F,B	B	F, B	F	D	D	D	D	F	**D**
	Means	15.00	15.00	14.00	14.00	14.50	13.50	15.00	14.50	14.00	14.00	13.00	**14.50**
	SD	1.00	0.50	0.50	1.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	**0.50**
	F	22.77	58.05	24.20	14.31	161.87	16.80	8.80	31.31	5.40	4.68	5.76	**23.65**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.008	0.013	0.006	**0.001**

B: ***Ecballium elaterium*****, D:** ***Rosmarinus officinalis,*** **F:** ***Ruta graveolens***

4.3.3.4. The essential oils:

Table 4.28 & Figures 4.40. A - L summarizes the synergistic effect of Essential oils against *K. pneumoniae, which* was extracted by using steam distillation apparatus for 4 hours. Among the six EOs (i.e. A. *Allium sativum* (bulbes) EO, B. *Ecballium elaterium* (fruite) EO, C. *Pelargonium graveolens* (shoots) EO, D. *Rosmarinus officinalis* (shoots) , E. *Phagnalon rupestre* (shoots) EO & F. *Ruta-graveolens*(leaf) EO), *Allium sativum* (bulbes) EO, *Pelargonium graveolen* (shoots) EO & *Rosmarinus officinalis* (shoots) EO when added as crude extracts of 50 µl / disc of 200 mg/ml concentration it was had the most synergistic effects with all antibiotics

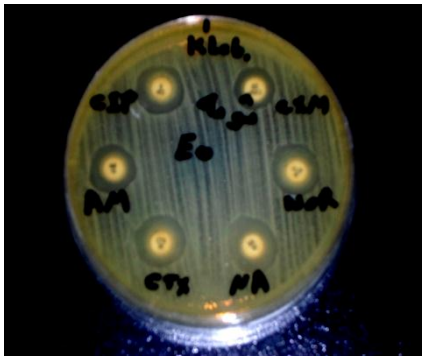


Figure 4.40. A. combination of *Allium sativum* EO with antibiotics against *K. pneumoniae*

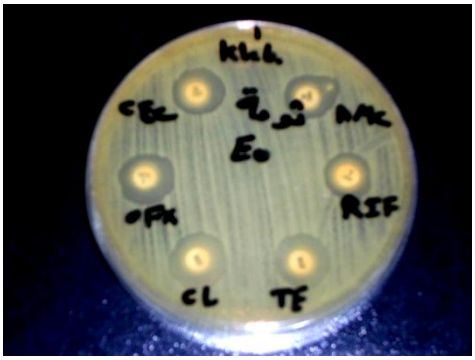


Figure 4.40. B. combination of *Allium sativum* EO with antibiotics against *K. pneumoniae*

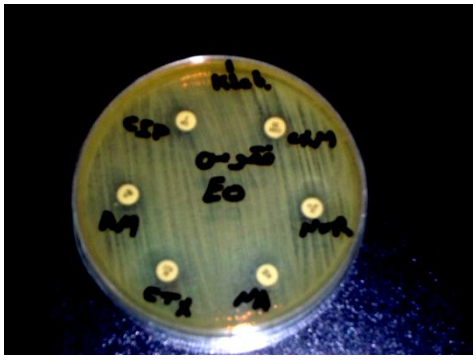


Figure 4.40. C. combination of *Ecballium elaterium* EO with antibiotics against *K. pneumoniae*

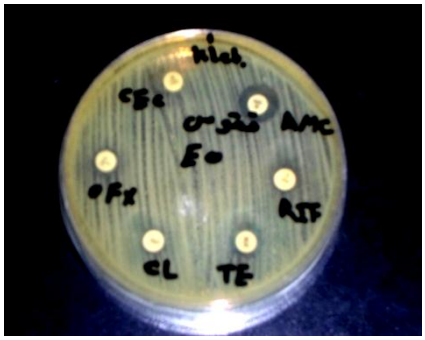


Figure 4.40. D. combination of *Ecballium elaterium* EO with antibiotics against *K. pneumoniae*

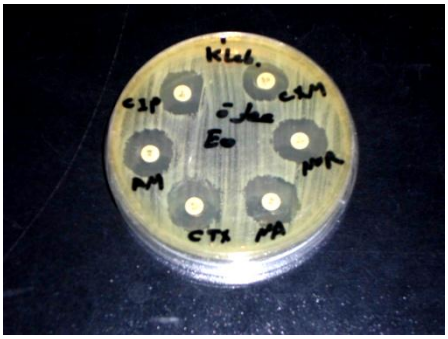


Figure 4.40. E. combination of. *Pelargonium graveolen* EO with antibiotics against *K. pneumoniae*

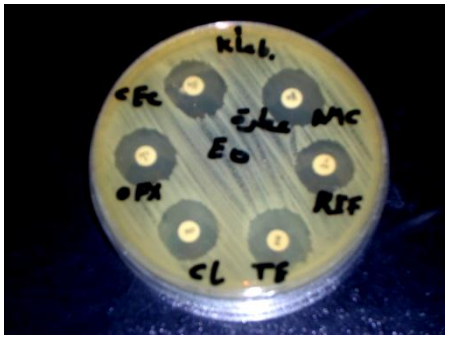


Figure 4.40. F. combination of. *Pelargonium graveolen* EO with antibiotics extract against *K. pneumoniae*

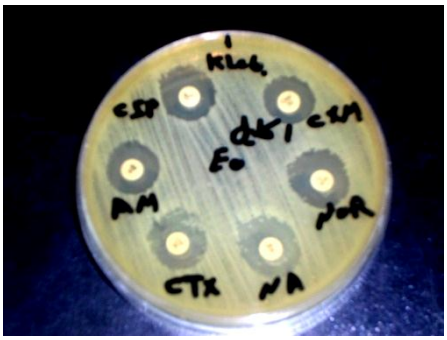


Figure 4.40. G. combination of *Rosmarinus officinalis* EO with antibiotics against *K. pneumoniae*

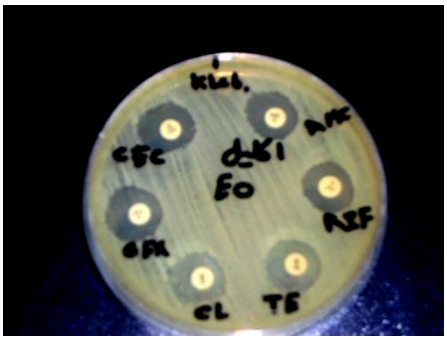


Figure 4.40. H. combination of *Rosmarinus officinalis* EO with antibiotics against *K. pneumoniae*

Figure 4.40. I. combination of *Phagnalon rupestre* EO with antibiotics against *K. pneumoniae*

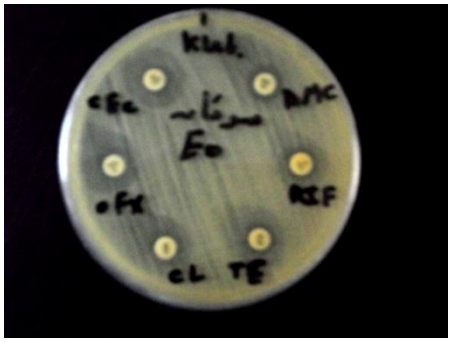


Figure 4.40. J. combination of *Phagnalon rupestre* EO with antibiotics against *K. pneumoniae*

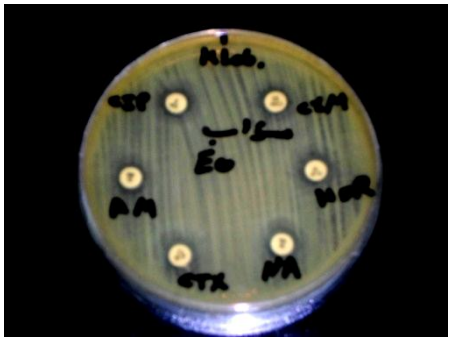


Figure 4.40. K. combination of *Ruta graveolen* EO with antibiotics against *K. pneumoniae*

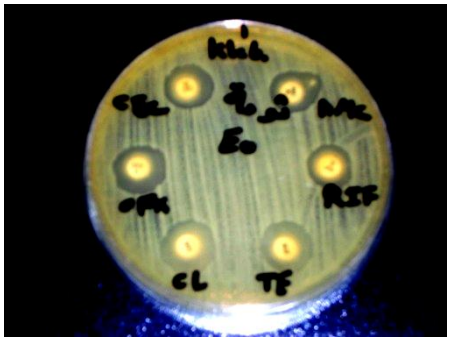


Figure 4.40. L. combination of *Ruta graveolen* EO with antibiotics against *K. pneumoniae*

and was able to suppress the *K. pneumoniae* growth, but the best synergism for *Allium sativum* was observed with CIP, NOR, OFX & CL with inhibition zones diameter of 14, 14, 14 & 15 respectively, and the best synergism for *Pelargonium graveolens* (shoots) EO was observed when 50µl/disc was added to AM, CTX, NA & OFX with inhibition zones diameter of 18, 18.5, 19 & 18 mm respectively, and the best synergism for *Rosmarinus officinalis* (shoots) EO was observed when combined with CIP, NOR & CF with inhibition zones diameter of 15.5, 15.5 & 16 mm respectively.

Table 4.28. Synergistic activity of different EOs with different antibiotics against *K. pneumoniae*:

Essential Oils		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
	Antibiotic alone / Extract	0	0	0	0	0	0	0	0	0	10	8	0
A	10.00	**14**	**12**	**13.5**	**13.5**	**14**	**12.5**	**13**	**14**	**15**	**14**	**12**	**12.5**
B	8.00	0	7	0	0	**8.5**	8	0	7	8	10	**9**	**12.5**
C	11.33	**17**	**18**	**18.5**	**19**	**17.5**	**16**	**17**	**18**	**17.5**	**18**	**16.5**	**17.5**
D	11.66	**15.5**	**15**	**12.5**	**14.5**	**15.5**	**14**	**16**	**15**	**14**	**15**	**14.5**	**14**
E	9.66	9	0	7	8	7	9	8	0	0	8.5	9	10
F	9.66	9.5	9	9	9	**11**	**10**	9.5	**10**	9	10	9	**12**

(A: *Allium sativum*, B: *Ecballium elaterium*, C: *Pelargonium graveolen*, D: *Rosmarinus officinalis*, E: *Phagnalon rupestre* & F: *Ruta-graveolens*).

The results in table 4.28 also showed that *Ecballium elaterium* (fruites) EO had a synergistic effects with NOR, RIF & AMC, and had antagonistic effects with AM & OFX, and had additive effects with the rest of antibiotics, while *Phagnalon rupestre* (shoots) EO when combined with the tested antibiotics had additive effects with AM, OFX & CL, and had antagonistic effects with the rest of antibiotics, and *Ruta graveolens* (leaves) EO when added as crude extracts of 50 µl /disc it was had a synergistic effects with NOR, CXM, OFX & AMC with inhibition zones diameter of 11, 10, 10 & 12 mm respectively and was able to suppress the *K. pneumoniae* growth, and had additive effect with TE, and had antagonistic effects with the rest of the tested antibiotics. Overall, the best synergism between the EOs and antibiotics against *K. pneumoniae* was presented in Table 4.29.

Table 4.29. The best synergism with EOs against *K. pneumoniae:*

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Essential Oil	**Group**	C	C	C	C	C	C	C	C	C	C	C	**C**
	Means	17.00	18.00	18.50	19.00	17.50	16.00	17.00	18.00	17.50	18.00	16.50	**17.50**
	SD	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	0.50	0.50	0.50	**0.50**
	F	251.78	265.53	262.74	222.00	81.00	38.56	256.85	234.09	205.93	60.47	42.27	**30.44**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**0.001**

C: *Pelargonium graveolen*

4.3.4. Synergistic effect against *pseudomonas aeruginosa*:

The plant extracts differed significantly in their synergistic ability to inhibit the growth of *P. aeruginosa*, which was extracted using socxhlet apparatus for 8 hours.

4.3.4.1. The aquatic extracts:

Table 4.30 & Figures 4.41. A - L summarizes the synergistic effect of plant extracts against *p. aeruginosa*, which was extracted using distilled water for 8 hours in soxhlet extractor. Among the six extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf) when adds as crude extracts of 50µl/ disc of 200 mg/ml concentration), *Allium sativum* (bulbes) aquatic extract was observed to be had additive effects with all tested antibiotics except with CIP, AM, CXM, TE, RIF & AMC which had a synergistic effects against *P. aeruginosa*, and was able to inhibit the *P. aeruginosa* growth, while *Ecballium elaterium* (fruite) aquatic extract when added as 50µl/disc it was observed to be had additive effects with all tested antibiotics except with TE, RIF & AMC which had a synergistic effects against *P. aeruginosa*.

Table 4.30. Synergistic activity of different plant extracts with different antibiotics against *p. aeruginosa*:

			CIP	**AM**	**CTX**	**NA**	**NOR**	**CXM**	**CF**	**OFX**	**CL**	**TE**	**RIF**	**AMC**
Aquatic	Antibiotic alone		0	0	0	0	0	0	0	0	0	8	8	7
	Extract													
	A	7.00	**10**	**9**	0	0	0	**9**	0	0	0	**10**	**9**	**13**
	B	7.83	0	0	0	0	0	0	0	0	0	**10**	**9**	0
	C	11.66	8	7	10	10	**13**	**13**	**13**	**16**	**15**	**12**	**12**	**15**
	D	10.00	9	7	0	8	7	**11.5**	8	0	0	8	9	8
	E	7.00	7	7	**8**	**8**	**12**	**8.5**	**9**	7	**8**	7	0	0
	F	11.00	**14**	**13**	**12**	**12**	**12**	10	10	**13**	**14**	11	**12**	**14**

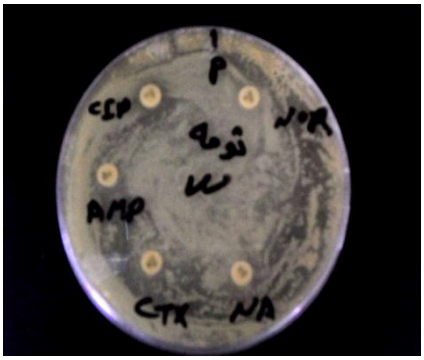


Figure 4.41. A. combination of *Allium sativum* aquatic extract with antibiotics against *P. aeruginosa*

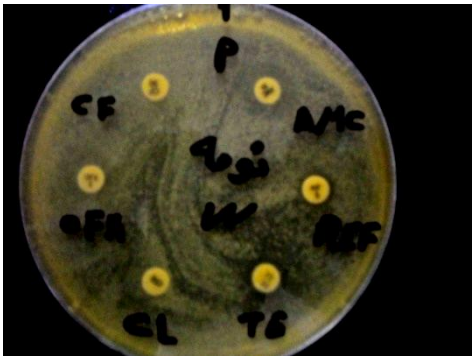


Figure 4.41. B. combination of *Allium sativum aquatic* extract with antibiotics against *P. aeruginosa*

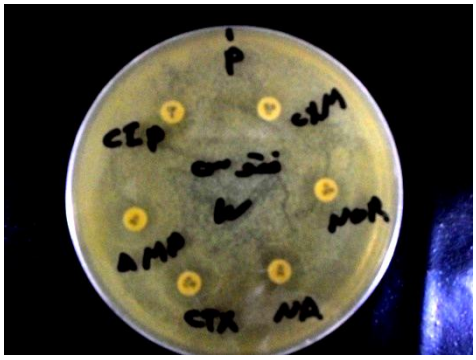


Figure 4.41. C. combination of *Ecballium elaterium aquatic* extract with antibiotics against *P. aeruginosa*

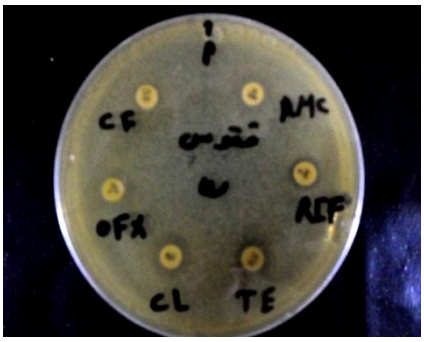


Figure 4.41. D. combination of *Ecballium elaterium* aquatic extract with antibiotics against *P. aeruginosa*

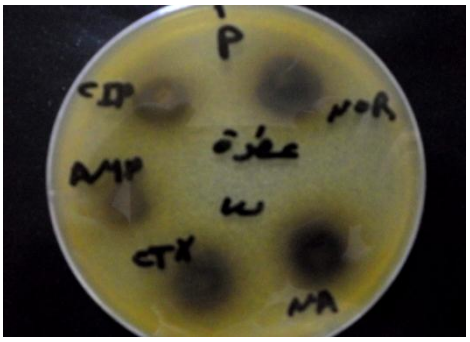


Figure 4.41. E. combination of. *Pelargoium graveolen* aquatic extract with antibiotics against *P. aeruginosa*

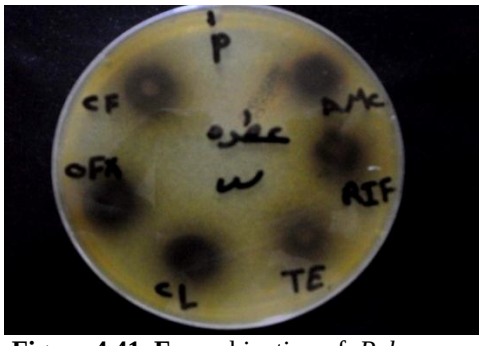


Figure 4.41. F. combination of. *Pelargonium graveolen* aquatic with antibiotics extract against *P. aeruginosa*

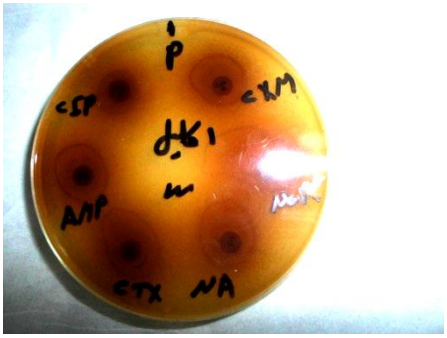


Figure 4.41. G. combination of *Rosmarinus officinalis* aquatic extract with antibiotics against *P. aeruginosa*

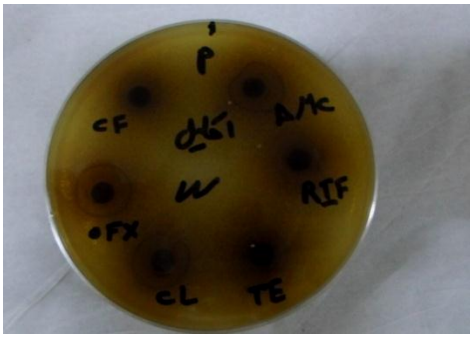


Figure 4.41. H. combination of *Rosmarinus officinalis* aquatic extract with antibiotics against *P. aeruginosa*

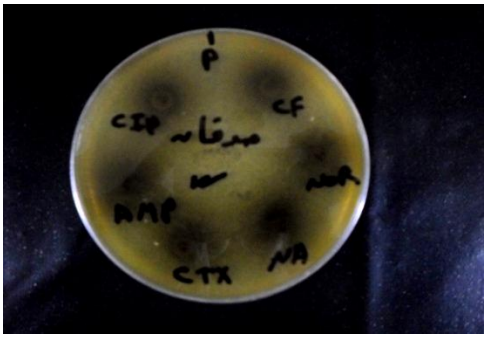


Figure 4.41. I. combination of *Phagnalon rupestre* aquatic extract with antibiotics against *P. aeruginosa*

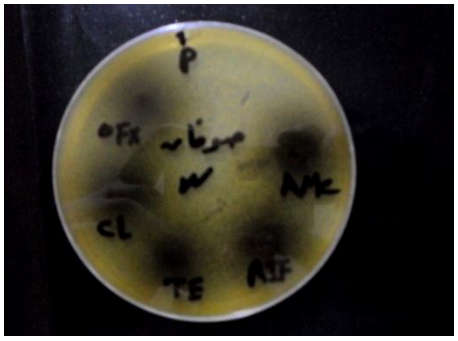


Figure 4.41. J. combination of *Phagnalon rupestre* aquatic extract with antibiotics against *P. aeruginosa*

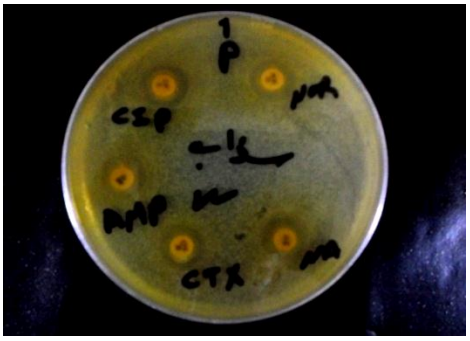


Figure 4.41. K. combination of *Ruta graveolen* aquatic extract with antibiotics against *P. aeruginosa*

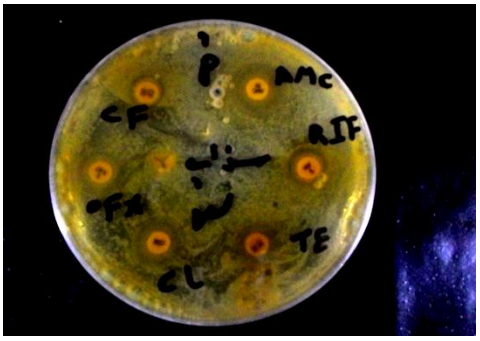


Figure 4.41. L. combination of *Ruta graveolen* aquatic extract with antibiotics against *P. aeruginosa*

The results in table 4.30 either showed that *Pelargonium graveolens* (shoots) aquatic extract had a synergistic effects with most the tested antibiotics including NOR, CXM, CF, OFX, CL, TE, RIF & AMC against *P. aeruginosa*, and was able to suppress the growth of this bacteria, but the best synergism was observed with OFX, CL & AMC with inhibition zones diameter of 16, 15 & 15 mm respectively. *Rosmarinus officinalis* (shoots) aquatic extract when combined with screened antibiotics it was had a synergistic effect with CXM with inhibition zones diameter of 11.50 mm against *P. aeruginosa*, and had additive effects when combined with CTX, OFX, CL & RIF, and had antagonistic effects with the rest of antibiotics. While *Phagnalon rupestre* (shoots) aquatic extract had a synergistic effects when combined with CTX, NA, NOR, CXM, CF & CL against *P. aeruginosa*, but the best synergism was observed with NOR with inhibition zones diameter of 12 mm, and had antagonistic effects with the rest of screened antibiotics. While *Ruta-graveolens* (leaf) when adds as crude extracts of 50µl/disc of 200 mg/ml concentration to the antibiotics it was had a synergistic effects when combined with all the tested antibiotics except with (CXM, CF) & TE which had antagonistic & additive effects respectively, but the best synergism was observed with CIP, AM, OFX, CL & AMC with inhibition zones diameter of 14, 13, 13, 14 & 14 mm respectively, and was able to suppress the *pseudomonas aeruginosa* growth. Overall, the best synergism between the EOs and antibiotics against *P. aeruginosa* was presented in Table 4.31.

