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The Antibacterial Effect of Some Medicinal Plant Extracts and their Synergistic Effect with Antibiotic and Non-antibiotic Drugs

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In the name of Allah, the Beneficent, the Merciful

DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains neither materials 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.

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ABSTRACT

The aim of the study was assess the antibacterial effect of some medicinal plant extracts and their synergistic antibiotic and non-antibiotic drugs against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The extract of medicinal plants were prepared using Soxhlet apparatus for alcoholic extract, and water reflux for aqueous extracts. The antibacterial activities of extracts were evaluated using the disk diffusion method as well as well diffusion method; the inhibitory zones were recorded in millimeters. The minimal inhibitory concentration (MIC) of the plant extracts against *E. coli*, *S. aureus* and *P. aeruginosa* were assessed using microdilution method. The synergistic effect between plants and extraction of antibiotics and / or Non-antibiotic drugs was assessed using disk diffusion method. The results of this study showed that ethanolic extracts used against *E. coli*, *S. aureus* and *P. aeruginosa* were showed antimicrobial and synergistic effect with most antibiotics better than methanolic and aquatic extracts.Water extracts were showed synergistic effect with the Paracetamol and Loperamide Hcl better than methanolic and ethanolic extracts against *E. coli* and *S. aureus*. Ethanolic extracts were showed synergistic effect with the Paracetamol and Loperamide Hcl better than methanolic and aquatic extracts against *P. aeruginosa*. The results of this study showed that there is a decrease in MIC in case of methanolic extract of *E. camaldulensis* against *E. coli* (3.125 mg/ml), and the methanol and aquatic extract of *F. sycomorus* (leaves) against *S.aureus* varying from 6.25 to 3.125 mg/ml, and the ethanol extract of *E. camaldulensis* against *P. areuginosa* (6.25 mg/ml). Thereby, our results indicate the possibility of using these extracts in the treatment of bacterial infections, and the results of this study was encouraging, despite the need for clinical studies to determine of the real effectiveness and potential toxic effects in vivo. These results was revealed the importance of plant extracts when associated with antibiotic and Non-antibiotic drugs in control of bacteria.

التأثير الضد بكتيري لبعض المستخلصات النباتية و تأثيرها التازري مع المضادات الحيوية والمضادات الغير حيوية من العقاقير

الملخـــــــــــص

تهدف الدراسة الى تقييم التأثير الضد بكتيري لبعض النباتات الطبية ، بالإضافة الى التأثير التازري لها مع بعض المضادات الحيوية و العقاقير (الغير مستخدمة في مكافحة الكائنات الحية الدقيقة) ضد الإشريكية القولونية و البكتريا المكورة العنقودية الذهبية و الزائفة الزنجارية . تم استخدام جهاز السوكسلت (Soxhlet) للحصول على المستخلصات الكحوليه، وجهاز التكثيف الراجع (water reflux) للحصول على المستخلصات المائية. وتم تقييم النشاط الضد بكتيري للمستخلصات النباتية، باستخدام طريقة الانتشار في القرص وكذلك طريقة الانتشار في الحفر. و قدرة قيمة أقل تركيز مثبيط من المستخلصات النباتية وحدها باستخدام طريقة التخفيف الجزئي. و تم تقييم التأثير التضافري بين المستخلصات النباتية و المضادات الحيوية و / أو المضادات الغير حيوية باستخدام طريقة الانتشار في القرص. أظهرت نتائج هذه الدراسة أن المستخلص الايثانولي من النباتات الطبية له تأثير ضد البكتريا المختبرة بالاضافة الى تأثير تضافري مع المضادات الحيوية أفضل من المستخلص الميثانولي والمائي. في حين أظهر المستخلص المائي تأثير تضافري مع الباراسيتامول و لبراميد هيدروكلورايد أفضل من المستخلص الميثانولي والايثانولي ضد كل من الإشريكية القولونية و بكتريا المكورة العنقودية الذهبية ولكن ضد الزائفة الزنجارية أظهر المستخلص الايثانولي تأثير متناغم مع الباراسيتامول و لبراميد هيدروكلورايد أفضل من المستخلص الميثانولي والمائي. في حين أظهرت النتائج أن هناك انخفاض في قيمة أقل تركيز مثبط للبكتريا (MIC) للمستخلصات النباتية، وكانت النتيجة الأكثر أهمية للمستخلص الميثانولي لنبات الكينيا ضد الإشريكية القولونية (٣,١٢٥ ملغ/مل)، و المستخلص الميثانولي و المائي لأوراق الجميز ضد المكورة العنقودية الذهبية (من ٦,٢٥ الى ٣,١٢٥ ملغ/مل)، و المستخلص الايثانولي لنبات الكينيا ضد الزائفة الزنجارية (٦,٢٥ ملغ/مل). وبالتالي،فإن نتائجنا تشير إلى إمكانية استخدام هذه المقتطفات في علاج الالتهابات البكتيرية، وكانت نتائج هذه الدراسة مشجعة، على الرغم من الحاجة إلى دراسات سريرية لتحديد الفاعلية الحقيقية والآثار السامة المحتملة في الجسم الحي .وقد كشفت هذه النتائج على أهمية المستخلصات النباتية عندما ترتبط مع المضادات الحيوية و الغير حيوية في السيطرة على البكتيريا.