Table 4.31. The best synergism with Aquatic extracts against *P. aeruginosa:*

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Aquatic	**Group**	F	F	F	F	C	C	C	C	C	C	C, F	**C**
	Means	14.00	13.00	12.00	12.00	13.00	13.00	13.00	16.00	15.00	12.00	12.00	**15.00**
	SD	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	**1.00**
	F	152.70	127.92	284.404	157.60	165.00	135.93	132.60	464.40	308.20	14.68	108.00	**379.73**
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	**0.001**

C: ***Pelargonium graveolen,*** **F:** ***Ruta-graveolens***

4.3.4.2. The ethanolic extracts:

Table 4.32 & Figures 4.42. A - L summarizes the synergistic effect of all plant extracts against *P. aeruginosa, which* was extracted using 80% ethanol for 8 hours in soxhlet apparatus.

The six plant extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf), had different degree of

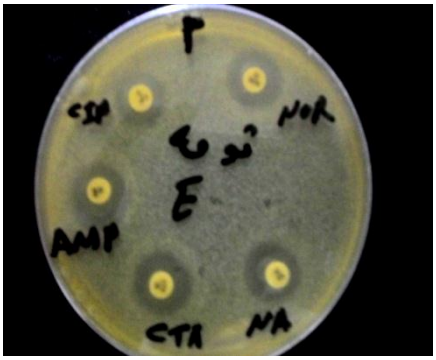


Figure 4.42. A. combination of *Allium sativum* ethanolic extract with antibiotics against *P. aeruginosa*

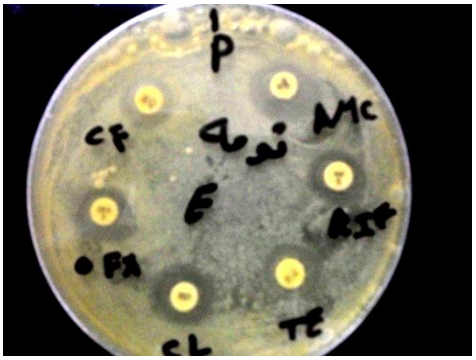


Figure 4.42. B. combination of *Allium sativum* ethanolic extract with antibiotics against *P. aeruginosa*

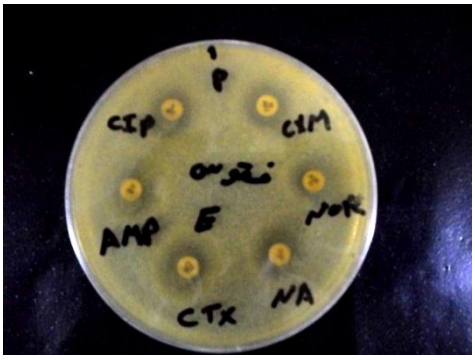


Figure 4.42. C. combination of *Ecballium elaterium* ethanolic extract with antibiotics against *P. aeruginosa*

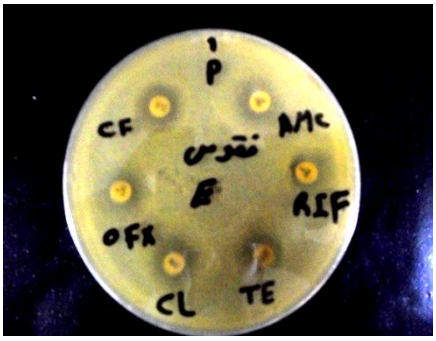


Figure 4.42 D. combination of *Ecballium elaterium* ethanolic extract with antibiotics against *P. aeruginosa*

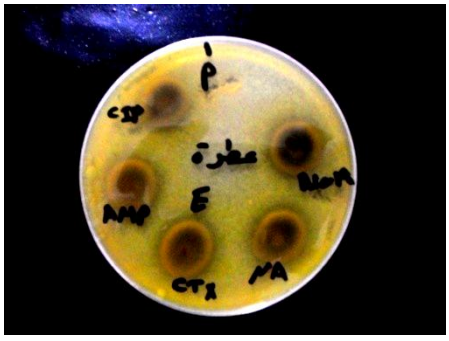


Figure 4.42. E. combination of. *Pelargonium graveolen* ethanolic extract with antibiotics against *P. aeruginosa*

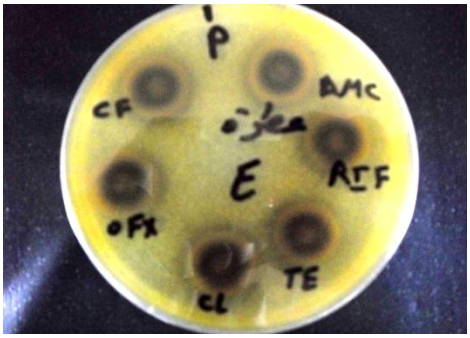


Figure 4.42. F. combination of. *Pelargonium graveolen* ethanolic with antibiotics extract against *P. aeruginosa*

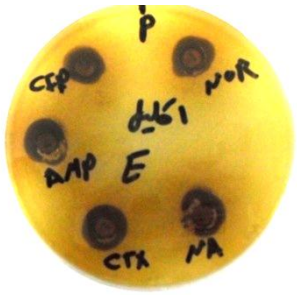


Figure 4.42. G. combination of *Rosmarinus officinalis* ethanolic extract with antibiotics against *P. aeruginosa*

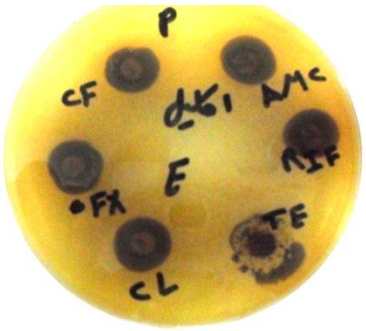


Figure 4.42. H. combination of *Rosmarinus officinalis* ethanolic extract with antibiotics against *P. aeruginosa*

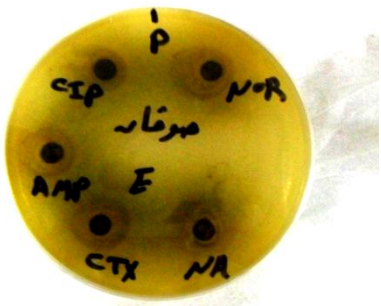


Figure 4.42. I. combination of *Phagnalon rupestre* ethanolic extract with antibiotics against *P. aeruginosa*

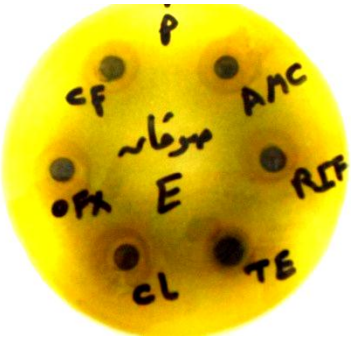


Figure 4.42. J. combination of *Phagnalon rupestre* ethanolic extract with antibiotics against *P. aeruginosa*

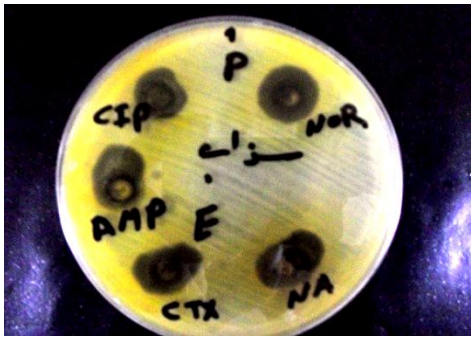


Figure 4.42. K. combination of *Ruta graveolen* ethanolic extract with antibiotics against *P. aeruginosa*

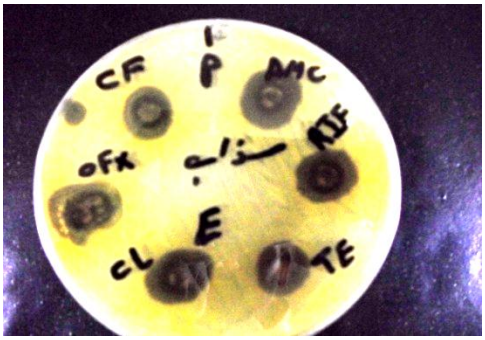


Figure 4.42. L. combination of *Ruta graveolen* ethanolic extract with antibiotics against *P. aeruginosa*

Table 4.32. Synergistic activity of different plant extracts with different antibiotics against *P. aeruginosa*:

			CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Ethanolic	Antibiotic alone		0	0	0	0	0	0	0	0	0	8	8	7
	Extract													
	A	12.66	**14**	**16**	**17**	**15**	**15**	12	**13**	**14**	**14**	**14**	**15**	**14**
	B	9.33	**12**	**11**	**11**	**11**	**11**	**11**	**12**	**11.5**	**11**	**12**	**11**	**11**
	C	15.33	14	**16**	**17**	**16**	**17**	15	**16**	**18**	**17**	**18**	**17**	**17**
	D	9.33	**11**	**13**	**13**	**13**	**12**	**10**	**10**	**12**	**13**	**10**	**13**	**15**
	E	8.66	**10**	**9**	**10**	**10**	**10**	**13**	**12**	**10**	**9**	**11**	**10**	**12**
	F	8.33	8	8	**10**	8	**10**	**10**	8	**10**	**10**	8	**11**	**13**

(**A:** ***Allium sativum,*** **B:** ***Ecballium elaterium,*** **C:** ***Pelargonium graveolen,*** **D:** ***Rosmarinus officinalis,*** **E:** ***Phagnalon rupestre*** **&** **F:** ***Ruta-graveolens*****).**

effect ranged between synergistic, antagonistic or additive effect on *pseudomonas aeruginosa* when added as crude extracts of 50µl / disc of 200 mg/ml concentration.

The results in table 4.19 showed that *Allium sativum* (bulbes) ethanolic extract when combined with the tested antibiotics had a synergistic effects with all the selected antibiotics against *pseudomonas aeruginosa*, except with CXM which had antagonistic effects, but the best synergism was observed with AM, CTX, NA & NOR with inhibition zones diameter of 16, 17, 15 & 15 mm respectively. While *Ecballium elaterium* (fruites), *Rosmarinus officinalis* (shoots) *& Phagnalon rupestre* (shoots) ethanolic extracts when combined with the tested antibiotics had the most synergistic effects with all the selected antibiotics against *pseudomonas aeruginosa*, but the best synergism for *Ecballium elaterium* (fruites) ethanolic extract was observed with CIP, CF & OFX with inhibition zones diameter of 12, 12 &11.5 mm respectively, while the best synergism for *Rosmarinus officinalis* (shoots) ethanolic extract was observed with AM, CTX, NA & CL with inhibition zones diameter of 13 mm for all of it, while the best synergism for *Phagnalon rupestre* (shoots) ethanolic extract was observed with CXM & CF with inhibition zones diameter of 13 & 12 mm respectively. *Pelargonium graveolen* (shoots) ethanolic extract had antagonistic effects with CIP & CXM, while it was observed to be had a synergistic effects with the rest of tested antibiotics and it was able to suppress the *pseudomonas aeruginosa* growth, but the best synergism was observed with OFX & TE with inhibition zones diameter of 18

mm for both of it. while *Ruta graveolens* (leaves) ethanolic extract when combined with the tested antibiotics had a synergistic effects with CTX, NOR, CXM, OFX, CL, RIF & AMC with inhibition zones diameter of 10mm for CTX, NOR, CXM, OFX & CL and 11 & 13 mm for RIF & AMC respectively and was able to suppress the *pseudomonas aeruginosa* growth, and had additive effects with TE only, and had antagonistic effects with the rest of antibiotics.

Overall, the best synergism between the ethanolic extracts and antibiotics against *P. aeruginosa* was presented in Table 4.33.

Table 4.33. The best synergism with ethanolic extracts against *P. aeruginosa*:

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Ethanolic	**Group**	A	A,C	A,C	C	C	E	C	C	C	C	C	C
	Means	14.00	16.00	17.00	16.00	17.00	13.00	16.00	18.00	17.00	18.00	17.00	17.00
	SD	1.00	1.00	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	F	22.00	40.34	55.54	44.96	28.45	15.16	25.26	31.69	36.71	43.71	25.26	18.67
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.114	0.003	0.004	0.001	0.001	0.001

A*: Allium sativum*, C: *Pelargonium graveolens*, E:*Phagnalon rupestre*

4.3.4.3. The methanolic extracts:

Table 4.34 & Figures 4.43. A - L summarizes the synergistic effect of all plant extracts against *P. aeruginosa, which* was extracted using HPLC methanol for 8 hours by using soxhlet apparatus.

The six plant extracts (i.e. A: Allium sativum (bulbes) , B: Ecballium elaterium (fruite) , C: Pelargonium graveolens (shoots) , D: Rosmarinus officinalis (shoots) , E: Phagnalon rupestre (shoots) & F: Ruta-graveolens(leaf), had different degree of effects ranged between synergistic, antagonistic or additive effect on *pseudomonas aeruginosa* when added as crude extracts of 50 µl / disc of 200 mg/ml concentration. The results in Table 4.34 showed that *Allium sativum* (bulbes) methanolic extract when added as 50µl/disc it was had a synergistic effects with all screened antibiotics against *pseudomonas aeruginosa,* except with CTX, which had antagonistic effect. While *Ecballium elaterium* (fruites) methanolic extract had antagonistic effects with NA, OFX, CL, RIF & AMC, and had additive effects with AM, CTX, CXM, CF & TE, and had a synergistic effects with CIP & NOR and was able to suppress the *pseudomonas aeruginosa* growth. The results in Table 4.34 either showed that *Pelargonium graveolen* (shoots) methanolic extract had synergistic effects with all screened antibiotics against *pseudomonas aeruginosa,* except with CIP & CXM.

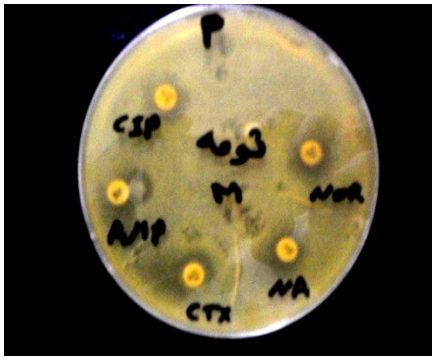


Figure 4.43. A. combination of *Allium sativum* methanolic extract with antibiotics against *P. aeruginosa*

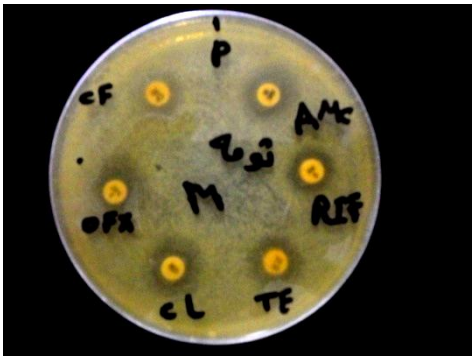


Figure 4.43. B. combination of *Allium sativum* methanolic extract with antibiotics against *P. aeruginosa*

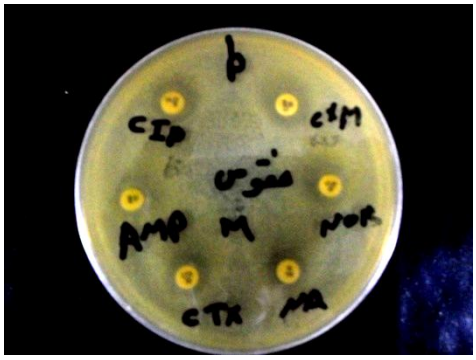


Figure 4.43. C. combination of *Ecballium elaterium* methanolic extract with antibiotics against *P. aeruginosa*

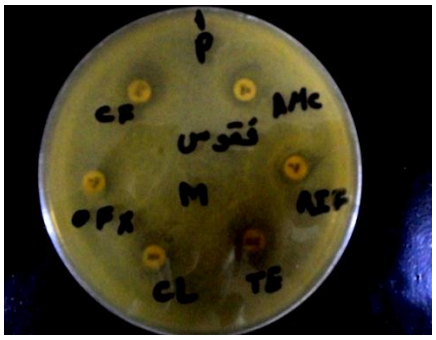


Figure 4.43. D. combination of *Ecballium elaterium* methanolic extract with antibiotics against *P. aeruginosa*

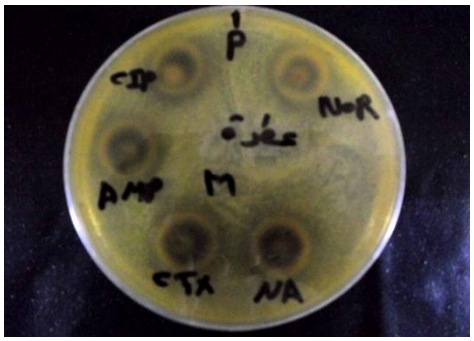


Figure 4.43. E. combination of. *Pelargonium graveolen* methanolic extract with antibiotics against *P. aeruginosa*

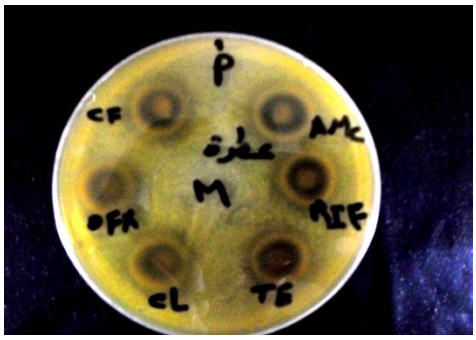


Figure 4.43. F. combination of. *Pelargonium graveolen* methanolic with antibiotics extract against *P. aeruginosa*

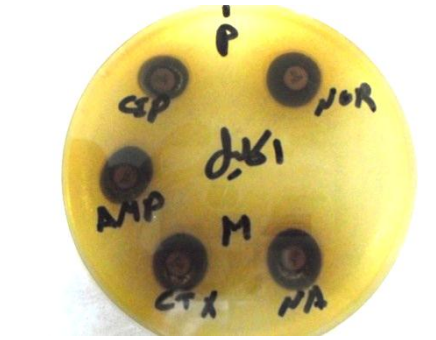


Figure 4.43. G. combination of *Rosmarinus officinalis* methanolic extract with antibiotics against *P. aeruginosa*

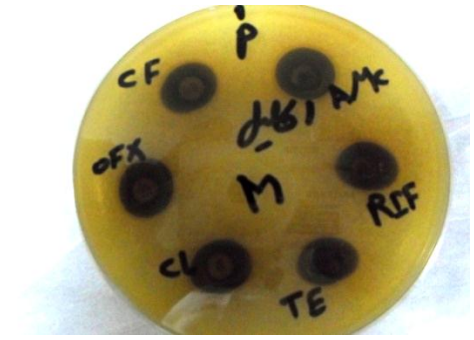


Figure 4.43. H. combination of *Rosmarinus officinalis* methanolic extract with antibiotics against *P. aeruginosa*

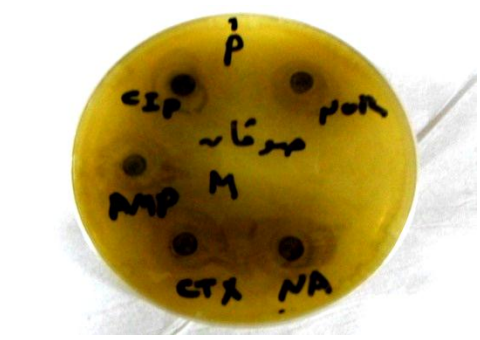


Figure 4.43. I. combination of *Phagnalon rupestre* methanolic extract with antibiotics against *P. aeruginosa*

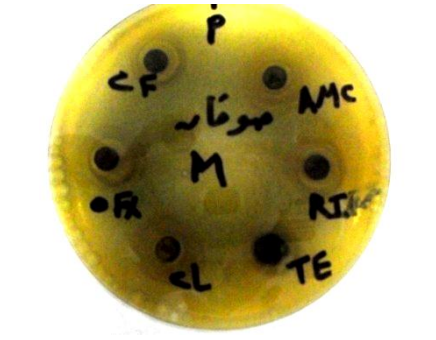


Figure 4.43. J. combination of *Phagnalon rupestre* methanolic extract with antibiotics against *P. aeruginosa*

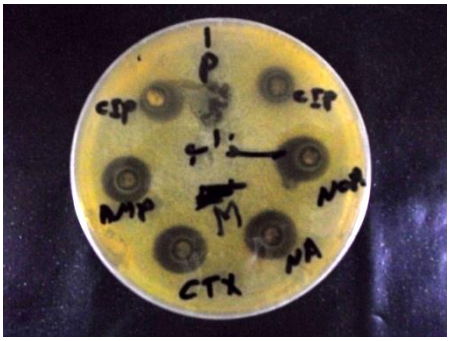


Figure 4.43. K. combination of *Ruta graveolen* methanolic extract with antibiotics against *P. aeruginosa*

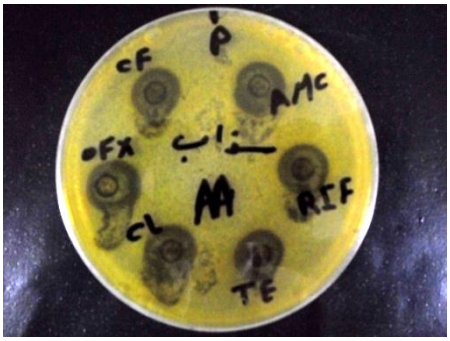


Figure 4.43. L. combination of *Ruta graveolen* methanolic extract with antibiotics against *P. aeruginosa*

which had additive & antagonistic effects respectively, but the best synergism was observed with CTX, NA & NOR with inhibition zones diameter of 18, 18 & 20 mm respectively and was able to suppress the *pseudomonas aeruginosa* growth. While *Rosmarinus officinalis* (shoots) *& Ruta graveolens* (leaves) methanolic extracts had a synergistic effects with all screened antibiotics against *pseudomonas aeruginosa*, but the best synergism with *Rosmarinus officinalis* (shoots) methanolic extract was observed with CTX, NA, NOR & CXM with inhibition zones diameter of 14, 14, 14 & 15 mm respectively, and the best synergism with *Ruta graveolens* (leaves) methanolic extract was observed with CXM with inhibition zones diameter of 10 mm and was able to suppress the *P. aeruginosa* growth. While *Phagnalon rupestres* (shoots) methanolic extract when combined with the screened antibiotics it was had a synergistic effects with all screened antibiotics except with AM, NA & NOR which had antagonistic effects, but the best synergism was observed with CIP, CTX & CXM with inhibition zones diameter of 12, 12 & 11.5 mm respectively and was able to suppress the *P. aeruginosa* growth.