Dedication

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

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

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List of Abbreviated Terms

MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
ESBLs	extended-spectrum β lactamases
UTIs	Urinary Tract Infections
ADME	Absorption, Distribution, Metabolism, and Excretion
TB	Tuberculosis Bacterial
MBC	Minimum Bactericide Concentration
CFU	Colony Forming Unit
BHI	Brain Heart Infusion
DMSO	Dimethyl sulfoxide
Ppm	parts per million
HIV	Human Immunodeficiency Virus
VRSA	Vancomycin-resistant *S. aureus*
OD	Optical Density
CLSI	Clinical and Laboratory Standards Institute
CAMHB	Cation Adjusted Mueller Hinton Broth
AFB	acid-fast bacilli
CLED	cystine-lactose-electrolyte deficient
milligram/milliliter	mg/ml
MHA	Muller-Hinton Agar
TTC	Tetrazolium chloride

Chapter 1

Introduction

1.1 Overview

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Gram positive bacteria such as *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Benayache *et al*., 2001). Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benhassaini et al., 2003; Benjilali et al.,1986). Different antibiotics exercise their inhibitory activity on different pathogenic organisms (Chanda and Rakholiya, 2011). Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (Abiramasundari *et al*., 2011).

These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as 'forgotten plants'. Taking into account the increasing demand for natural ingredients that might be used as food additives, components of functional foods, preventing plant diseases and nutraceuticals as well as for other applications. It is reasonable to revise the 'forgotten plants' by assessing their applicability and benefits using modern scientific analysis methods (Abdel Rahman *et al*., 2011). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Gislene *et al*., 2000).

1.2 Antibiotic resistance

From these microbes resistant to antibiotics, Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections. MRSA infections are very difficult to cure because MRSA strains are resistance against almost all clinically available antibiotics. For most MRSA strains, glycopeptide-type drugs such as vancomycin are the only effective antimicrobial agents. However, vancomycin-resistant S. aureus (VRSA) has been reported (Adwan and Mhanna, 2008). *Pseudomonas aeruginosa* also causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments and resistance to many antibiotics and antiseptics. A main problem is the emergence of multidrug-resistant *P. aeruginosa* strains resistant to different antimicrobial agent classes. Perhaps, this high degree of multidrug resistance related to the presence of antibiotic efflux systems which provide resistance to multiple antimicrobial agents (Adwan *et al.*, 2009).

Multidrug-resistant Enterobacteriaceae, mostly Escherichia coli, produces extended-spectrum β lactamases (ESBLs) such as the CTX-M enzymes. These enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates such as ceftazidime, ceftriaxone, or cefepime have emerged within the community setting as an important cause of urinary tract infections (UTIs). Recent reports have also described ESBL-producing *E. coli* as a cause of bloodstream infections associated with these community-onsets of UTI (Darwish and Aburjai, 2010).