Table 4.34. Synergistic activity of different plant extracts with different antibiotics against *P. aeruginosa*:

			CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Methanolic	Antibiotic alone		0	0	0	0	0	0	0	0	0	8	8	7
	Extract													
	A	9.66	**13**	**11**	9	**10**	**12**	**13**	**12**	**12**	**11**	**12**	**12**	**14**
	B	11.00	**12**	11	11	10	**12**	11	11	7	9	11	9	8
	C	15.00	15	**16**	**18**	**18**	**20**	10	**17**	**16**	**17**	**17**	**17**	**18**
	D	7.66	**13**	**13.5**	**14**	**14**	**14**	**15**	**12**	**13**	**13.5**	**11**	**13**	**14**
	E	8.66	**12**	7	**12**	8	8	**11.5**	**10**	**10**	**9**	**10**	**11**	**11**
	F	8.66	**9**	**9**	**9**	**9**	**9**	**10**	**9**	**9**	**9**	**9**	**9**	**9**

(A: *Allium sativum*, B: *Ecballium elaterium*, C: *Pelargonium graveolen*, D: *Rosmarinus officinalis*, E: *Phagnalon rupestre* & F: *Ruta-graveolens*).

Overall, the best synergism between the EOs and antibiotics against *P. aeruginosa* was presented in Table 4.35.

Table 4.35. The best synergism with methanolic extracts against *P. aeruginosa*:

		CIP	AM	CTX	CF	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Methanolic	**Group**	A,D	C	C	C	C	D	C	C	C	C	C	C
	Means	13.00	16.00	18.00	18.00	20.00	15.00	17.00	16.00	17.00	17.00	17.00	18.00
	SD	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	F	40.35	52.33	40.34	73.54	62.74	15.10	27.96	30.50	36.48	23.60	53.80	41.60
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

A: *Allium sativum*, C: *Pelargonium graveolen*, D: *Rosmarinus officinalis*

4.3.4.4. The essential oils:

Table 4.36 & Figures 4.44. A - L summarizes the synergistic effect of Essential oils against *P. aeruginosa*, which was extracted by using steam distillation apparatus for 4 hours. Among the six EOs (i.e. A. *Allium sativum* (bulbes) EO , *B. Ecballium elaterium* (fruites) EO , *C. Pelargonium graveolen* (shoots) EO , *D; Rosmarinus officinalis* (shoots) EO, *E. Phagnalon rupestre* (shoots) EO & *F. Ruta-graveolens*(leaves) EO), adds as crude extracts of 50 µl / disc of 200 mg/ml concentration, *Allium sativum* (bulbes) EO when combined with the screened antibiotics it was observed to be had a synergistic effects with all antibiotics and was

Table 4.36. Synergistic activity of different EOs with different antibiotics against *p. aeruginosa*:

Essential Oils		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
	Antibiotic alone / Extract	0	0	0	0	0	0	0	0	0	8	8	7
A	11.33	**16**	**16**	**15**	**17**	**16.5**	**15.5**	**15.5**	**16**	**16**	**13**	**14**	**17**
B	9.33	0	0	0	0	0	0	8	0	0	9	9	0
C	12.66	**15**	**15**	**16**	**15**	**16**	**15**	**14**	**14**	**15**	12	**15**	**15**
D	12.00	**16**	**16**	**15**	**14**	**14**	**15**	12	**15**	**16**	12	**14**	**15.5**
E	8.66	**10**	**10**	8	8	8	8	8	7	7	8	**9**	8
F	12.33	10	8	7	7	7	8	9	9	9	11	11	8

(A: *Allium sativum*, B: *Ecballium elaterium*, C: *Pelargonium graveolen*, D: *Rosmarinus officinalis*, E: *Phagnalon rupestre* & F: *Ruta-graveolens*).

able to suppress the growth of *P. aeruginosa*, but the best synergism was observed with NA & NOR with inhibition zones diameter of 17 & 16.5 mm respectively. While *Ecballium elaterium* (fruites) EO had additive effects with all tested antibiotics except with CF, TE & RIF which had antagonistic effects.

While *Pelargonium graveolens* (shoots) EO had a synergistic effects with all antibiotics against *P. aeruginosa*, except with TE which had antagonistic effect, but the best synergism was observed with CTX & NOR with inhibition zones diameter of 16 mm for both. Either *Rosmarinus officinalis* (shoots) EO when combined with the screened antibiotics it was observed to be had a synergistic effects with all antibiotics against *P. aeruginosa* except with CF & TE which had additive effects, but the best synergism was observed with CIP, AM & CL with inhibition zones diameter of 16 mm for all and was able to suppress the growth of *P. aeruginosa.*

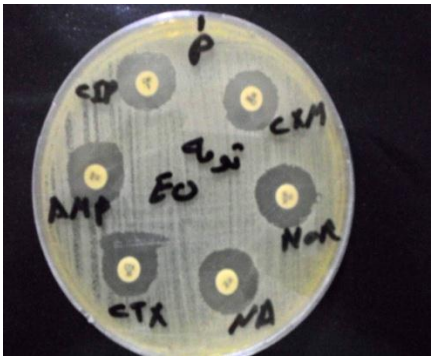


Figure 4.44. A. combination of *Allium sativum* EO with antibiotics against *P. aeruginosa*

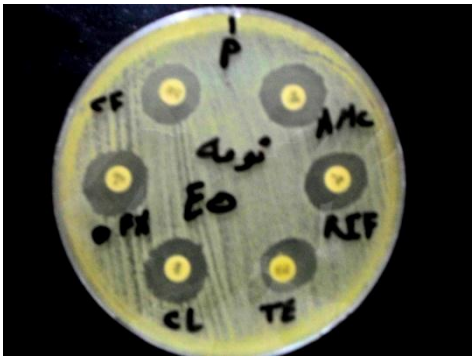


Figure 4.44. B. combination of *Allium sativum* EO with antibiotics against *P. aeruginosa*

Figure 4.44. C. combination of *Ecballium elaterium* EO with antibiotics against *P. aeruginosa*

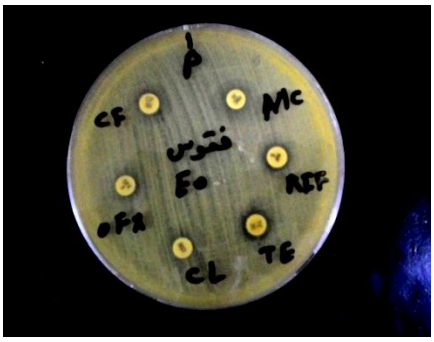


Figure 4.44. D. combination of *Ecballium elaterium* EO with antibiotics against *P. aeruginosa*

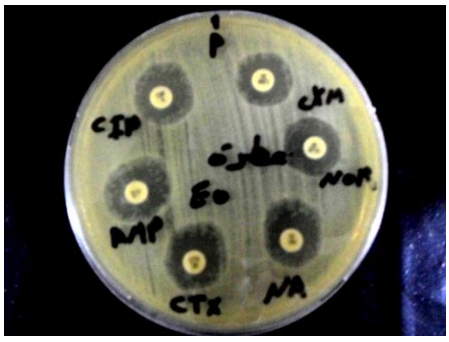


Figure 4.44. E. combination of. *Pelargon-ium graveolen* EO with antibiotics against *P. aeruginosa*

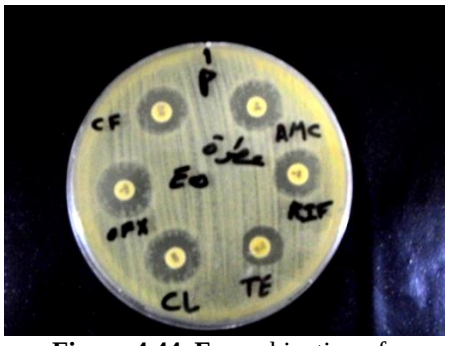


Figure 4.44. F. combination of. *Pelargon-ium graveolen* EO with antibiotics extract against *P. aeruginosa*

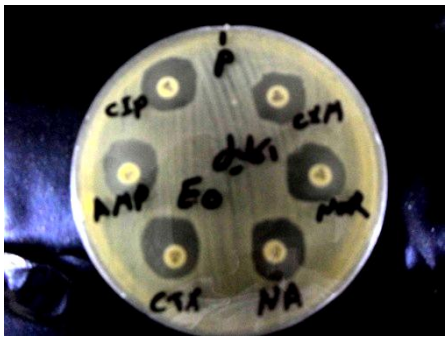


Figure 4.44. G. combination of *Rosmar-inus officinalis* EO with antibiotics against *P. aeruginosa*

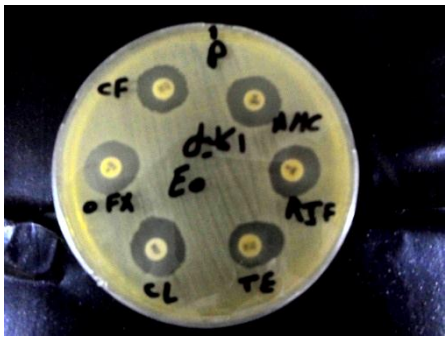


Figure 4.44. H. combination of *Rosmarinus officinalis* EO with antibiotics against *P. aeruginosa*

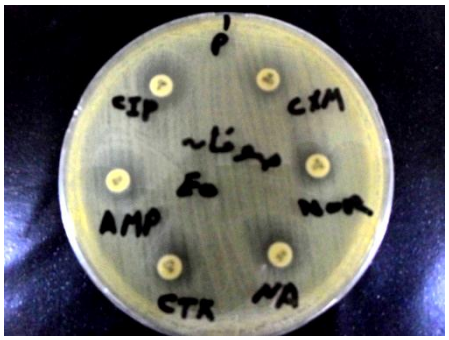


Figure 4.44. I. combination of *Phagnalon rupestre* EO with antibiotics against *P. aeruginosa*

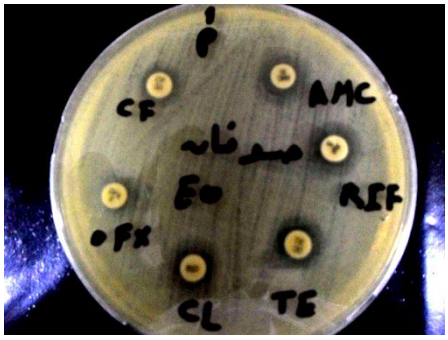


Figure 4.44. J. combination of *Phagnalon rupestre* EO with antibiotics against *P. aeruginosa*

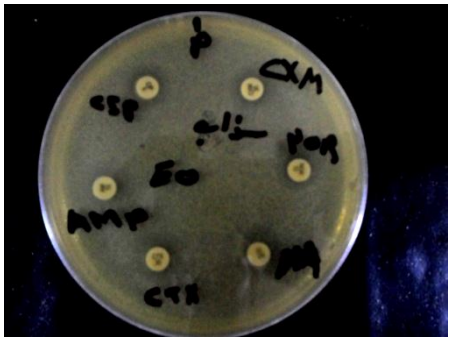


Figure 4.44. K. combination of *Ruta graveolen* EO with antibiotics against *P. aeruginosa*

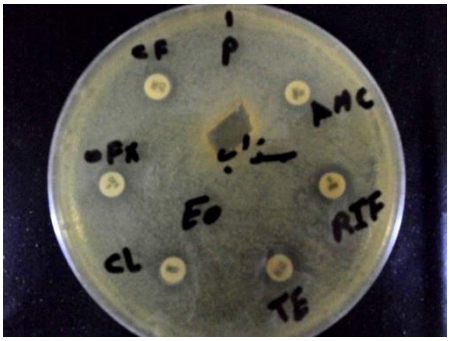


Figure 4.44. L. combination of *Ruta graveolen* EO with antibiotics against *P. aeruginosa*

The results in table 4.21 also showed that *Phagnalon rupestre* (shoots) EO when adds as crude extract of 50µl/disc it was observed to be had a synergistic effect with CIP, AM & RIF against *pseudomonas aeruginosa*, and had additive effect with TE, and had antagonistic effect when combined with the rest of screened antibiotics. While *Ruta graveolens* (leaves) EO) when adds as crude extracts of 50 µl/disc it was observed to be had antagonistic effect with all antibiotics.

Overall, the best synergism between the EOs and antibiotics against *P. aeruginosa* was presented in Table 4.37.

Table 4.37. The best synergism with EOs against P. aeruginosa

		CIP	AM	CTX	NA	NOR	CXM	CF	OFX	CL	TE	RIF	AMC
Essential Oil	**Group**	A, D	A, D	C	A	A, C	A	A	A	A, D	A	C	A
	Means	16.00	16.00	16.00	17.00	1600	15.16	15.50	16.00	1600	13.00	15.00	17.00
	SD	1.00	1.00	1.00	1.00	0.50	0.57	0.50	1.00	1.00	1.00	1.00	1.00
	F	159.95	166.73	318.13	208.63	206.57	185.33	44.22	168.15	184.95	30.13	25.92	336.33
	Sig	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

A: ***Allium sativum*****, C:** ***Pelargonium graveolen*****, D:** ***Rosmarinus officinalis***

4.4. The minimum inhibitory concentration (MIC) & minimum bactericidal concentrations (MBC) of plant extracts against isolated bacteria:

Extracts were tested against the bacterial isolates for their inhibitory activity, using a common broth microdilution method in 96 multiwell microtiter plates in two fold dilution series of these extracts was prepared. 200, 100, 50, 25, 12.5. 6.25, 3.125, 1.562, 0.781 & 0.390 (mg/ml for the aquatic, ethanolic & methanolic extracts and µl/ml for the essential oils), in duplicate and the average of the obtained minimum inhibitory concentrations (MICs) & minimum bactericidal concentrations (MBCs) is listed in Tables 4.38 - 4.43. The micro-titre plate or broth microdilution method has provided a potentially useful technique for determining MICs & MBCs of large numbers of test samples**.**

4.4.1. The MICs & MBCs of *Allium sativum* extracts against isolated bacteria:

As the results in Table 4.38 & Figures 4.45. A - D, shown the MIC of the aquatic extract of *Allium sativum* against *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa* was 50, 25, 50 & 100 mg/ml respectively, and had lethal concentration of 200, 100

mg/ml against both of *S. aureus, E. coli* respectively, and 100 & >200 mg/ml for both *K. pneumoniae* & *P. aeruginosa* respectively. While the ethanolic extract showed inh-

Table 4.38. The MICs & MBCs of *Allium sativum* extracts against isolated bacteria:

Scientific name of the plant used		*S. aureus*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Allium sativum	W	50	200	25	100	50	100	100	> 200
	E	25	200	25	100	50	100	50	100
	M	25	200	25	100	50	200	25	200
	EO	25	200	3.125	200	50	100	50	100

W: Aquatic, E: Ethanolic, M: Methanolic & EO: Essential oil. W, E & M expressed as mg/ml, while EO expressed as µl/ml.

ibition property against *S. aureus & E. coli*, having MIC values of 25 mg/ml for both, and having MIC values of 50 mg/ml for both of *K. pneumoniae* & *P. aeruginosa*, while having MBC values of 100 mg/ml for *E. coli, K. pneumoniae* & *P. aeruginosa* and having MBC value of 200 mg/ml for *S. aureus*. While the MIC of methanolic extract was found to be 25 mg/ml for *S. aureus, E. coli* & *P. aeruginosa*, and 50 mg/ml for *K. pneumoniae*. While both of *S. aureus, K. pneumoniae* & *P. aeruginosa* required about 200 mg/ml of the crude extract for lethal activity. *E. coli* was killed at the dose of 100 mg/ml of the crude extract. The EO either showed inhibitory & lethal property against *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa*, having MIC values of 25 & 3.152 µl/ml for both of *S. aureus & E. coli* respectively, and having lethal activity at MBC values of 200 µl/ml for both of them. While both of *K. pneumoniae* & *P. aeruginosa* required about 50 µl/ml of the crude extract for inhibitory activity, and about 100 µl/ml of the EO for lethal activity.

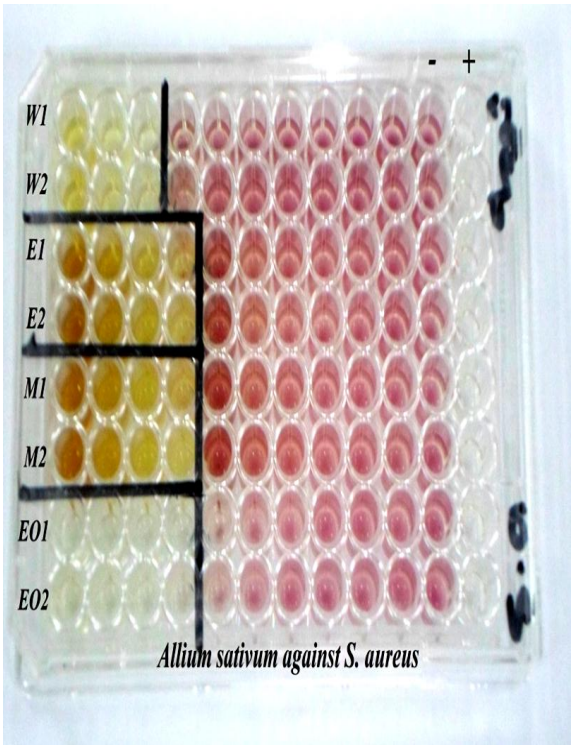


Figure. 4.45.A. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Allium sativum* extracts against *S. aureus*.

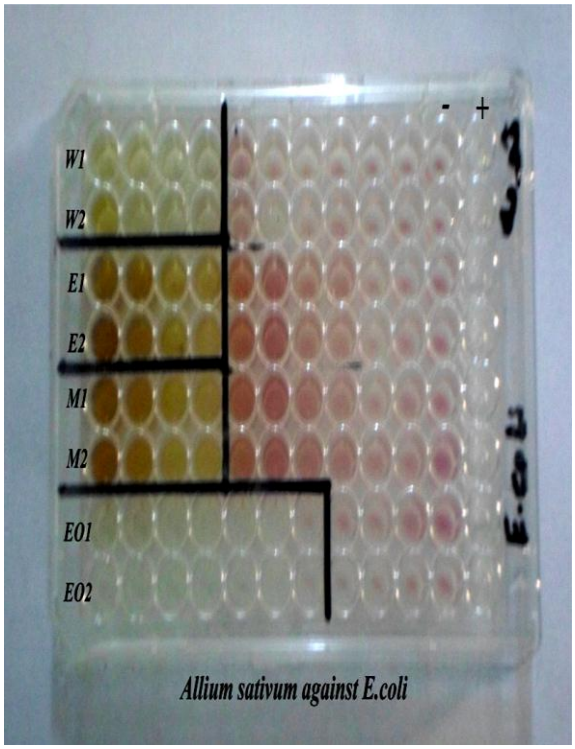


Figure. 4.45.B. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Allium sativum* extracts against *E. coli*.

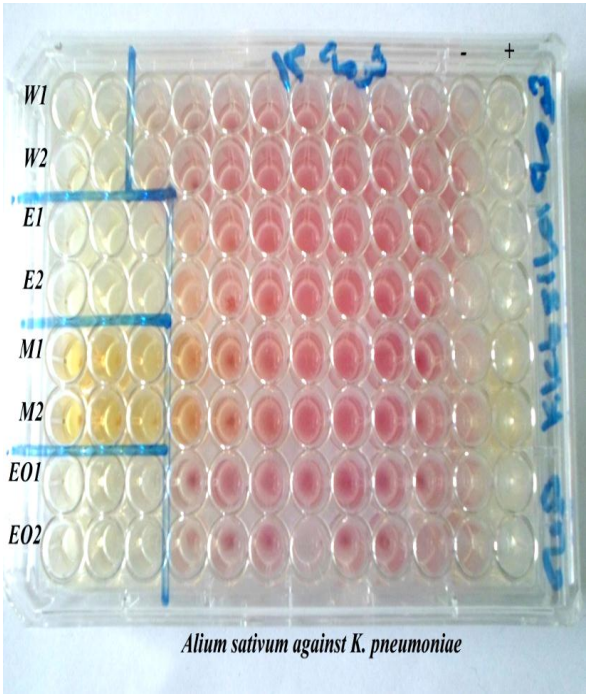


Figure. 4.45.C. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Allium sativum* extracts against *K. pneumonia***e.**

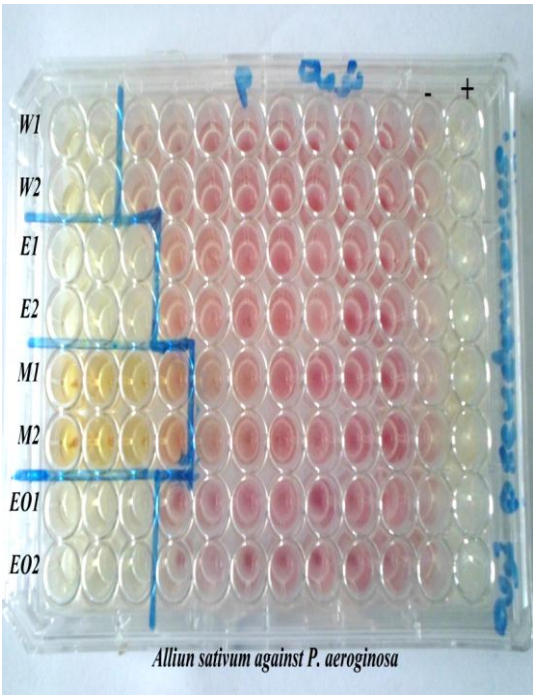


Figure. 4.45.D. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Allium sativum* extracts against *P. aeruginosa*.

4.4.2. The MICs & MBCs of *Ecballium eleterium* extracts against isolated bacteria.

As the results in Table 4.39 & as shown in Figures 4.46. A- D, the MIC & MBC of *Ecballium eleterium* aquatic extract was (25 & 200) mg/ml for both *S. aureus* & *P. aeruginosa*, while for *E. coli* & *K. pneumoniae* was (50 & 100) & (25 & 100) mg/ml respectively.

Table 4.39. The MICs & MBCs of *Ecballium eleterium* extracts against isolated bacteria:

Scientific name of the plant used		*S. aureus*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ecballium eleterium	W	25	200	50	100	25	100	25	200
	E	25	200	25	50	25	100	25	200
	M	25	200	25	50	25	200	25	200
	EO	50	> 200	50	100	50	200	25	> 200

W: Aquatic, E: Ethanolic, M: Methanolic & EO: Essential oil. W, E & M expressed as mg/ml, while EO expressed as µl/ml.

The growth of *S. aureus & P. aeruginosa* was inhibited by the ethanolic extract of *Ecballium eleterium* at concentration of 25 mg/ml for both and died at concentration of 200 mg/ml for both, while *E. coli* & *K. pneumoniae* inhibited at concentration of 25 mg/ml for both, and was died at concentration of 50 & 100 mg/ml respectively. The MIC of *Ecballium eleterium* methanolic extract for *S. aureus*, *E. coli*, *K. pneumoniae & P. aeruginosa* was 25 mg/ml for all isolates, while MBC was 200 mg/ml for all tested bacteria except for *E. coli*, which was 50 mg/ml. The growth of *S. aureus*, *E. coli* & *K. pneumoniae* was inhibited by the EO of *Ecballium eleterium* at concentration of 50 µl/ml for all, and 25 µl/ml for *P. aeruginosa*, and was died at concentration of > 200, 100, 200 & > 200 µl/ml for *S. aureus*, *E. coli*, *K. pneumoniae & P. aeruginosa* respectively.

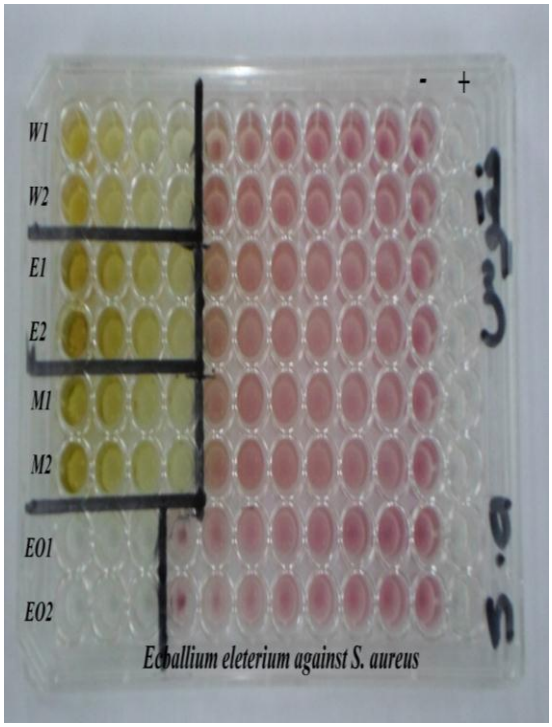


Figure. 4.46.A. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Ecballium eleterium* extracts against *S. aureus*.

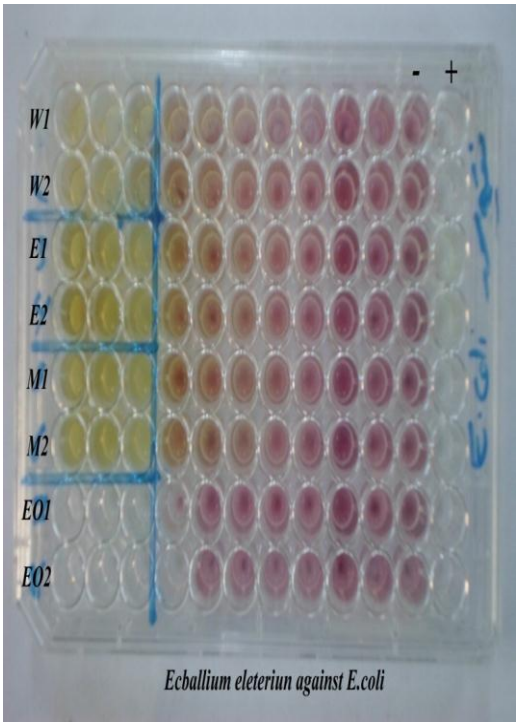


Figure. 4.46.B. W. Aquatic, E. Ethanolic, M. ethanolic & EO. Essintial oil. The MIC & MBC *Ecballium eleterium* extracts against *E. coli*.

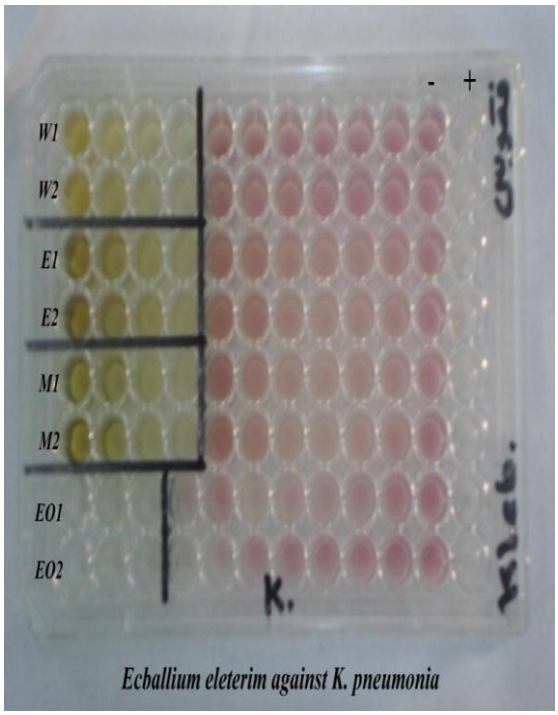


Figure. 4.46.C. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Ecballium eleterium* extracts against *K. pneumoniae*.

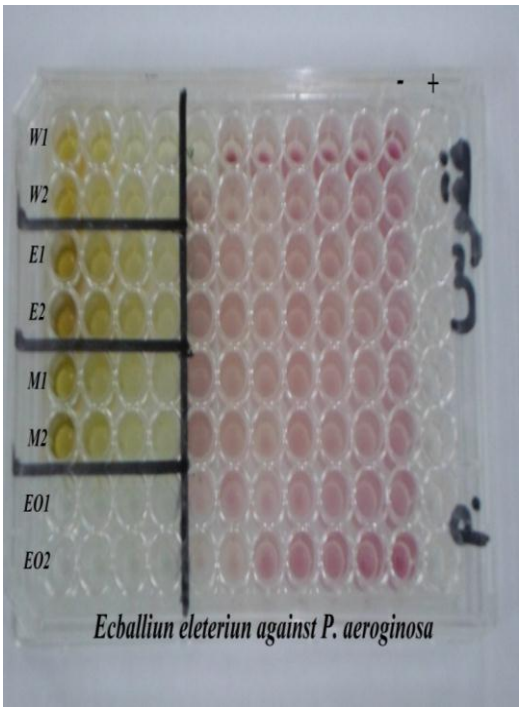


Figure. 4.46.D. W. Aquatic, E. Ethanolic, M. ethanolic & EO. Essintial oil. The MIC & MBC of *:ballium eleterium* extracts against *P. aeruginosa*.

4.4.3. The MICs & MBCs of *Pelargonium graveolens* extracts against isolated bacteria:

Table 4.40. The MICs & MBCs of *Pelargonium graveolens* extracts against isolated bacteria:

Scientific name of the plant used		*S. aureus*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Pelargonium graveolens	W	12.5	> 200	3.125	25	1.562	25	6.25	200
	E	1.562	100	1.562	100	1.562	100	1.562	100
	M	1.562	100	0.390	200	1.562	100	1.562	100
	EO	25	200	50	100	25	200	25	200

W: Aquatic, E: Ethanolic, M: Methanolic & EO: Essential oil. W, E & M expressed as mg/ml, while EO expressed as µl/ml.

As the results in Table 4.40 & as shown in Figures 4.47. A - D, aqueous extract from *Pelargonium graveolens* showed antibacterial activity against the tested bacteria, and the strongest activity was seen against *E. coli* (MIC= 3.125 mg/ml & MBC= 25 mg/ml), it was active against *Staphylococcus aureus (*MIC=12.5 & MBC= >200 mg/ml). The extract also manifested activity against *K. pneumoniae & P. aeruginosa* at an MIC of 1.562 & 6.25 mg/ml respectively, and MBC of 25 & 200 mg/ml respectively. The minimum inhibitory concentrations (MICs) of the *Pelargonium graveolens* ethanolic extract against the test organisms are shown in Table 4.40. *S. aureus*, *E. coli*, *K. pneumoniae & P. aeruginosa* was inhibited within the MIC values of 1.562 mg/ml. The MBC was 100 mg/ml against the clinical isolates of *S. aureus*, *E. coli*, *K. pneumoniae & P. aeruginosa*. The results in Table 4.40 either shown that *Pelargonium graveolens* methanolic extract is sensitive against the clinical isolates of *S. aureus, K. pneumoniae & P. aeruginosa* (MIC = 1.526 mg/ml for all & MBC= 100 mg/ml for all) and had the strongest activity against *E. coli* (MIC = 0.390 mg/ml & MBC= 200 mg/ml). The MIC values of *Pelargonium graveolens* EO was (25 µl/ml) for *S. aureus*, *K. pneumoniae & P. aeruginosa* and (50 µl/ml) for *E. coli*, the extract also shown lethal activity against *S. aureus*, *K. pneumoniae & P. aeruginosa* with MBC values of 200 µl/ml for all, and 100 µl/ml for *E. coli* bacteria.

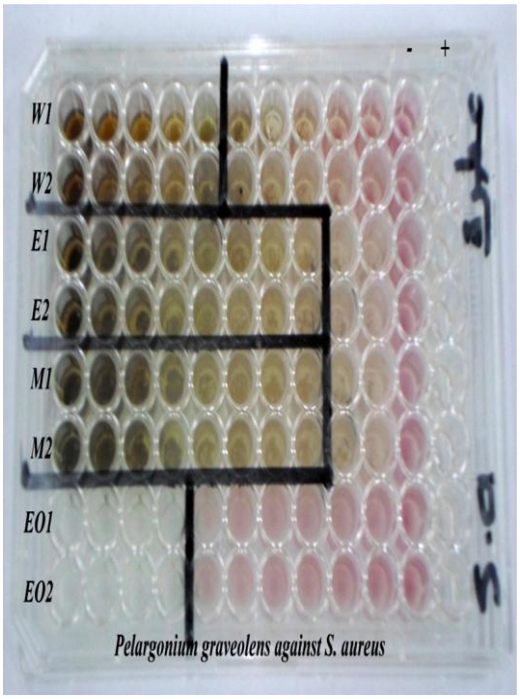


Figure. 4.47.A. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Pelargonium graveolens* extracts against *S. aureus.*

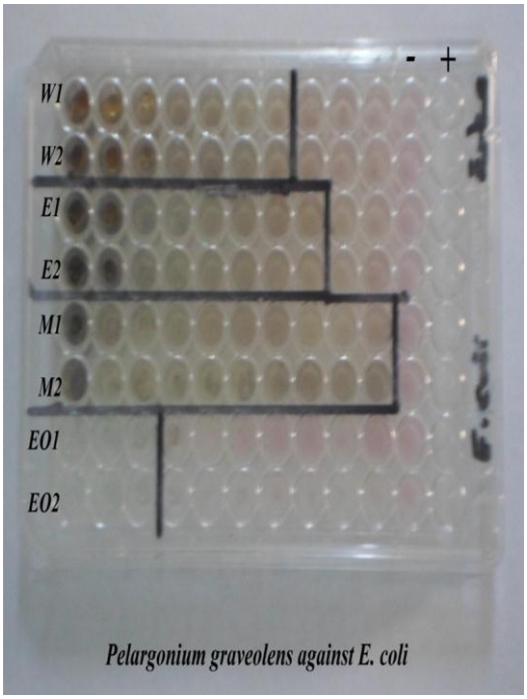


Figure. 4.47.B. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Pelargonium graveolens* extracts against *E. coli*.

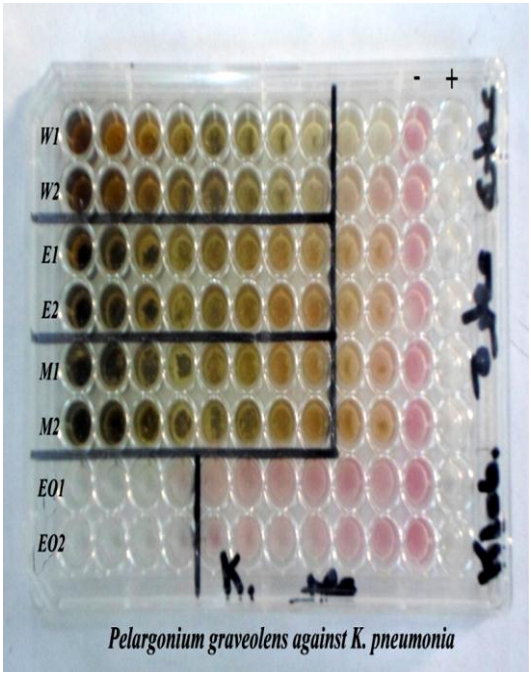


Figure. 4.47.C. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Pelargonium graveolens* extracts against *K. pneumoniae***.**

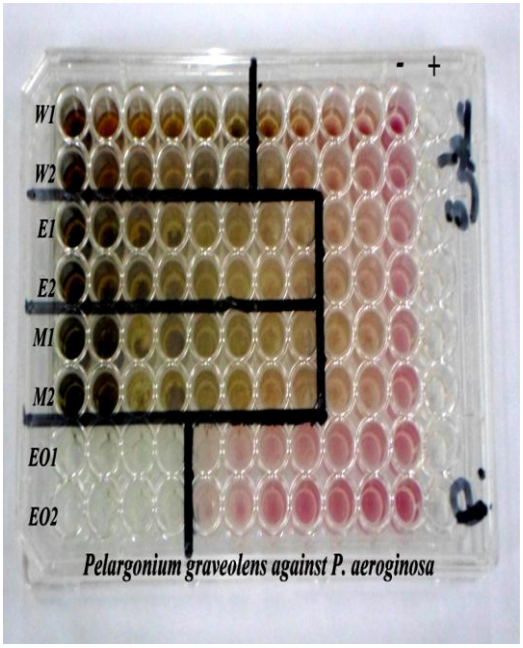


Figure. 4.47.D. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Pelargonium graveolens* extracts against *P. aeruginosa*.

4.4.4. The MICs & MBCs of *Rosmarinus officinalis* extracts against isolated bacteria:

Table 4.41. The MICs & MBCs of *Rosmarinus officinalis* extracts against isolated bacteria:

Scientific name of the plant used		*S. aureus*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Rosmarinus officinalis	W	12.5	50	6.25	25	6.25	100	6.25	100
	E	6.25	> 200	12.5	25	6.25	100	1.562	25
	M	1.562	25	3.125	25	3.125	25	1.562	100
	EO	50	200	50	100	100	200	25	200

W: Aquatic, E: Ethanolic, M: Methanolic & EO: Essential oil. W, E & M expressed as mg/ml, while EO expressed as µl/ml.

As can be seen in Table 4.41 & as shown in Figures 4.48. A - D. MIC assay for *Rosmarinus officinalis* aqueous extracts revealed that the MIC value for *E. coli*, *K. pneumoniae & P. aeruginosa* was 6.25 mg/ml for all of it, and was 12.5 mg/ml for *S. aureus* clinical isolates. while MBC assay for *Rosmarinus officinalis* aqueous extracts revealed that the MBC value for *S. aureus* & *E. coli* was 50 & 25 mg/ml respectively, and for both *K. pneumoniae & P. aeruginosa* was 100 mg/ml. Ethanolic extract of *Rosmarinus officinalis* showed antibacterial activity against the tested bacteria, and the strongest activity was seen against *S. aureus* (MIC= 6.25 mg/ml & MBC= >200 mg/ml), it was active against *E. coli (*MIC=12.5 & MBC= 25 mg/ml). The extract also manifested activity against *K. pneumoniae & P. aeruginosa* at an MIC= 100 & 25 mg/ml respectively & MBC= 100 & 25 mg/ml respectively. The MIC of *Rosmarinus officinalis* methanolic extract for both *S. aureus & P. aeruginosa* was 1.562 mg/ml, while MBC was 25 & 100 mg/ml for both of it, while MIC for both *E. coli* & *K. pneumoniae* was 3.125 mg/ml and MBC of 25 mg/ml for both. The MIC values of *Rosmarinus officinalis* EO was (50 µl/ml) for *S. aureus* & *E. coli* and (100 & 25 µl/ml) for *K. pneumoniae & P. aeruginosa* respectively, the extract also shown lethal activity against *S. aureus, K. pneumoniae & P. aeruginosa* with MBC values of 200 µl/ml for all, and 100 µl/ml for *E. coli* bacteria.

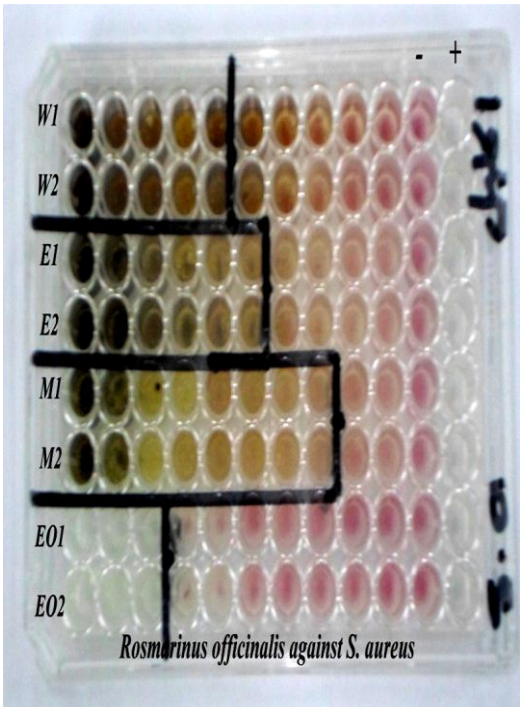


Figure. 4.48. A. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Rosmarinus officinalis* extracts against *S. aureus*.

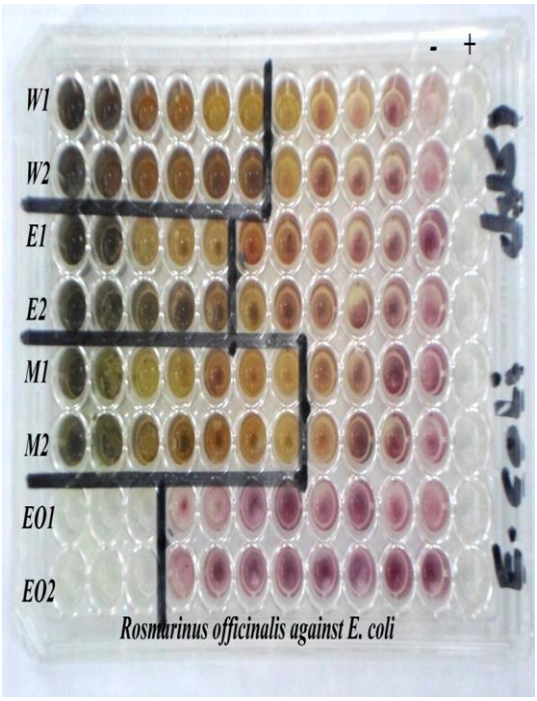


Figure. 4.48. B. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Rosmarinus officinalis* extracts against *E. coli*.