Some Palestinian plants exhibit significant potency against human bacterial pathogens. However, at present, plant extracts are rarely used as antimicrobials or as a systemic antibiotics and this may be due to their low level of activity, especially against gram-negative bacteria (Adwan and Mhanna, 2008).

Wadi Gaza is an essential part of natural life in Palestine and has a rich biodiversity in terms of fauna and flora. As many as 70 plant species belonging to 32 families and 24 orders were identified in Wadi Gaza. The aster or daisy family (Compositae) is the largest found family which composed of 14 plant species (20%) of the recorded species. The natural flora of Wadi Gaza was commonly used in different ways as a source of food, herbal medicine, fodder for grazing animals, timber and fuel production (Abd Rabou *et al*., 2008).

1.3 Aim of the Study

To assess the antimicrobial and synergistic effect of some medicinal plant extracts with antibiotic and non-antibiotic drugs against isolates *E. coli*, *S. aureus* and *P. aeruginosa*.

1.4 Specific objectives

The following specific objectives were achieved:

1. To collect and to identify of medicinal plants.
2. To extract the selected medicinal plants using different solvents such as methanol, ethanol and water reflux.
3. To find out the synergistic effect of these plant extracts with antibiotics and non-antibiotic drugs against *E. coli*, *S. aureus* and *P. aeruginosa*.
4. To measure the minimum inhibitory concentration (MIC) of the selected plant extracts against isolates *E. coli*, *S. aureus* and *P. aeruginosa*.

1.5 Significance

To my knowledge this study will be the first in Palestine, to deal with the synergistic effect of medicinal plant extracts in combination with antibiotics and non-antibiotic drugs against *S. aureus, E. coli* and *P. aeruginosa*.

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 researchs a synergistic effect of various plant extracts with antibiotic and non antibiotic drugs against some resistant bacteria, therefore we will check this possibility in our study by using palestinian traditional plants.

Chapter 2

Literature review

2.1 Ethnobotanical

Ethnobotany is the study of the relationship between plants and people: From "ethno" - study of people and "botany" - study of plants. Ethnobotany is considered a branch of ethnobiology. Ethnobotany studies the relationships between (uses of) plants and cultures. The complex focus of ethnobotany is on how plants have been or are used, managed and perceived in human societies and includes plants used for food, medicine, divination cosmetics, dyeing, textiles, for building, tools, currency,clothing, rituals, social life and music. Ethnobotany is a multidisciplinary science defined as the interaction between plants and people. The relationship between plants and human cultures is not limited to the use of plants for food, clothing and shelter but also includes their use for ornamentation and health care (Choudhary, 2008).

2.1.1 Medicinal plants

Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Over 50% of all modern clinical drugs are of natural product origin (Kirbag *et al.,* 2009).

Phytochemicals such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activity (Madhuri and Pandey, 2009).

The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases. Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (Shakeri *et al*., 2012).

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 and Chowdhry, 2006). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Nascimento *et al*., 2000).

In Palestine, there are numerous medicinal plants described for treatment of many diseases. Herbal medicine is considered an integral part of the Palestinian culture and plays a pivotal and indispensable role in the current public healthcare. The hills and mountains of Palestine are covered with more than 2600 plant species of which more than 700 are noted for their uses as medicinal herbs or as botanical pesticides (Jaradat, 2005). The following are some of the medicinal plants that have been studying its effect against some clinically isolated bacteria.

2.1.1.1 *Nerium oleander*

Nerium oleander linn belongs to Apocynaceae family (Table 1) commonly known as Gandeera, which is a large glabrous evergreen shrub with milky juice (Hussain and Gorsi, 2004).

Figure 2.1 Leaves and flower of *N. oleander*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Gentianales
Family:	Apocynaceae
Genus:	Nerium
Species:	*oleander*

Table 2.1 Classification of *Nerium oleander*

In history this plant has been used in medicine. It is popularly used as an ornamental plant, for its evergreen nature. Although it's toxic to human and animals , but it is also proved to contain medicinal value like antibacterial activity and Anti-inflammatory activity, and with these considerations, this plant is now being studied for its uses medicine with caution (Lokesh, 2010).