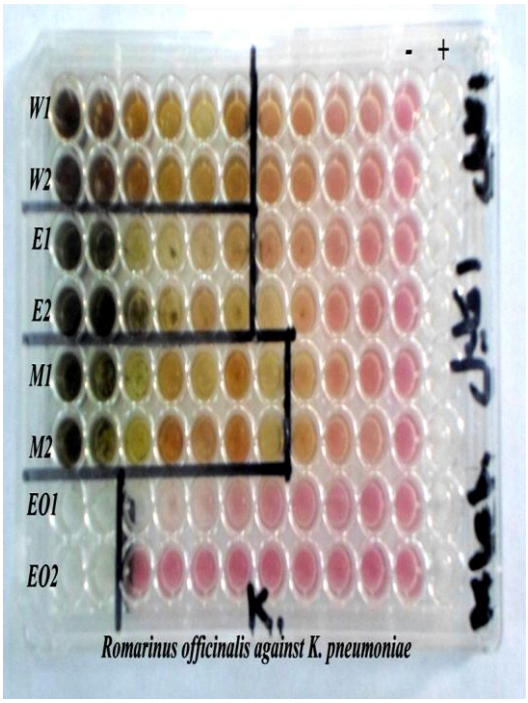


Figure. 4.48. C. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Rosmarinus officinalis* extracts against *K. pneumoniae*.

Figure. 4.48. D. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Rosmarinus officinalis* extracts against *P. aeruginosa*.

4.4.5 The MICs & MBCs of *Phagnalon rupestre* extracts against isolated bacteria:

Table 4.42. The MICs & MBCs of *Phagnalon rupestre* extracts against isolated bacteria:

Scientific name of the plant used		*S. aureus*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Phagnalon rupestre	W	12.5	200	50	100	6.25	100	50	100
	E	3.125	200	12.5	50	3.125	200	12.5	25
	M	3.125	200	12.5	50	1.562	200	12.5	50
	EO	25	200	25	50	25	200	25	200

W: Aquatic, E: Ethanolic, M: Methanolic & EO: Essential oil. W, E & M expressed as mg/ml, while EO expressed as µl/ml.

As can be seen in Table 4.42 & as shown in Figures 4.49. A - D. The growth of *S. aureus, E. coli, K. pneumoniae & P. aeruginosa* was inhibited by the aquatic extract of *Phagnalon rupestre* at concentration of 12.5, 50, 6.25 & 50 mg/ml respectively, and had lethal concentration of 200 mg/ml for *S. aureus*, while *E. coli, K. pneumoniae & P. aeruginosa* killed at concentration of 100 mg/ml for both. Ethanolic extract from *Phagnalon rupestre* showed antibacterial activity against all the tested bacteria, and the strongest activity was seen against *E. coli* (MIC= 12.5 mg/ml & MBC= 50 mg/ml), it was active against both *S. aureus & K. pneumoniae* (MIC=3.125 & MBC= 200 mg/ml for both). The extract also manifested activity against *P. aeruginosa* at an MIC= 12.5 & MBC= 25 mg/ml. The minimum inhibitory concentrations (MICs) of the *Phagnalon rupestre* methanolic extract against the test organisms are shown in Table 4.42, *S. aureus* & *K. pneumoniae* was inhibited within the MIC values of 3.125 & 1.562 mg/ml respectively, and died at the MBC was 200 mg/ml for both. While MIC against the clinical isolates of *E. coli & P. aeruginosa* was of 12.5 mg/ml for both & MBC values of 50 mg/ml for both. The MIC values of *Phagnalon rupestre* EO was (25 µl/ml) for *S. aureus, E. coli, K. pneumoniae & P. aeruginosa*, the extract also shown lethal activity against *S. aureus, K. pneumoniae & P. aeruginosa* with MBC values of 200 µl/ml for all, and 50 µl/ml for *E. coli* bacteria.

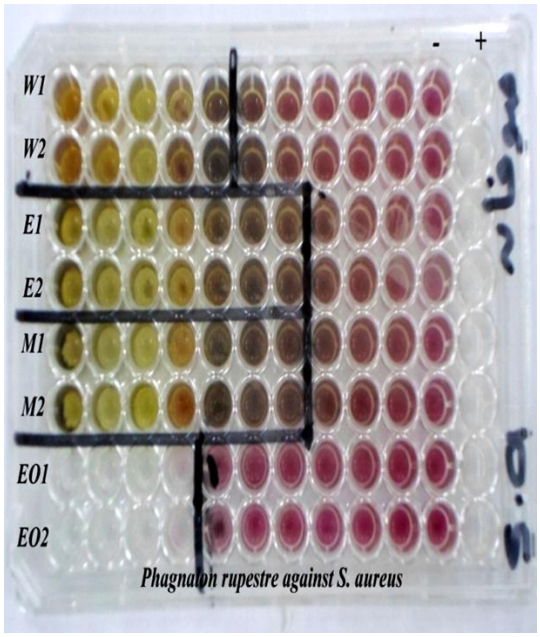


Figure. 4.49. A. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Phagnalon rupestre* extracts against *S. aureus*.

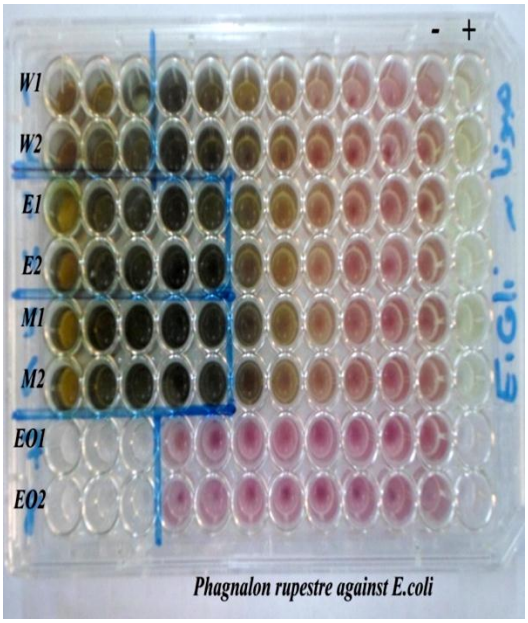


Figure. 4.49. B. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Phagnalon rupestre* extracts against *E. coli*.

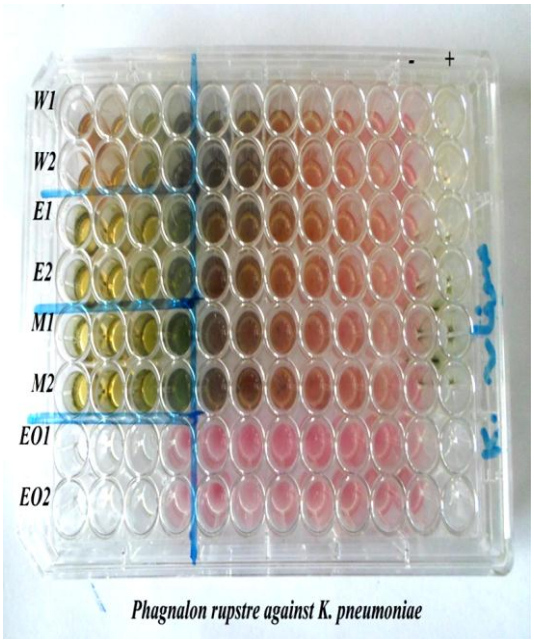


Figure. 4.49. C. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Phagnalon rupestre* extracts against *K.pneumoniae*.

Figure. 4.49. D. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Phagnalon rupestre* extracts against *P. aeruginosa*.

4.4.6. The MICs & MBCs of *Ruta graveolens* extracts against isolated bacteria:

Table 4.43. The MICs & MBCs of *Ruta graveolens* extracts against isolated bacteria:

Scientific name of the plant used		*S. aureus*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ruta-graveolens	W	25	200	12.5	100	25	50	25	200
	E	3.125	100	1.562	200	1.562	25	3.125	> 200
	M	3.125	100	1.562	50	1.562	25	3.125	100
	EO	50	> 200	25	200	50	100	12.5	> 200

W: Aquatic, E: Ethanolic, M: Methanolic & EO: Essential oil. W, E & M expressed as mg/ml, while EO expressed as µl/ml.

As represented in Table 4.43 & as shown in Figures 4.50. A - D, The growth of *S. aureus, K. pneumoniae & P. aeruginosa* was inhibited by the aquatic extract of *Ruta graveolens* at concentration of 25 mg/ml for all of it, while the growth of *E. coli* was inhibited at concentration of 12.5 mg/ml, and had lethal activity at concentration of 200 mg/ml for both of *S. aureus* & *P. aeruginosa*, while both of *E. coli* & *K. pneumoniae* was died at concentration of 100 & 50 mg/ml respectively. Ethanolic extract from *Ruta graveolens* showed antibacterial activity against all the tested bacteria, and the strongest activity was seen against *S. aureus* (MIC= 3.125 mg/ml & MBC= 100 mg/ml), it was active against both *E. coli & K. pneumoniae* (MIC=1.562 mg/ml for both and MBC= 200 & 25 mg/ml respectively). The extract also manifested activity against *P. aeruginosa* at an MIC= 3.125 & MBC= >200 mg/ml. The MIC of *Ruta graveolens* methanolic extract for both *S. aureus & P. aeruginosa* was 3.125 mg/ml, while MBC was 100 mg/ml for both of it, while MIC for both *E. coli* & *K. pneumoniae* was 1.562 mg/ml for both, and MBC of 50 & 25 mg/ml respectively. As can be seen in Table 4.43. MIC assay for *Ruta graveolens* EO revealed that the MIC value for *S. aureus* & *K. pneumoniae* was 50 µl/ml for both, and was 25 & 12.5 µl /ml for both of *E. coli & P. aeruginosa* clinical isolates. while MBC assay for *Ruta graveolens* EO extract revealed that the MBC value for *S. aureus* & *P. aeruginosa* was >200 µl/ml for both of it, and for both *E. coli & K. pneumoniae* was 200 & 100 µl /ml respectively.

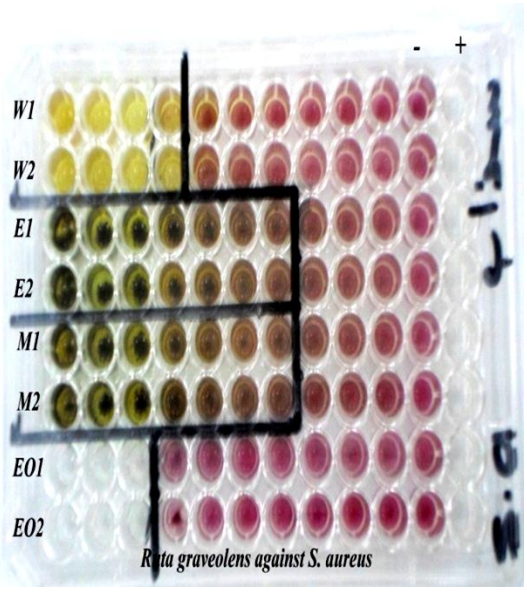


Figure. 4.50. A. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Ruta graveolens* extracts against *S. aureus*.

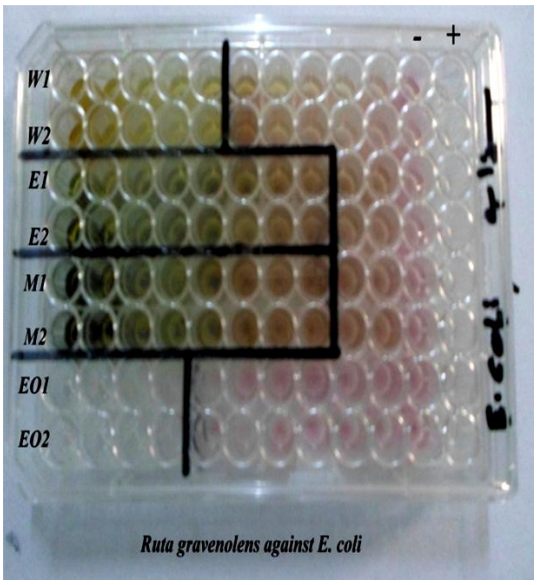


Figure. 4.50. B. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Ruta graveolens* extracts against *E. coli*.

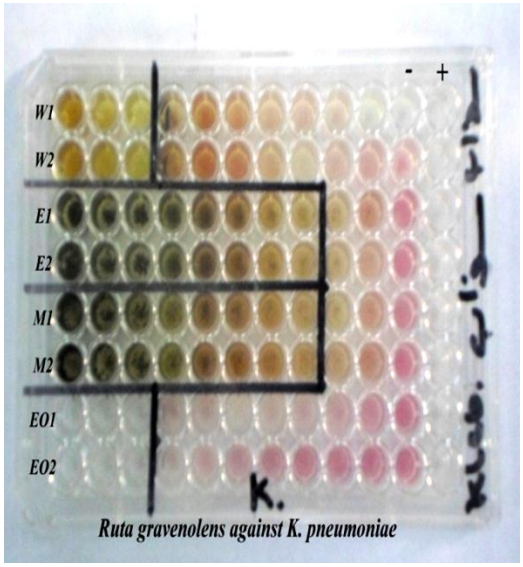


Figure. 4.50. C. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Ruta graveolens* extracts against *K.pneumoniae*.

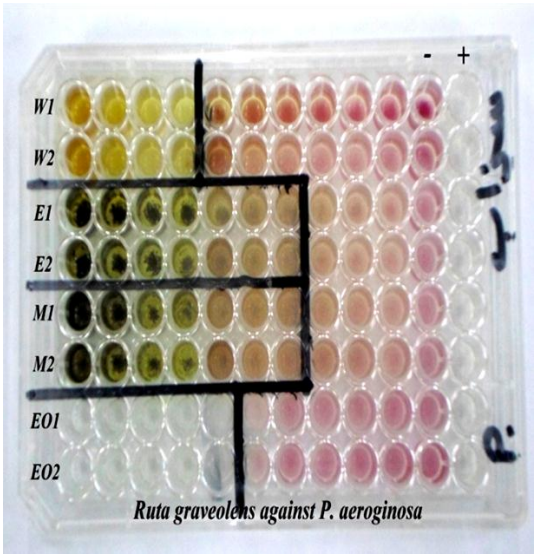


Figure. 4.50. D. W. Aquatic, E. Ethanolic, M. Methanolic & EO. Essintial oil. The MIC & MBC of *Ruta graveolens* extracts against *P. aeruginosa*.

Chapter 5

Discussion

The usage of medicinal plants for primary health care needs by millions of people in developing world is still occupying a prominent position (WHO, 2002). The folk remedies are considered readily available, cheap and time tested (Pandikumar *et al*., 2011).

Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics; some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria. The results of this indicate the need for further research into traditional health systems. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity. The need of the hour is to screen a number of medicinal plants for promising biological activity (Parekh & Chanda., 2007).

The aim of this study was the evaluation of the antibacterial activity and synergistic effects of six plants extracts using different method of extraction against multi-drug-resistant (MDR) *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa* bacteria. In this study different extracts of *Allium sativum, Ecballium elaterium, Pelargonium graveolens, Rosmarinus officinalis, Phagnalon rupestre* & *Ruta graveolens* were evaluated for exploration of their antibacterial activity against clinically isolates pathogenic bacteria.

Susceptibility of each plant extracts were tested by serial microdilution method for MIC & MBC and agar disc diffusion method.

5.1. Antibacterial activity:

The presence of antibacterial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Parekh & Chanda., 2007). The traditional healers use primarily water as the solvent but we found in this study the plant extracts by methanol provided more

consistent antimicrobial activity compared to those extracted by water, ethanol & steam distillation. This might have resulted from the differing of solubility of the active constituents in aqueous & ethanol solutions while methanol extract showed some degree of antibacterial activity (Parekh & Chanda., 2007).

In this study, different extracts of *Allium sativum, Ecballium elaterium, Pelargonium graveolens, Rosmarinus officinalis, Phagnalon rupestre & Ruta graveolens* showed significant antibacterial activity against multi-drug-resistant (MDR) gram positive (*S. aureus*) and gram negative (*E. coli, K. pneumoniae* & *P. aeruginosa*) bacteria as assessed by the diameter of zone of inhibition of the extracts. Although, the low values recorded for some plant extracts may be attributed to the fact that the extracts being in crude form, contain very small amounts of bioactive compounds. At the same time, several workers have reported bioactivity of crude extracts of medicinal plants within such range of diameter zone of inhibition (Karmegam *et al*., 2008).

5.1.1. Antibacterial activity of *Allium sativum*:

Allium sativum has been studied for its antibacterial activity against multi-drug-resistant (MDR) *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa*.

5.1.1.1. *Allium sativum* aquatic extract:

The results of this work provide evidence that the aqueous extract of *Allium sativum* possesses significant antibacterial activity against MDR clinical isolates of *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa* bacteria. Similar results were obtained by Gupta *et al* (2015). Our results was compatible with the results obtained by Palaksha *et al* (2010), where they showed a strong antimicrobial activity of *Allium sativum* aquatic extract against streptomycin-resistant *S. aureus* and *E. coli*. In addition, the results in our study are in good agreement with the results that obtained by Chandra *et al* (2010), regarding *S. aureus, E. coli* & *K. pneumoniae*. Either our results agreed with the results that obtained by Abubakar (2009), regarding *S. aureus, E. coli* & *P. aeruginosa* bacteria.

5.1.1.2. *Allium sativum* ethanolic extract:

The results of this study showed that the ethanolic extracts of *Allium sativum* had a higher inhibitory effect against the cited bacteria than that of the water extract.

This could be as a result of better extraction with alcohol solvents. This is in conformity with the work of Arekemase *et al* (2013) & Karuppiah, P., & Rajaram., (2012), regarding *S. aureus, E. coli, P. aeruginosa and K. pneumoniae* bacteria. They discovered that ethanolic extract of garlic produced higher antimicrobial activity than the wateric extract of the plant against *E. coli, P. aeruginosa and K. pneumoniae*, while *S. aureus and Bacillus subtilis* showed least sensitivity. In addition, the results in our study are compatible with the results that obtained by Akintobi OA *et al* (2013), regarding *S. aureus* & *P. aeruginosa* Ethanolic extract of the garlic, while our results incompatible regarding *E. coli*. Although the reason for this variation is not clear, it could be assumed to be as a result of difference in the extraction method & genetic differences between the plant and microbial strains used in this study. In addition, the results are agreement with the results that obtained by Abubakar (2009), regarding *S. aureus, E. coli & P. aeruginosa* bacteria.

5.1.1.3. *Allium sativum* methanolic extract:

The methanolic extract of garlic bulb was also effective against all the four MDR bacteria under study. The results obtained by Gaherwal *et al* (2014), was compatible with our results regarding *E. coli, S. aureus.* In addition, similar results were obtained by Meriga *et al* (2012), where they showed that the methanolic extract of garlic bulb showed antimicrobial activity against *E. coli* and *K. pneumoniae* except *S. aureus*. Also Similar results were obtained by Gull *et al* (2012), in which the inhibitory effect of aqueous, methanolic & ethanolic extracts of *Allium sativum* had been assayed separately against drug resistant *E. coli, P. aeruginosa, K. pneumoniae* & *S. aureus.* Where they showed that all tested strains was susceptible to garlic aqueous, methanolic and ethanolic extracts.

5.1.1.4. *Allium sativum* essential oil:

In general, the inhibitory activity of essential oils was greater than that of aquatic & ethanolic extracts. The EO of *Allium sativum* bulbs was also effective against drug resistant *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa* bacteria under study. Our results agreement with the results obtained by Sharma *et al* (2013), regarding *S. aureus & E. coli* bacteria. Either our results are agreement with the result obtained by Casella *et al* (2013), against *S. aureus, P. aeruginosa* and *E. coli*. This activity was

attributed to the presence of diallyl sulfides (Disulfide, Trisulfide & Tetrasulfide) and increased according to the number of sulfur atoms in the compounds. Either our results are in good agreement with the result obtained by Al-Mariri, & Safi (2014), regarding *K. pneumoniae*. In addtion, our results are agreement with the result were obtained by Viswanathan *et al* (2014), in which the antibacterial activity of *Allium sativum* essential oil showed a potent antimicrobial activity against *S. aureus*, *E. coli and MRSA.*

The antimicrobial activity of *A. sativum* bulbs extracts suggested by Meriga *et al* (2012), suggest that, these extracts contain effective phytochemicals responsible for the inhibition of microorganisms. The antibacterial activity of garlic is widely attributed to its phytochemicals. Allicin, an important constituent of garlic interferes with RNA production and lipid synthesis.

5.1.2. Antibacterial activity of *Ecballium eleterium*:

5.1.2.1. *Ecballium eleterium* aquatic extract:

The aquatic extract of *Ecballium eleterium* shown a good *in-vitro* inhibitory activity against *S. aureus*, *E. coli*, *K. pneumoniae* & *P. aeruginosa*. Our results are not agreement with that result obtained by **Dogruoz *et al*** (2008), in which the antibacterial activity of the aqueous extract of *Ecballium elaterium* was not active against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* at concentration of 0.001mg/ml. Although the reason for this variation is not clear, it could be assumed to be as a result of low concentration of the aquatic extract of *Ecballium eleterium* and difference in the extraction method & genetic differences between the plant and microbial strains used in this study.

5.1.2.2. *Ecballium eleterium* ethanolic extract:

The ethanolic extract of *Ecballium eleterium* fruits showed a good *in-vitro* antibacterial inhibitory activity against multi-drug resistant *S. aureus*, *E. coli*, *K. pneumoniae* & *P. aeruginosa*. The results observed in our study are consistent with previous reports on related plants regarding Gram-positive *S. aureus* bacteria (Adwan *et al*., 2011). In addition, ethanolic extract of *Ecballium elaterium* fruits had antibacterial inhibitory activity against multi-drug resistant *K. pneumoniae* (Koca et al., 2010). Either our results was compatible with previous reports on related plant

regarding multi-drug resistant *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa* (Oskay *et al*., 2009). Either ethanolic extract of *Ecballium elaterium* leaves & fruits had antibacterial inhibitory activity against MRSA & E. *coli* (Oskay & Sarı., 2007). This works confirmed what is reported in the literature that ethanolic extract of *Ecballium eleterium* fruits show antibacterial activity.

5.1.2.3. *Ecballium eleterium* methanolic extract:

The methanolic extract of *Ecballium eleterium* fruits showed a good *in-vitro* antibacterial inhibitory activity against multi-drug resistant *S. aureus, E. coli, K. pneumoniae* & P. *aeruginosa*. Our results are in agreement with the result obtained by **Sasmakov *et al*** (2012), in which the antibacterial activity of *Ecballium elaterium* methanolic extract showed moderate antibacterial activity against *S. aureus*. While the *E. coli* was not sensitive to methanolic extract of *Ecballium elaterium*, the difference in the result against *E. coli* is not clear, it could be assumed to be as a result of difference in the extraction method & genetic differences between the plant and in concentration of the methanolic extract and microbial strains used in this study.

5.1.2.4. *Ecballium eleterium* essential oil:

This is the first time where the antibacterial activity of *Ecballium eleterium* fruits essential oil were estimated against the clinically isolates bacterial strains.

No articles on antimicrobial activity of *Ecballium eleterium* essential oil was published in Pub Med. In this study, the antibacterial activity of essential oil of *Ecballium eleterium* fruits was assessed *in-vitro* when used against multi-drug resistant *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa* clinically isolates. Essential oil of *Ecballium eleterium* fruits showed a good *in-vitro* antibacterial inhibitory activity against cited microorganisms.

5.1.3. Antibacterial activity of *Pelargonium graveolens*

The antibacterial activity of *Pelargonium graveolens* extracts & EO, both by direct contact through agar disk diffusion method, was assessed by the presence or absence of inhibition zone. The diameter of the inhibition zone is given in Tables 4.2- 5. To the best of our knowledge, the antibacterial activity of *Pelargonium graveolens*

extracts & EO growing in Palestine has never been reported. This work is therefore the first report on the extracts & EO from this aromatic plant in Palestine.

5.1.3.1. *Pelargonium graveolens* aquatic extract:

The aquatic extract of *Pelargonium graveolens* in general shown a high *in-vitro* inhibitory activity against *S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa*.

The aquatic extract of *Pelargonium graveolens* had antibacterial inhibitory activity against *S. aureus, E. coli* & *P. aeruginosa* (Boukhris *et al*., 2013). This work confirmed what is reported in the literature that aquatic extract of *Pelargonium graveolens* show antibacterial activity. While other article reported that, the aquatic extracts remained inactive against *S. aureus & K. pneumoniae* in the range of the used concentration 4 mg/wells (Hsouna & Hamdi., 2012). This work is not confirmed what is reported in the literature that aquatic extract of *Pelargonium graveolens* show antibacterial activity. These differences seem to depend on low concentration of aquatic extract & climate changes and conditions and methods of extraction.

5.1.3.2. *Pelargonium graveolens* ethanolic extract:

The results obtained from the disc diffusion method, indicated that the ethanolic extract of *Pelargonium graveolens* exhibited a high *in-vitro* antibacterial inhibitory activity against multi-drug resistant Gram-positive (*S. aureus*) and three Gram-negative (*E. coli, K. pneumoniae* & *P. aeruginosa*) clinically isolated bacteria at the concentration of 10µl/disc. This is the first time where the antibacterial activity of ethanolic extract of *Pelargonium graveolens* was estimated against the bacterial strains. No articles on antimicrobial activity of ethanolic extract of *Pelargonium graveolens* was published in Pub Med.

5.1.3.3. *Pelargonium graveolens* methanolic extract:

The results obtained from the disc diffusion method, indicated that the methanolic extract of *Pelargonium graveolens* in general exhibited a high *in-vitro* antibacterial inhibitory activity against multi-drug resistant Gram-positive (*S. aureus*) and three Gram-negative (*E. coli, K. pneumoniae* & *P. aeruginosa*) clinically isolated bacteria at the concentration of 10µl / disc. Our conclusions were in agreement with the results of an earlier study (Boukhris *et al*., 2013), in which the antibacterial activity of

Pelargonium graveolens methanolic extract against *P. aeruginosa*, *E. coli*, *S. aureus* exhibit bactericidal effects. Also our results are in agreement with the result obtained by Hsouna & Hamdi (2012), where they showed that The methanolic extract of *Pelargonium graveolens* exhibited a promising antimicrobial inhibitory activity against a panel of microorganisms (including within of it *S. aureus* & *K. pneumoniae*).

5.1.3.4. *Pelargonium graveolens* essential oil:

In this study, the antibacterial activity of essential oil of *Pelargonium graveolens* was assessed *in-vitro* when used against multi-drug resistant *S. aureus*, *E. coli*, *K. pneumoniae* & *P. aeruginosa* clinically isolates. Essential oil of *Pelargonium graveolens* showed a good *in-vitro* antibacterial inhibitory activity against cited bacteria. Similar results were obtained by Boukhris *et al* (2013), Ghannadi A *et al* (2012), regarding *P. aeruginosa*, *E. coli* & *S. aureus*. Either our results agreed with that results were obtained by Hsouna & Hamdi (2012), regarding *S. aureus* & *K. pneumoniae* bacteria. Furthermore similar results were obtained by Rosato *et al* (2007), where they showed that, the *Pelargonium graveolens* EO had antibacterial effect against *S. aureus* & *E. coli*. In addition, the results obtained by Dorman & Deans (2000) support our finding regarding *S. aureus*, *K. pneumoniae* & *P. aeruginosa*, while incompatible with our results regarding *E. coli* bacteria. Although the reason for this variation is not clear, it could be assumed to be as a result of low concentration of the EO of *Pelargonium graveolens* and difference in the extraction method & genetic differences between the plant and microbial strains used in this study. Either the results obtained by Hammer *et al* (1999) revealed that, *Pelargonium graveolens* herb EO had antibacterial activity against *E. coli*, *K. pneumoniae*, *P.* aeruginosa & *S. aureus*, this finding confirmed what is reported in our study.

5.1.4. Antibacterial activity of *Rosmarinus officinalis*:

The *in-vitro* antibacterial activity of the *Rosmarinus officinalis* extracts and EO estimated by the diameter of inhibition zones, which varied according to extraction varieties and bacterial strains.

5.1.4.1. *Rosmarinus officinalis* aquatic extract:

Our work showed that, the *in-vitro* antibacterial activity of the *Rosmarinus officinalis* aquatic extract had high antibacterial activities towards the Gram-positive bacteria (MRSA) and moderate antibacterial activities towards the Gram-negative bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*). These results are consistent with previous reports on related plants regarding Gram-positive (*S. aureus*) and Gram- negative (*E. coli*) bacteria (Jordan *et al* (2012). Either the results was obtained by Abu-Shanab *et al*., 2004, revealed that, the aquatic extract of *Rosmarinus officinalis* had a moderate antibacterial activity against MRSA and no effects were detected against EHE.coli or *P. aeruginosa* at concentration of 100 mg/ml. It is thought that the observed dissimilar results may be attributed to low concentration of the extract & to the genetic differences between the plant and microbial strains used in this study.

5.1.4.2. *Rosmarinus officinalis* ethanolic extract:

Antibacterial activity of *Rosmarinus officinalis* ethanolic extract has been evaluated *in-vitro* against Gram-positive bacteria (MRSA) and Gram-negative bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*), that are known to cause infections in humans. *Rosmarinus officinalis* ethanolic extract showed antibacterial activity against all tested microorganisms. According to Qabaha (2013), *Rosmarinus offcinalis* ethanolic extract exhibit significant antimicrobial activity against *S. aureus* & MDR. (*E. coli, P. aeruginosa* & *K. pneumoniae*) (Palestinian medicinal plants) these results were compatible with our result. Moreover, our results are consistent with previous reports on related plants regarding Gram-positive and Gram- negative bacteria (Oskay *et al*., 2009). Either according to Oskay & Sarı (2007), the antibacterial activity of *Rosmarinus officinalis* ethanolic extract showed broad-spectrum antimicrobial activity against Gram-positive (MRSA) and Gram-negative (*E. coli*) bacteria that are resistant to some antibiotics. Our similar results confirm this situation. furthermore the results that obtained by Abu-Shanab *et al*., 2004 support what is reported in our study regarding MRSA but from other hand our results was incompatible with the finding of this report regarding *E. coli* & *P. aeruginosa*. It is thought that the observed dissimilar results may be attributed to low concentration of the extract & to the genetic differences between the plant and microbial strains used in this study.

5.1.4.3. *Rosmarinus officinalis* methanolic extract:

The results of antibacterial assays revealed that this methanolic plant extract exhibited varying degree of antibacterial activities against all tested bacterial strains. In general, Gram-positive bacteria were more susceptible to plant extracts when compared to Gram-negative bacteria. As in the current study, previously studies have reported the antibacterial activity of various extracts of *Rosmarinus officinalis* on different microorganisms (Irshaid *et al* .,2014), in which the antibacterial activity of *Rosmarinus officinalis* methanolic extract exhibit high values of inhibition effects against *P. aeruginosa*, *E. coli* & *S. aureus* bacteria. But our results were not consistent with previous *in-vitro* studies (Celiktas *et al*., 2007) which reported antibacterial activity of *Rosmarinus officinalis* methanolic extract, which exhibited low activity against *S. aureus* whereas the extracts were inactive against *E. coli*, *K. pneumoniae* & *P. aeruginosa* at concentration ranging from 125 to 15.6 mg/ml of the extracts. Moreover the results that obtained by Abu-Shanab *et al*., 2004, revealed that, the methanolic extract of *Rosmarinus officinalis* had a significant antibacterial activity against MRSA and no effects were detected against *E. coli* or *P. aeruginosa* at concentration of 100 mg/ml. It is thought that the observed dissimilar results may be attributed to low concentration of the extract and to differences in techniques and extracts because different methods were used and the variable sensitivity of different microorganisms to chemical substances relates to different resistance levels between the strains.

5.1.4.4. *Rosmarinus officinalis* essential oil:

The results of antibacterial assays revealed that this *Rosmarinus officinalis* essential oil exhibited moderate antibacterial activities against all tested bacterial strains. This results was in agreement with many other studies reported on this plant (Barbosa *et al*., 2015 & Hussain *et al*., 2010), regarding ***S. aureus***, *E. coli* & ***P. aeruginosa***. In addition, in agreement with our results, Hosni *et al* (2013), which reported that, the essential oil from *Rosmarinus officinalis* inhibited the growth of *S. aureus* & *E. coli*. In addition our results was in agreement with Jordan *et al* (2013), which reported that, the essential oil from *Rosmarinus officinalis* inhibited the growth of *S. aureus* but had no effect against *E. coli*. It is thought that the observed dissimilar results may be attributed to differences in genetic differences between the plant and microbial

strains used in this study. Also as is in the current study, previously studies have reported the antibacterial activity of various extracts of *Rosmarinus officinalis* on different microorganisms (Zaoual *et al*., 2010; Celiktas *et al*., 2007; Hammer *et al*., 1999 Baratta *et al.,* 1998), in which the tested bacteria (*S. aureus*, *E. coli, K. pneumonia* & *P. aeruginosa*) were sensitive to the essential oils of *Rosmarinus officinalis*. In addition, our results are consistent with previous reports on related plants regarding Gram-positive (*S. aureus*) and Gram-negative (*E. coli* & *K. pneumoniae*) bacteria (Okoh *et al*., 2010). Furthermore our results are consistent with previous reports on related plants regarding Gram-positive (*S. aureus*) and Gram-negative (*K. pneumoniae*) bacteria (Van Vuuren *et al*., 2009).

5.1.5. Antibacterial activity of *Ruta-graveolens*:

The *in-vitro* antibacterial activity of the *Ruta graveolens* extracts and essential oil estimated by the diameter of inhibition zones varied according to extraction varieties and bacterial strains.

5.1.5.1. *Ruta graveolens* aquatic extract:

This is the first time where the antibacterial activity of *Ruta graveolens* aquatic extract were estimated against the clinically isolates bacterial strains.

No articles on antimicrobial activity of *Ruta graveolens* aquatic extract was published in Pub Med. In this study, the antibacterial activity of aquatic extract of *Ruta graveolens* was assessed *in-vitro* when used against multi-drug resistant *S. aureus*, *E. coli, K. pneumoniae* & *P. aeruginosa* clinically isolates. The aquatic extract of *Ruta graveolens* showed a low *in-vitro* antibacterial inhibitory activity against multi-drug resistant Gram-positive bacteria (MRSA) and Gram-negative bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*).

5.1.5.2. *Ruta graveolens* ethanolic extract:

Antibacterial activity of *Ruta graveolens* ethanolic extract has been evaluated *in-vitro* against Gram-positive bacteria (MRSA) and Gram-negative bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*), that are known to cause infections in humans. *Ruta graveolens* ethanolic extract showed good antibacterial activity against all the tested microorganisms. Our founding are incompatible with results of study of Jalali

Moghadam *et al* (2012) in which *Ruta graveolens* ethanolic extract had insignificant antimicrobial activity against 10 pathogenic bacteria including within of it *S. aureus, E. coli, K. pneumonia* and *P. aerogenosa* is up to concentration 5 mg/ml. It is thought that the observed dissimilar results may be attributed to low concentration of the extract and to differences in extraction method because different methods were used and to genetic differences between the plant and microbial strains used in this study. Moreover our results are consistent with previous reports on related plants regarding Gram-positive (*S. aureus*) and Gram-negative (*E. coli & P. aeruginosa*) bacteria, by using agar diffusion method (Valsaraj *et al.,* 1997) but from other hand our finding was incompatible regarding *E. coli*. It is thought that the observed dissimilar results may be attributed to low concentration of the extract and to differences in extraction method because different methods were used and to genetic differences between the plant and microbial strains used in this study.

5.1.5.3. *Ruta graveolens* methanolic extract:

The results of antibacterial assays revealed that this methanolic plant extract exhibited varying degree of antibacterial activities against all tested bacterial strains. In general, *Ruta graveolens* methanolic extract has a moderate antibacterial activities against Gram-positive bacteria (MRSA) and Gram-negative bacteria (*E. coli & K. pneumoniae*) except against *P. aeruginosa* that had a low sensitivity to *Ruta graveolens* methanolic extract. As is in the current study, previously studies have reported the antibacterial activity of various extracts of *Ruta graveolens* on different microorganisms (Ivanova *et al*., 2005), in which the antibacterial activity of *Ruta graveolens* methanolic extract exhibit a good antibacterial activity against *S. aureus* bacteria, and the extracts showed no activity against the Gram-negative *E. coli*. It is thought that the observed dissimilar results may be attributed to low concentration of the extract and to differences in techniques and extracts because different methods were used and the variable sensitivity of different microorganisms to chemical substances relates to different resistance levels between the strains. In addition, our results were consistent with previous *in-vitro* studies (Ojala *et al*., 2000) which reported antibacterial activity of *Ruta graveolens* methanolic extract, which revealed moderate antimicrobial activity against *S. aureus & P. aeruginosa* whereas the extracts were inactive against *E. coli* at concentration of 0.126 mg/ml of the

methanolic crude extracts. It is thought that the observed dissimilar results may be attributed to low concentration of the extract and to genetic differences between the plant and microbial strains used in this study and the variable sensitivity of different microorganisms to chemical substances relates to different resistance levels between the strains. No articles on antimicrobial activity of *Ruta graveolens* methanolic extract against *K. pneumoniae* was published in Pub Med.

5.1.5.4. *Ruta graveolens* essential oil:

The results of antibacterial assays revealed that this *Ruta graveolens* EO exhibited moderate antibacterial activities against all tested bacterial strains, except against *P. aeruginosa* that had a low antibacterial activity. These results was in agreement with many other studies reported on this plant (Al-Shuneigat *et al*., 2015) regarding MRSA, *E. coli & P. aeruginosa* bacteria. In addition, in agreement with our results Orlanda & Nascimento (2015) regarding *S. aureus*, *P. aeruginosa* and *E. coli*. Furthermore, our results in agreement with Haddouchi *et al* (2013), which reported that, the essential oils of the *Ruta graveolens* when applied at 10 µl / disc had low *in-vitro* potential of antibacterial activity against *S. aureus*, *E. coli*, *K. pneumoniae* & *P. aeruginosa*.

5.1.6. Antibacterial activity of *Phagnalon rupestre*:

The in vitro antibacterial activity of the *Phagnalon rupestre* extracts and EO estimated by the diameter of inhibition zone, which are varied according to extraction varieties and bacterial strains.

5.1.6.1. *Phagnalon rupestre* aquatic extract:

Our work showed that, the *in-vitro* antibacterial activity of the *Phagnalon rupestre* aquatic extract had in general good antibacterial activities towards the Gram-positive bacteria (MRSA) and the Gram-negative bacteria (*E. coli*, *K. pneumoniae* and *P. aeruginosa*). These results are consistent with previous reports on related plants regarding Gram-positive (*S. aureus*) and Gram- negative (*E. coli*, *K. pneumoniae* and *P. aeruginosa*) bacteria (Ali-Shtayeh *et al*., 1998).

5.1.6.2. *Phagnalon rupestre* ethanolic extract:

Antibacterial activity of *Phagnalon rupestre* ethanolic extract has been evaluated *in-vitro* against Gram-positive bacteria (MRSA) and Gram-negative bacteria (*E. coli, K. pneumoniae* and *P. aeruginosa*), that are known to cause infections in humans. *Phagnalon rupestre* ethanolic extract showed good antibacterial activity against all the tested microorganisms. Our founding are compatible with results of study of **Ali-Shtayeh *et al*** (1998), in which *Phagnalon rupestre* ethanolic extract had a good antimicrobial activity against *S. aureus*, *K. pneumoniae* & *P. vulgaris*, while Our founding are incompatible with results of this study regarding the *E. coli* & *P. aeruginosa* which it was resistant to the *Phagnalon rupestre* ethanolic extract at concentration 200 mg/ml in this study. It is thought that the observed dissimilar results may be attributed to differences in techniques of extraction because different methods were used and to genetic differences between the plant and microbial strains used in this study and the variable sensitivity of different microorganisms to chemical substances relates to different resistance levels between the strains.

5.1.6.3. *Phagnalon rupestre* methanolic extract:

This is the first time where the antibacterial activity of *Phagnalon rupestre* methanolic extract were estimated against the clinically isolates bacterial strains. The results of antibacterial assays revealed that this methanolic plant extract exhibited varying degree of antibacterial activities against all tested bacterial strains.

In general, *Phagnalon rupestre* methanolic extract has a good antibacterial activity against Gram-negative bacteria (*E. coli & K. pneumoniae*) except against *P. aeruginosa* and Gram-positive bacteria (MRSA) that had a low sensitivity to *Phagnalon rupestre* methanolic extract. There are no articles on antimicrobial activity of *Phagnalon rupestre* methanolic extract published in Pub Med.

5.1.6.4. *Phagnalon rupestre* essential oil:

This is the first time where the antibacterial activity of *Phagnalon rupestre* EO were estimated against the clinically isolates bacterial strains.

The results of antibacterial assays of *Phagnalon rupestre* EO revealed that *Phagnalon rupestre* EO exhibited low antibacterial activities against Gram-positive bacteria (MRSA) and Gram-negative bacteria (*E. coli*), and had good antibacterial activities

against *K. pneumoniae* & *P. aeruginosa* bacteria. There are no articles on the antibacterial activity of *Phagnalon rupestre* EO published in Pub Med.

In general, it was interesting to note that antibiotic-resistant bacteria showed more sensitivity to the investigated extracts. This has clearly indicated that antibiotic resistance does not interfere with the anti-microbial action of plant extracts, and these extracts might have different modes of action on test organisms.

5.2. Synergistic effects of plant extracts and antibiotics:

Combined antibiotic therapy has been shown to delay the emergency of bacterial resistance and may produce desirable synergistic effects in the treatment of bacterial infection. Drug synergism between known antibiotics and bioactive plant extracts is a novel concept, and could be beneficial (synergistic or additive interaction) or deleterious (antagonistic or toxic outcome). Despite the abundant literature about the antimicrobial properties of plant extracts, none of plant-derived chemicals has successfully been used for clinical use as antibiotics (Adwan & Mhanna., 2009).

In our experiments, despite that, some plant extracts showed weak antibacterial effect using agar disk diffusion method, the interactions between antibiotics and plant extracts were some time additive against the four stains of tested bacteria. This could be attributed to the inability of higher concentration of plant extracts to diffuse through the Mueller-Hinton Agar Media. The association of natural products such as plant extracts and antibiotics constitutes an alternative in the fight against MDR bacteria. This impairment in drug diffusion is a major limitation in the evaluation of the antimicrobial effects of plant extracts using the agar diffusion method (Adwan & Mhanna., 2009). The improvement in the activity of those antibiotics is probably due to accumulation of inhibitory concentrations at the target sites or due to the additional inhibitory effect of the tested plant material. Survival of MRSA strains in the presence of plant material could probably be due to the cell membrane permeability or other genetic factors (El-Kalek & Mohamed., 2012).

5.2.1. Synergistic effects of plant extracts and antibiotics against *Staphylococcus aureus*:

In this study, synergism effect resulting from the combination of antimicrobial agents with crude plant extracts was verified for most plants.

Significant synergistic effects were noted with both *Pelargonium graveolen* and *Rosmarinus officinalis* extracts when they were associated with several antibiotics. Such effects might be due to the action of the active compounds or possible inhibition of one or more mode of bacterial resistance mechanisms by other compounds of the extracts. The plants, *Pelargonium graveolen* and *Rosmarinus officinalis* followed by *Ruta graveolens* ethanolic extracts alone or in combination, are promising in the development of phytomedicines, which may be used alone or in combination with the antibiotics against MRSA infections, which had the most synergistic inhibitory effect against MRSA, which presented synergism with most drugs. The synergistic effects that resulting from the combination of *Rosmarinus officinalis* aquatic extract with CL & P against MRSA are compatible with results of study of Adwan & Mhanna., (2009), in which *Rosmarinus officinalis* aquatic extract had synergistic effects when combined with CL & P against MRSA & MSSA bacteria which showed a decrease in MIC of tested antimicrobial agents and this could be referred to that these crude extract have many different phyto-chemicals, which might inhibit bacteria by different mechanisms. The lowest synergistic effects was observed with DNA synthesis inhibitors (CIP when combined with *Ecballium elaterium* ethanolic extract and *Phagnalon rupestre* methanolic and *Ruta graveolens* aquatic & methanolic extracts and OFX when combined with *Ruta graveolens* ethanolic extract) and was observed with cell wall synthesis inhibitors (AM when combined with *Ruta graveolens* methanolic extract & AMC when combined with *Ruta graveolens* methanolic extract and EO & was observed with P & OX when combined with *Ruta graveolens* methanolic extract), this is obviously due to the fact their target are localized in the bacterial cell coat. However, the synergistic effects observed indicate that active compounds of the extracts could also present different mode(s) of action from those of the studied antibiotics. However, other researcher's studies the effect of combination of plant extracts with antimicrobial agents against MRSA like El-Kalek & Mohamed., (2012) which Screenings of Synergistic effect of certain medicinal plants (nine medicinal plants. three essential oils and six methanolic herbal extracts) and amoxicillin against some clinical isolates of MRSA. Results revealed that, all of tested plants possess a degree of antibacterial activity toward MRSA strains under study, but each of *lemon grass* oil (LGEO), *Cardamom* oil and *thymus vulgaris* extract possess highly degree of antibacterial activity towards (MRSA), (LGEO) showed better inhibitory effects than others. The activity of Amoxacillin against

MSSA (C.I), MSSA (ATCC) and 15 MRSA strains tested were from 15 to 28 mm (inhibition zone), three strains of MRSA (M2, M16 and M18) showed less susceptibility to amoxicillin. When Amoxicillin was combined with LGEO, *Cardamom oil* and *Thymus vulgaris* extracts, the inhibition zones were increased. The results also revealed that, M2, M16 and M18 became sensitive to each combination; the results showed significantly increase in activity of Amoxacillin when combined with tested plant material.

5.2.2. Synergistic effects of plant extracts and antibiotics against gram negative bacteria:

Many studies have shown that active efflux can be a mechanism of resistance for almost all antibiotics. The majority of the efflux systems in bacteria are non-drug-specific proteins that can recognize and export a broad range of chemically and structurally unrelated compounds from bacteria without drug alteration or degradation. Antibiotic efflux is a major mechanism of antibiotic resistance mainly those clinically described as AcrAB-TolC pump in *Enterobacteriaceae* or MexAB-OprM pump in *P. aeruginosa*, are associated with a major human health problem as they play a central role in multidrug resistance of pathogenic Gram-negative bacteria. Resistance to β–lactams and non-β-lactam antibiotics has been attributed to efflux by the MexAB-OprM pump in *P. aeruginosa*. Other Mex efflux proteins mediating multidrug resistance have also been identified in *P. aeruginosa*. Efflux pump inhibitors (phenylalanine arginine β-naphthylamide was used as efflux pumps inhibitor) combined with antibiotics strategy is an effective way to solve the problem caused by resistant bacteria. The majority of plant derived antimicrobial compounds generally have higher MICs than bacterial or fungal produced antibiotics, thus limiting their therapeutic potential (Voukeng *et al*., 2012 & Adwan *et al*., 2010).

5.2.2.1. Synergistic effects of plant extracts and antibiotics against *Esherichia coli*:

The results obtained by combining the antibiotic with the extracts of *Allium sativum* methanolic extracts & EO, *Pelargonium graveolen* EO, *Rosmarinus officinalis* aquatic, methanolic extracts & EO and *Ruta graveolens* methanolic extract which had the most synergistic inhibitory effect against *E. coli* which presented synergism with

most drugs, indicate that these extracts contain chemical compounds that can modulate the activity of antibiotics against bacteria expressing MDR phenotypes. There are no articles about the synergism effect of these plant extracts were published in the Pub Med, But other researchers studies the effect of combination of plant extracts with antimicrobial agents against *E. coli* and other bacteria like Sasidharan *et al*., 2014 which evaluate the in vitro synergistic effect of curcumin in combination with third generation cephalosporins (Cefaclor, Cefodizime & Cefotaxime) against bacteria associated with infectious diarrhea (*S. aureus ,B. subtilis, E. coli, P. aeruginosa & V. cholerae*). The results revealed that CUR-1 significantly lowered the MICs of antibiotics (cefaclor, cefodizime, and cefotaxime) against the tested bacteria.

5.2.2.3. Synergistic effects of plant extracts and antibiotics against *K. pneumoniae*:

The therapeutic properties of *Rosmarinus officinalis* include the treatment of bronchitis, sinusitis, as an expectorant, as a mucolytic and an antiseptic. The administration by inhalation has been used by aromatherapists, traditional Chinese medicinal practitioners as well as more recently, used in the fumigation of French hospitals. *K. pneumoniae* has been identified as one of the major causes of septicaemia in pediatric wards (Van Vuuren *et al*., 2009). In this study, synergism effect resulting from the combination of antimicrobial agents with crude plant extracts was verified for most plants. Significant synergistic effects were noted with both *Pelargonium graveolen* (EO), *Rosmarinus officinalis, Ecballium elaterium* & *Ruta graveolens* extracts when they was associated with most tested antibiotics had the most favourable synergistic pattern . Such effects might be due either to the action of the active compounds or possible inhibition of one or more mode of bacterial resistance mechanisms by other compounds of the extracts. The plants, *Pelargonium graveolen* (EO) and *Rosmarinus officinalis* followed by *Allium sativum* & *Ruta graveolens* extracts alone or in combination, are promising in the development of phytomedicines, which may be used, alone or in combination with the antibiotics against *K. pneumoniae* infections, which had the most synergistic inhibitory effect against *K. pneumoniae*, which presented synergism with most drugs. The synergistic effects that resulting from the combination of *Rosmarinus officinalis* EO with CIP against *K. pneumonia*e is compatible with results of study of Van Vuuren *et al*.,

(2009), in which *Rosmarinus officinalis / ciprofloxacin* combination against *K. pneumoniae* displayed the most favourable synergistic pattern.

5.2.2.4. Synergistic effects of plant extracts and antibiotics against *P. aeruginosa*:

In this study, synergism effect resulting from the combination of antimicrobial agents with crude plant extracts was verified for most plants. Significant synergistic effects was noted with both *Pelargonium graveolen* & *Ruta graveolens* aquatic extracts, when they were associated with most tested antibiotics had the most favourable synergistic pattern. Also significant synergistic effects were noted with both *Pelargonium graveolen* & *Allium sativum* ethanolic extracts, when they was associated with most tested antibiotics had the most favourable synergistic pattern. In addition, significant synergistic effects were noted with both *Pelargonium graveolen, Rosmarinus officinalis* & *Allium sativum* methanolic extracts and EOs, when they were associated with most tested antibiotics had the most favourable synergistic pattern. Such effects might be due either to the action of the active compounds or possible inhibition of one or more mode of bacterial resistance mechanisms by other compounds of the extracts. The plants, *Allium sativum* (ethanolic, methanolic extracts & EO) and *Pelargonium graveolen* (aquatic, ethanolic, methanolic extracts & EO) followed by *Rosmarinus officinalis* (methanolic extracts & EO) alone or in combination, are promising in the development of phytomedicines, which may be used, alone or in combination with the antibiotics against *P. aeruginosa* infections which had the most synergistic inhibitory effect against *P. aeruginosa* which presented synergism with most drugs. There are no articles about the synergism effect of these plant extracts was published in the Pub Med. But other researchers studies the effect of combination of plant extracts with antimicrobial agents against *P. aeruginosa* like Adwan *et al*.,2010 which evaluate the possible *in-vitro* interaction between ethanolic extracts of *Rhus coriaria* (seed), *Sacropoterium spinosum* (seed), *Rosa damascena* (flower) and certain known antimicrobial drugs including oxytetracycline HCl, penicillin G, cephalexin, sulfadimethoxine as sodium, and enrofloxacin. This synergy study was carried out against three clinical strains of multidrug-resistant *P. aeruginosa*. The results of this study showed that there is a

decrease in the MIC in case of combination of ethanolic plant extracts and test antimicrobial agents.

5.3. MIC & MBC of plant extracts:

The micro-titre plate or broth microdilution method has provided a potentially useful technique for determining MICs & MBCs of large numbers of test samples. It is advantages over diffusion techniques include increased sensitivity for small quantities of extract which is important if the antibacterial is scarce as in the case for many natural products, some researchers however, have reported MICs & MBCs values obtained by the agar diffusion method, although high activity in the disk diffusion assay does not necessarily correlate to low MIC & MBC values in the microtitre plate method (Ncube *et al.,* 2008). This finding were agreed with our results as shown in Table (4.27), figure (4.50) in which the MIC of *Ruta graveolens* methanolic extract against *S. aureus* was 3.125 mg/ml while the inhibition zone diameter was 8.33 mm (Table (4.2), figure (4.10). Also as shown in Table (4.26), figure (4.49), in which the MIC of *Ruta graveolens* methanolic extract against *S. aureus* was 3.125 mg/ml while the inhibition zone diameter was 8.33 mm (Table (4.4), figure (4.21).

The results of MIC and MBC values showed that *Allium sativum, Ecballium elaterium, Pelargonium graveolen, Rosmarinus officinalis, Phagnalon rupestre & Ruta graveolens* had potential inhibitory activity against MDR clinically isolated pathogenic bacteria (*S. aureus, E. coli, K. pneumoniae* & *P. aeruginosa*). This activity could be attributed to the components present in plant extracts, which might be involved in some type of antibacterial synergism with the other active compounds. The MBC value of the *Allium sativum* aquatic extract against *P. aeruginosa*, *Ecballium elaterium* EO against *S. aureus* and *P. aeruginosa, Pelargonium graveolen* aquatic extract against *S. aureus, Rosmarinus officinalis* ethanolic extract against *S. aureus* and *Ruta graveolens* ethanolic extract against *P. aeruginosa* & *Ruta graveolens* EO against *S. aureus* & *P. aeruginosa* used in the present study was not possible to determined against these microorganisms, because the concentration of stock solution of extracts was beyond the concentration of MBC.

Chapter 6

Conclusion & Recommendations

- The overall results of the present work provide baseline information for the possible use of the studied plant extracts in the treatment of bacterial infections involving MDR phenotypes. In addition to these antibacterial activities, the data reported herein indicated that possible combinations of the extracts of *Allium sativum, Ecballium elaterium, Pelargonium graveolen, Rosmarinus officinalis, Phagnalon rupestre & Ruta graveolens* plants with several antibiotics could be used in the control of bacterial infections involving MDR phenotypes. Our results support the use of these plants in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search for new drugs.
- The tested crude extracts from *Allium sativum, Ecballium elaterium, Pelargonium graveolen , Rosmarinus officinalis, Phagnalon rupestre & Ruta graveolens* have proved to be promising treating agents against the tested pathogenic microbes, but it needs to be concentrated and furthermore evaluated. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes.
- A wider study is needed to identify the effective components, the mode of action and the possible toxic effect *in-vivo* of these ingredients. The maximum benefit can be achieved when the pharmacokinetics of natural product and the antibiotic combination match. The optimal ratio and dosing regimens should be explored for higher efficacy and decreased toxicological profiles.

REFERENCES:

Abu-Al-Basal, M. A. (2010). Healing potential of Rosmarinus officinalis L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice. *Journal of ethnopharmacology*, *131*(2), 443-450.

Abbassi, F., Ayari, B., Mhamdi, B & Toumi, L. (2014). Phenolic contents and antimicrobial activity of squirting cucumber (*Ecballium elaterium*) extracts against food-borne pathogens. *Pakistan journal of pharmaceutical sciences*, 27(3), 475-479.

Abubakar, E. M. M. (2009). Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniea and Pseudomonas aeruginosa*. *Journal of Medicinal Plants Research*, *3*(4), 179-185.

Abu-Shanab, B., Adwan, G., Abu-Safiya, D., Jarrar, N., & Adwan, K. (2004). Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. *Turkish Journal of Biology*, *28*(2-4), 99-102.

Abd El-Kalek, H. H., Mohamed, E. A. (2012). Synergistic effect of certain medicinal plants and amoxicillin resistant *staphylococcus aureus* (MRSA). *International Journal of Pharmaceutical Applications*, *3*(3), 387-398.

Adwan, G., Abu-Shanab, B., & Adwan, K. (2010). Antibacterial activities of some plant extracts alone and in combination with different antimicrobials against multidrug–resistant *Pseudomonas aeruginosa* strains. *Asian Pacific Journal of Tropical Medicine*, *3*(4), 266-269.

Adwan, G., Salameh, Y., & Adwan, K. (2011). Effect of ethanolic extract of *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans*. *Asian Pacific journal of tropical biomedicine*, *1*(6), 456-460.

Adwan G. & Mhanna M. (2009). Synergistic effects of plant extracts and antibiotics on *staphylococcus aureus* isolates isolated from clinical specimens. *Asian Pacific journal of tropical biomedicine*, *2*(3), 46-51.

Ahmad, N., Faisal, M., Anis, M., &Aref, I. M. (2010). In vitro callus induction and plant regeneration from leaf explants of *Rutagraveolens* L. *South African Journal of Botany*, *76*(3), 597-600.

Ahmad, I., & Aqil, F. (2007). In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. *Microbiological Research*, *162*(3), 264-275.

Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of ethnopharmacology*, *74*(2), 113-123.

Akintobi OA1, Nwanze JC2, Ogele JO1, Idowu AA3, Onianwa O4, Okonko IO4. (2013). Antimicrobial Activity of *Allium sativum* (Garlic) Extract against Some Selected Pathogenic Bacteria. *Nature and Science*, *11*(1), 1-6.

Albayrak, S., Aksoy, A., Sagdic, O., & Hamzaoglu, E. (2010). Compositions, antioxidant and antimicrobial activities of *Helichrysum* (Asteraceae) species collected from Turkey. *Food Chemistry*, *119*(1), 114-122.

Al-Shuneigat, J. M., Al-Tarawneh, I. N., Al-Qudah, M. A., Al-Sarayreh, S. A., Al-Saraireh, Y. M., & Alsharafa, K. Y. (2015). The Chemical Composition and the Antibacterial Properties of *Ruta graveolens* L. Essential Oil Grown in Northern Jordan. *Jordan Journal of Biological Sciences*, *8*(2), 139-143.

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

Al-Mariri, A., & Safi, M. (2014). In vitro antibacterial activity of several plants extracts and oils against some gram-negative bacteria. *Iranian journal of medical sciences*, *39*(1), 36-43.

Al-Sereiti MR, Abu-Amer KM, Sen P. (1999). Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. *Indian journal of experimental biology*, *37*(2), 124-131.

Al-Shuneigat, J. M., Al-Tarawneh, I. N., Al-Qudah, M. A., Al-Sarayreh, S. A., Al-Saraireh, Y. M., & Alsharafa, K. Y. (2015). The Chemical Composition and the Antibacterial Properties of *Ruta graveolens* L. Essential Oil Grown in Northern Jordan. *Jordan Journal of Biological Sciences*, *8*(2), 139-143.

Al-Sokari, S. S., & El Sheikha, A. F. (2015). In vitro antimicrobial activity of crude extracts of some medicinal plants from Al-Baha region in Saudi Arabia. *Journal of Food and Nutrition Sciences*, *3*(1-2), 74-78.

Alzoreky, N. S., & Nakahara, K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International journal of food microbiology*, *80*(3), 223-230.

Alonso-Castro, A. J., Maldonado-Miranda, J. J., Zarate-Martinez, A., del Rosario Jacobo-Salcedo, M., Fernández-Galicia, C., Figueroa-Zuñiga, L. A., & Carranza-Alvarez, C. (2012). Medicinal plants used in the Huasteca Potosina, Mexico. *Journal of ethnopharmacology*, *143*(1), 292-298

Arekemase, M. O., Adetitun, D. O., & Oyeyiola, G. P. (2013). In-vitro Sensitivity of Selected Enteric Bacteria to Extracts of *Allium sativum* L. *Notulae Scientia Biologicae*, *5*(2), 183-188.

Arora, S., &Tandon, S. (2015). DNA fragmentation and cell cycle arrest. A hallmark of apoptosis induced by *Ruta graveolens* in human colon cancer cells. *Homeopathy*, *104*(1), 36-47.

Azizi, I. G., & Karouei, S. M. H. (2012). Effect of Aquatic, Methanolic and Ethanolic Extracts of *Ruta graveolens* on Some Mycotoxigenic Fungi. *American-Eurasian Journal. Agric. & Environ. science*, 12(6). 729-732, 2012.

Barbosa, L. N., Probst, I. S., Teles, A. B., Bérgamo, A. F., Albano, M., Ribeiro, D. S. D. C. M., ... & Júnior, A. F. (2015). In vitro Antibacterial and Chemical Properties of Essential Oils Including Native Plants from Brazil against Pathogenic and Resistant Bacteria. *Journal of oleo science*, *64*(3), 289-298.

Baratta, M. T., Dorman, H. D., Deans, S. G., Biondi, D. M., & Ruberto, G. (1998). Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. *Journal of Essential Oil Research*, *10*(6), 618-627.

Barreto, H. M., Silva Filho, E. C., Lima, E. D. O., Coutinho, H. D., Morais-Braga, M. F., Tavares, C. C., ... & Lopes, J. A. D. (2014). Chemical composition and possible use as adjuvant of the antibiotic therapy of the essential oil of *Rosmarinus officinalis* L. *Industrial Crops and Products*, *59*, 290-294.

Bhunia A., Ray B (2008). Fundamental food microbiology. 4th edition. United States of America. *Taylor and Francis Group*, 429-432.

Biology - Flora. Classification of *Rosmarinus officinalis* Retrived December 25,2014from http.//ikon.altervista.org/biologia/flora af/index.php taxanorm Rosmarinus officinalis.

Biology - Flora. Classification of *Pelargonium graveolens* Retrived December 25, 2014 from http.//ikon.altervista.org/biologia/flora af/index.php taxanorm Pelargonium graveolens.

Biology - Flora. Classification of *Allium sativum* Retrived December 25, 2014 from http.//ikon.altervista.org/biologia/flora af/index.php taxanorm Allium sativum.

Biology - Flora. Classification of *Ecballium elaterium* Retrived December 25, 2014 from http.//ikon.altervista.org/biologia/floraaf/index.php taxanorm Ecballium elaterium.

Biology - Flora. Classification of *Ruta graveolens* Retrived December 25, 2014 from http.//ikon.altervista.org/biologia/floraaf/index.php taxanorm Ruta graveolens.

Biology - Flora. Classification of *phagnalon rupestre* Retrived December 25, 2014 from http.//ikon.altervista.org/biologia/floraaf/index.php recn 38397&scientificname phagnalon rupestre subsp. illyricum.

Boukhris, M., Ben Nasri-Ayachi, M., Mezghani, I., Bouaziz, M., Boukhris, M., & Sayadi, S. (2013). Trichomes morphology, structure and essential oils of *Pelargonium graveolens* L'Hér. (Geraniaceae). *Industrial Crops and Products, 50*, 604-610.

Boukhatem, M. N., Kameli, A., & Saidi, F. (2013). Essential oil of Algerian rose-scented geranium (*Pelargonium graveolens*). Chemical composition and antimicrobial activity against food spoilage pathogens. *Food Control*, *34*(1) (2013), pp. 208–213

Boukhris, M., Simmonds, M. S., Sayadi, S., & Bouaziz, M. (2013). Chemical composition and biological activities of polar extracts and essential oil of rose-scented geranium, *Pelargonium graveolens*. *Phytotherapy Research*, *27*(8), 1206-1213.

Casella, S., Leonardi, M., Melai, B., Fratini, F., & Pistelli, L. (2013). The role of diallyl sulfides and dipropyl sulfides in the in vitro antimicrobial activity of the essential oil of garlic, *Allium sativum* L., and leek, *Allium porrum* L. *Phytotherapy Research*, *27*(3), 380-383.

Celiktas, O. Y., Kocabas, E. H., Bedir, E., Sukan, F. V., Ozek, T., & Baser, K. H. C. (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chemistry*, *100*(2), 553-559.

Ghannadi A, Bagherinejad MR, Abedi D, Jalali M, Absalan B, Sadeghi N. 2012. Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L'Her and Vitex agnus-castus L. *Iranian journal of microbiology*, *4*(4), 171-176.

Chandra, H., Singh, A., Srivastava, J., Bishnoi, P., & Nautiyal, A. R. (2010). Antibacterial Activity of *Allium sativum* (L.) Against Bacteria Isolated from Upper Respiratory Tract. *IUP Journal of Life Sciences*, *4*(4), 43-49.

Chaudharia, G. M., & Mahajanb, R. T. (2015). Comparative Antioxidant Activity of Twenty Traditional Indian Medicinal Plants and its Correlation with Total Flavonoid and Phenolic Content. *International Journal of Pharmaceutical Sciences Review and Research*, *30*(1), 105-111.

Cheraif, I., Ben Jannet, H., Hammami, M., Khouja, M. L., & Mighri, Z. (2007). Chemical composition and antimicrobial activity of essential oils of *Cupressus arizonica* Greene. *Biochemical Systematics and Ecology*, *35*(12), 813-820.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews*, *74*(3), 417-433.

Davies, J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. *Science*, *264*(5157), 375-382.

De Feo, V., De Simone, F., &Senatore, F. (2002). Potential allelochemicals from the essential oil of *Ruta graveolens*. *Phytochemistry*, *61*(5), 573-578.

Diwan, R., &Malpathak, N. (2009). Furanocoumarins. Novel topoisomerase I inhibitors from *Ruta graveolens* L. *Bioorganic & medicinal chemistry*, *17*(19), 7052-7055.

Dogruoz, N., Zeybek, Z., & Karagoz, A. (2008). Antibacterial activity of some plant extracts. *IUFS Journal of Biology*, *67*(1), 17-21.

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants. antibacterial activity of plant volatile oils. *Journal of applied microbiology*, *88*(2), 308-316.

Drancourt, M., Bollet, C., Carta, A., & Rousselier, P. (2001). Phylogenetic analyses of Klebsiella species delineate *Klebsiella* and *Raoultella* gen. nov., with description of Raoultella ornithinolytica comb. nov., Raoultella terrigena comb. nov. and Raoultella planticola comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, *51*(3), 925-932.

Elbashiti. T, Elmanama. A and Masad. A (2011) The Antibacterial and Synergistic Effects of Some Palestinian Plant Extracts on *Escherichia coli* and *Staphylococcus aureus. Functional Plant Science and Biotechnology*, *5*(1), 57-62.

El-Kalek, H. H. A., & Mohamed, E. A. (2012). Synergistic effect of certain medicinal plants and amoxicillin against some clinical isolates of methicillin–Resistant *Staphylococcus aureus* (MRSA). *International Journal of Pharmaceutical Applications*, *3*(3), 387-398.

Elkhair, E. K. A. (2014). Antidermatophytic Activity of Essential Oils against Locally Isolated Microsporum canis—Gaza Strip. *Natural Science*, *6*(9), 676-684.

Elkhair, E. A., Fadda, H., & Mohsen, U. A. (2010). Antibacterial activity and Phytochemical analysis of some medicinal plants from Gaza Strip-Palestine. *Journal of Al Azhar University-Gaza*, *12*, 45-54.

Fleischauer, A. T., Poole, C., & Arab, L. (2000). Garlic consumption and cancer prevention. meta-analyses of colorectal and stomach cancers. *The American journal of clinical nutrition*, *72*(4), 1047-1052.

Gaherwal, S., Johar, F., Wast, N., & Prakash, M. M. (2014). Anti-Bacterial Activities of *Allium sativum* Against *Escherichia coli, Salmonella Ser. Typhi and Staphylococcus aureus*. *International Journal of Microbiological Research*, *5*(1), 19-22.

Gensini, G. F., Conti, A. A., & Lippi, D. (2007). The contributions of Paul Ehrlich to infectious disease. *Journal of Infection*, *54*(3), 221-224.

Ghannadi A, Bagherinejad MR, Abedi D, Jalali M, Absalan B, Sadeghi N. 2012. Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L'Her and *Vitex agnus-castus* L. *Iranian journal of microbiology, 4(4), 171-176*

Ghazanfari, T., Hassan, Z. M., & Khamesipour, A. (2006). Enhancement of peritoneal macrophage phagocytic activity against Leishmania major by garlic (Allium Sativum) treatment. *Journal of ethnopharmacology*, *103*(3), 333-337.

Gilbert. B and Alves. L.F. (2003). Synergy in plant medicines. *Current Medicinal Chemistry*, *10*(1), 13–20.

Góngora, L., Máñez, S., Giner, R. M., Recio, M. C., Gray, A. I., & Ríos, J. L. (2002). Phenolic glycosides from *Phagnalon rupestre*. *Phytochemistry*, *59*(8), 857-860.

Góngora, L., Giner, R. M., Manez, S., Recio, M. D. C., & Rios, J. L. (2002). *Phagnalon rupestre* as a source of compounds active on contact hypersensitivity. *Planta medica*, *68*(6), 561-564

Grosvenor, P. W., Supriono, A., & Gray, D. O. (1995). Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2. antibacterial and antifungal activity. *Journal of Ethnopharmacology*, *45*(2), 97-111.

Gupta, N., Mittal, M., Parashar, P., Mehra, V., &Khatri, M. (2014). Antibacterial Potential of *Elletariacardamomum, Syzygium aromaticum and Piper nigrum*, their synergistic effects and phytochemical determination. *Journal of Pharmacy Research*, *8*(8), 1-7.

Gupta, S., Kapur, S., Padmavathi, D. V., & Verma, A. (2015). Garlic. An effective functional food to combat the growing antimicrobial resistance. *Pertanika Journal of Tropical Agricultural Science*, *38*(2), 271-278.

Gull, I., Saeed, M., Shaukat, H., Aslam, S. M., Samra, Z. Q., & Athar, A. M. (2012). Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. *Annals of clinical microbiology and antimicrobials*, *11*(8), 1- 6.

Haddouchi, F., Chaouche, T. M., Zaouali, Y., Ksouri, R., Attou, A., & Benmansour, A. (2013). Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria. *Food chemistry*, *141*(1), 253-258.

Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*, *86*(6), 985-990.

Harat, Z. N., Sadeghi, M. R., Sadeghipour, H. R., Kamalinejad, M., &Eshraghian, M. R. (2008). Immobilization effect of *Ruta graveolens* L. on human sperm: a new hope for male contraception. *Journal of ethnopharmacology*, *115*(1), 36-41.

Hemaiswarya, S., Kruthiventi, A. K., & Doble, M. (2008). Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine*, *15*(8), 639-652.

Hemalatha. N and P. Dhasarathan (2010). Multi-Drug Resistant Capability of *Pseudomonas Aeruginosa* Isolates from Nasocomal and Non-Nasacomal Sources. *Journal of Biomedical Science*, *2*(4), 236-239.

Hosni, K., Hassen, I., Chaâbane, H., Jemli, M., Dallali, S., Sebei, H., & Casabianca, H. (2013). Enzyme-assisted extraction of essential oils from *thyme (Thymus capitatus* L.) and *rosemary (Rosmarinus officinalis* L.). Impact on yield, chemical composition and antimicrobial activity. *Industrial Crops and Products*, *47*, 291-299.

Hosni, K., Hassen, I., Chaâbane, H., Jemli, M., Dallali, S., Sebei, H., & Casabianca, H. (2013). Enzyme-assisted extraction of essential oils from thyme (*Thymus capitatus* L.) and rosemary (*Rosmarinus officinalis* L.). Impact on yield, chemical composition and antimicrobial activity. *Industrial Crops and Products*, *47*, 291-299.

Hsouna, A. B., & Hamdi, N. (2012). Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from *pelargonium graveolens* growing in Tunisia. *Lipids in health and disease*, *11*(1), 167-173.

Hussain, A. I., Anwar, F., Chatha, S. A. S., Jabbar, A., Mahboob, S., & Nigam, P. S. (2010). *Rosmarinus officinalis* essential oil. antiproliferative, antioxidant and antibacterial activities. *Brazilian Journal of Microbiology*, *41*(4), 1070-1078.

Ibrahim, T. A., Opawale, B. O., & Oyinloye, J. M. A. (2011). Antibacterial activity of herbal extracts against multi drug resistant strains of bacteria from clinical origin. *Life Sciences Leaflets*, *15*, 490-498.

Irshaid, F. I., Tarawneh, K. A., Jacob, J. H., & Alshdefat, A. M. (2014). Phenol content, antioxidant capacity and antibacterial activity of methanolic extracts derived from four Jordanian medicinal plants. *Pakistan Journal of Biological Sciences*, *17*(3), 372.

Ivanova, A., Mikhova, B., Najdenski, H., Tsvetkova, I., &Kostova, I. (2005). Antimicrobial and cytotoxic activity of *Ruta graveolens*. *Fitoterapia*, *76*(3), 344-347.

Jalali Moghadam, M. A., Honarmand, H., Falah-Delavar, S., & Saeidinia, A. 2012. Study on antibacterial effect of *Ruta graveolens* extracts on pathogenic bacteria. *Annals of Biological Research, 3*(9).4542-4545.

Jameela. M, Mohideen. A, Sunitha. K and Narayanan. M (2011) Antibacterial Activities of Three Medicinal Plants Extract against Fish Pathogens. *International Journal of Biological Technology 2*(2).57-60.

Johnson. L (2006). Antibiotic resistance. National Center for Competency Testing, Ver 6.0

Jordan, M. J., Lax, V., Rota, M. C., S. Lorán, S., Sotomayor, J. A. (2013). Effect of the phenological stage on the chemical composition, and antimicrobial and antioxidant properties of *Rosmarinus officinalis* L essential oil and its polyphenolic extract. *Industrial Crops and Products, 48*, 144 – 152

Jordan, M. J., Lax, V., Rota, M. C., Loran, S., & Sotomayor, J. A. (2012). Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. *Food Control*, *30*(2), 463-468.

Karmegam, N., Karuppusamy, S., Prakash, M., Jayakumar, M., & Rajasekar, K. (2008). Antibacterial potency and synergistic effect of certain plant extracts against food-borne diarrheagenic bacteria. *International journal of biomedical and pharmaceutical sciences*, *2*(2), 88-93.

Kavalci C., Durukan P., Cevik Y& Ozer M. (2007). Angioedema due to *Ecbalium elaterium*. case report. *Akademik Acil Tipdegrisi*, *5*(3), 39 - 40.

Kennedy, D. O., Scholey, A. B., & Wesnes, K. A. (2002). Modulation of cognition and mood following administration of single doses of Ginkgo biloba, ginseng, and a ginkgo/ginseng combination to healthy young adults. *Physiology & behavior*, *75*(5), 739-751.

Kirbag. S, Zengin. F and Kursat. M (2009). Antimicrobial Activities of Extracts of some Plants. *Pakistan Journal of Botany* 41(4). 2067-2070.

Karuppiah, P., & Rajaram, S. (2012). Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. *Asian Pacific journal of tropical biomedicine*, *2*(8), 597-601.

Ko, K.N., Lee, K.W., Lee, S.E., Kim, E.S. 2007. Development and ultrastucture of glan-dular trichomes in *Pelargonium fragrans* 'Mabel Grey' (Geraniaceae). *journal of Plant Biol*. 50(3), 362–368.

Koca, U., Ozcelik, B., & Ozgen, S. (2010). Comparative in vitro activity of medicinal plants Arnebia densiflora and *Ecballium elaterium* aginst isolated strains of Klebsiella pneumoniae. *Turkish Journal of Pharmaceutical Sciences*, *7*(3), 197-204.

Kumar, V. G., Surendranathan, K. P., Umesh, K. G., Devi, D. G., & Belwadi, M. R. S. (2003). Effect of onion (*Allium cepa* Linn.) and garlic (Allium sativum Linn.) on plasma triglyceride content in Japanese quail (*Coturnix coturnix japonicum*). *Indian journal of experimental biology*, *41*(1), 88-90.

Langeveld, W. T., Veldhuizen, E. J., & Burt, S. A. (2014). Synergy between essential oil components and antibiotics. A review. *Critical reviews in microbiology*, *40*(1), 76-94.

Lievre, K., Hehn, A., Tran, T. L. M., Gravot, A., Thomasset, B., Bourgaud, F., &Gontier, E. (2005). Genetic transformation of the medicinal plant *Ruta graveolens* L. by an Agrobacterium tumefaciens-mediated method. *Plant science*, *168*(4), 883-888.

Mabrouk, M. I. (2012). Synergistic and antibacterial activity of six medicinal plants used in folklore medicine in Egypt against *E. coli* O157. H7. *Jornal of Application science Research*, *8*(2), 1321-1327.

Marin, M., Koko, V., Duletić-Laušević, S., Marin, P. D., Rančić, D., & Dajic-Stevanovic, Z. (2006). Glandular trichomes on the leaves of *Rosmarinus officinalis*. Morphology, stereology and histochemistry. *South African Journal of Botany*, *72*(3), 378-382.

Meepagala, K. M., Schrader, K. K., Wedge, D. E., & Duke, S. O. (2005). Algicidal and antifungal compounds from the roots of *Ruta graveolens* and synthesis of their analogs. *Phytochemistry*, *66*(22), 2689-2695.

Mangena, T., & Muyima, N. Y. O. (1999). Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra, Pteronia incana and Rosmarinus officinalis* on selected bacteria and yeast strains. *Letters in applied microbiology*, *28*(4), 291-296.

Meriga, B., Mopuri, R., & MuraliKrishna, T. (2012). Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum. Asian Pacific Journal of Tropical Medicine*, *5*(5), 391-395.

Montes Moreno, N., Garcia i Jacas, N., Sáez, L., & Benedí, C. (2013). Phylogenetic studies in Gnaphalieae (Compositae). The genera Phagnalon Cass. and Aliella Qaiser & Lack. *Recent Advances in Pharmaceutical Sciences III, 2013, Transworld Research Network. Editors. Diego Muñoz Torrero, Amparo Cortés & Eduardo L. Mariño. ISBN. 978-81-7895-605-3. Chapter 7, p. 109-130.*

Nascimento. G, Locatelli. P, Freitas. C and Silva. G (2000). Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic resistant Bacteria. *Brazilian Journal of Microbiology* , *31*(4).247-256.

Nelson.T(2008). *Escherichia Coli*. http.//www.bettycjung.net

Ojala, T., Remes, S., Haansuu, P., Vuorela, H., Hiltunen, R., Haahtela, K., & Vuorela, P. (2000). Antimicrobial activity of some coumarin containing herbal plants growing in Finland. *Journal of ethnopharmacology*, *73*(1), 299-305.

Okoh, O. O., Sadimenko, A. P., & Afolayan, A. J. (2010). Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. *Food chemistry*, *120*(1), 308-312.

Oldfield, E., & Feng, X. (2014). Resistance-resistant antibiotics. *Trends in pharmacological sciences*, *35*(12), 664-674.

Olmos, A., Máñez, S., Giner, R. M., del Carmen Recio, M., & Ríos, J. L. (2005). Isoprenylhydroquinone glucoside. a new non-antioxidant inhibitor of peroxynitrite-mediated tyrosine nitration. *Nitric Oxide*, *12*(1), 54-60.

Orlanda, J. F., & Nascimento, A. R. (2015). Chemical composition and antibacterial activity of *Ruta graveolens* L. (Rutaceae) volatile oils, from São Luís, Maranhão, Brazil. *South African Journal of Botany*, *99*, 103-106.

Oskay, M., Oskay, D., & Kalyoncu, F. (2009). Activity of Some Plant Extracts against Multi-Drug Resistant Human Pathogens. *Iranian Journal of Pharmaceutical Research*, *8*(4), 293-300.

Oskay, M., & Sarı, D. (2007). Antimicrobial screening of some Turkish medicinal plants. *Pharmaceutical Biology*, *45*(3), 176-181.

Ovesná, J., Kučera, L., Horníčková, J., Svobodová, L., Stavělíková, H., Velíšek, J., & Milella, L. (2011). Diversity of S-alkenyl cysteine sulphoxide content within a collection of garlic (*Allium sativum* L.) and its association with the morphological and genetic background assessed by AFLP. *Scientia Horticulturae*, *129*(4), 541-547.

Palaksha, M. N., Ahmed, M., & Das, S. (2010). Antibacterial activity of garlic extracts on streptomycin-resistant *Staphylococcus aureus* and *Escherichia coli* solely and in synergism with streptomycin. *Journal of natural science, biology, and medicine*, *1*(1), 12-15.

Pandikumar, P., Chellappandian, M., Mutheeswaran, S., & Ignacimuthu, S. (2011). Consensus of local knowledge on medicinal plants among traditional healers in Mayiladumparai block of Theni District, Tamil Nadu, India. *Journal of ethnopharmacology*, *134*(2), 354-362.

Pandey, A.K. and Chowdhry, P.K (2006). Propagation techniques and harvesting time on productivity and root quality of *Withania somnifera*. *Journal of Tropical Medicinal Plants*, 7(1), 79-81.

Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, *10*(2), 175 - 181.

Piddock, L. J. V., & Wise, R. (1989). Mechanisms of resistance to quinolones and clinical perspectives. *Journal of Antimicrobial Chemotherapy*, *23*(4), 475-480.

Pokhrel, S., Singh, R., Gautam, P., Dixit, V. K., & Das, A. J. (2012). Comparison of antimicrobial activity of crude ethanolic extracts and essential oils of spices against five strains of diarrhoea causing *Escherichia coli*. *International Journal of Pharmacy & Life Sciences*, *3*(4), 1624 - 1627.

Preedy, V. R., Watson, R. R., & Patel, V. B. (Eds.). (2011). *Nuts and seeds in health and disease prevention*. Academic Press. Chapter 128.

Qabaha, K. I. (2013). Antimicrobial and free radical scavenging activities of five Palestinian medicinal plants. *African Journal of Traditional, Complementary and Alternative Medicines*, *10*(4), 101-108.

Quirke, V. M. 2007. Penicillin (The History). *Van Nostrand's Scientific Encyclopedia*.

Raghav, S. K., Gupta, B., Shrivastava, A., & Das, H. R. (2007). Inhibition of lipopolysaccharide-inducible nitric oxide synthase and IL-1β through suppression of NF-κB activation by 3-(1′-1′-dimethyl-allyl)-6-hydroxy-7-methoxy-coumarin isolated from Rutagraveolens L. *European journal of pharmacology*, *560*(1), 69-80.

Raghav, S. K., Gupta, B., Agrawal, C., Goswami, K., & Das, H. R. (2006). Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells. *Journal of ethnopharmacology*, *104*(1), 234-239.

Rosato, A., Vitali, C., De Laurentis, N., Armenise, D., & Milillo, M. A. (2007). Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. *Phytomedicine*, *14*(11), 727-732.

Rubin, R. 2007. a Brief history of great discoeries in pharmacology. In Celebration of the Centennial Anniversary of the Founding of the american society of pharmacology and Experimental Therapeutics. *Pharmacol Reviews, 59*(4), 289-359

Saieed, P., Reza, R. M., Abbas, D., Seyyedvali, R., &Aliasghar, H. (2006). Inhibitory effects of *Ruta graveolens* L. extract on guinea pig liver aldehyde oxidase. *Chemical and pharmaceutical bulletin*, *54*(1), 9-13.

Santoyo, S., Cavero, S., Jaime, L., Ibanez, E., Senorans, F. J., & Reglero, G. (2005). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction.*Journal of Food Protection*, *68*(4), 790–795.

Sasidharan, N, K., Sreekala, S, R., Jacob, J & Nambisan, B. (2014). *In-Vitro* Synergistic Effect of Curcumin in Combination with Third Generation Cephalosporins against Bacteria Associated with Infectious Diarrhea. *BioMed Research International*, *2014*, 1-9.

Sasmakov, S. A., Putieva, Z. M., Azimova, S. S., & Lindequist, U. *In-vitro* screening of the cytotoxic, antibacterial and antioxidant activities of some Uzbek plants used in folk medicine. *Asian Journal of Traditional Medicines*, 7(2), 73-80.

Saxena, V. K., & Sharma, R. N. (1999). Antimicrobial activity of the essential oil of Lantana aculeata. *Fitoterapia*, *70*(1), 67-70.

Sharma, A., Bajpai, V. K., & Baek, K. H. (2013). Determination of antibacterial mode of action of *Allium sativum* essential oil against foodborne pathogens using membrane permeability and surface characteristic parameters. *Journal of Food Safety*, *33*(2), 197-208.

Shihabudeen. M, Priscilla. H, Thirumurugan. D (2010) Antimicrobial Activity and Phytochemical Analysis of Selected Indian Folk Medicinal Plants. *International Journal of Pharma Sciences and Research*, *1*(10). 430-434.

Silva, O., Duarte, A., Cabrita, J., Pimentel, M., Diniz, A., & Gomes, E. (1996). Antimicrobial activity of Guinea-Bissau traditional remedies. *Journal of ethnopharmacology*, *50*(1), 55-59.

Sundaram, S. G., & Milner, J. A. (1996). Diallyl disulfide inhibits the proliferation of human tumor cells in culture. *Biochimica et Biophysica Acta -Molecular Basis of Disease*, *1315*(1), 15-20.

Tang, J. Y., &Ren, M. X. (2011). Sex allocation and functional bias of quaternary and quinary flowers on same inflorescence in the hermaphrodite Rutagraveolens. *ActaOecologica*, *37*(5), 449-454.

Tadeg, H., Mohammed, E., Asres, K., & Gebre-Mariam, T. (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of ethnopharmacology*, *100*(1), 168-175.

Tortora Gerard J., Funke Berdell R., Case Christian L. (2010) Microbiology an introduction, 10th edition. United States of America. Pearson Education.

Tsao, S. M., & Yin, M. C. (2001). In-vitro antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oils. *Journal of medical microbiology*, *50*(7), 646-649.

Uslu, C., Karasen, R. M., Sahin, F., Taysi, S., & Akcay, F. (2006). Effect of aqueous extracts of *Ecballium elaterium* rich, in the rabbit model of rhinosinusitis. *International journal of pediatric otorhinolaryngology*, *70*(3), 515-518.

Valsaraj, R., Pushpangadan, P., Smitt, U. W., Adsersen, A., & Nyman, U. (1997). Antimicrobial screening of selected medicinal plants from India. *Journal of Ethnopharmacology*, *58*(2), 75-83.

Viswanathan, V., Phadatare, A. G., & Mukne, A. (2014). Antimycobacterial and antibacterial activity of *Allium sativum* bulbs. *Indian journal of pharmaceutical sciences*, *76*(3), 256-261.

Van Vuuren, S. F., Suliman, S., & Viljoen, A. M. (2009). The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials. *Letters in applied microbiology*, *48*(4), 440-446.

Voukeng, I. K., Kuete, V., Dzoyem, J. P., Fankam, A. G., Noumedem, J. A., Kuiate, J. R., & Pages, J. M. (2012). Antibacterial and antibiotic-potentiation activities of the methanol extract of some Cameroonian spices against Gram-negative multi-drug resistant Phenotypes. *BioMed Central research notes*, *5*(1), 299-308.

Zaouali, Y., Bouzaine, T., & Boussaid, M. (2010). Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. *Food and chemical toxicology*, *48*(11), 3144-3152.