All parts of the plant are poisonous, from roots to stems, from leaves to flowers and seeds, including the smoke if we try to burn them. Many experiments have been made in time, and there is now common knowledge that chewing or simply biting the leaves a couple of times can lead to severe intoxication (in extreme cases followed by death), that even dry leaves are toxic, that cattle, horses and sheep being experimentally poisoned have died, etc. Humans have even died after eating meat (Zimer, 2009).

The leaves and the flowers are cardiotonic, diaphoretic (is excessive sweating commonly associated with shock and other medical emergency conditions), diuretic, anticancer, antibacterial , antifungal and expectorant. And also the flowers, leaves, leaf juice , bark and roots have been used against corns, warts, cancerous ulcers, carcinoma,ulcerating or hard tumors (Zibbu and Batra, 2010).

The root is better; aphrodisiac, tonic good for chronic pain in the abdomen and pain in the joints, very poisonous, but an antidote to snake-venom. The juice of the young leaves is poured into eyes in ophthalmia with copious lachrymation (Hussain and Gorsi, 2004).

Essential oils and their components are widely used in medicine as constituents of different medical products, in the food industry as flavouring additives and also in cosmetics as fragrances and pharmaceutical industries and also are generally used in the cosmetic, medical and food industries. The essential oil of *Nerium oleander* has been the object of several studies antifungal, antibacterial, molluscicidal, antioxidant, antihyperglycemic, antifungal, cytotoxial and insecticidal activity (Table 8). (Derwich *et al.*, 2010).

2.1.1.2 *Artemisia herba-alba*

The genus *Artemisia* L. (family Asteraceae, tribe Anthemideae), comprises a variable number of species from 200 to over 400, (depending on the authors) found throughout the northern half of the world. The genus may be divided into sections Artemisia and Dracunculus (Table 2) (Mohsen and Ali, 2008).

Figure 2.2 Leaves of *A. herba-alba*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Asterales
Family:	Asteraceae
Tribe:	Anthemideae
Genus:	*Artemisia*
Species:	*herba-alba*

Table 2.2 Classification of *A. herba-alba*

The genus *Artemisia* is known to contain many bioactive compounds; artemisinin exerts not only antimalarial activity but also profound cytotoxicity against tumor cells and arglabin is employed for treating certain types of cancer (Mohamed *et al*., 2010).
Artemisia is used for the treatment of diabetes mellitus in Iraq, and for hypertension and diabetes in oriental Morocco (Seddik *et al.,* 2010).
Many Artemisia species have a high economic value in several fields, as food plants and as antihelminthic and antimalaria in medicine (Mohamed *et al.,* 2009).

This species of sagebrush is widely used in folk and traditional medicine for its antiseptic, vermifuge and antispasmodic properties. Artemisia herba-alba was reported as a traditional remedy of enteritis, and various intestinal disturbances, among the Bedouins in the Negev desert. In fact, essential oil showed antibacterial activity, as well as, antispasmodic activity on rabbits (Yashphe *et al*., 1987; Yashphe *et al*., 2006).

The antibacterial activity of *Artemisia herba-alba*. Only its essential oil was active against some Gram-positive and Gram-negative bacteria (Table 8) (Yashphe *et al.,* 2006).

2.1.1.3 *Withania somnifera*

Withania somnifera belongs to Solanaceae family (Table 3) commonly known as Ashwagandha/Indian ginseng/winter cherry (Chatterjee *et al*., 2010).

Figure 2.3 Leaves of *W. somnifera*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Solanales
Family:	Solanaceae
Genus:	*Withania*
Species:	*somnifera*

Table 2.3 Classification of *Withania somnifera*

The main active constituents of *Withania somnifera* are steroidal lactones, alkaloids, flavonoids, tannin etc.The major chemical constituents of these plants, withanolides, are mainly localized in leaves (Kapoor, 2001; Rastogi and Mehrotra, 1998).

Numerous studies indicated that ashwagandha possesses antioxidant, antitumor, antistress, anti-inflammatory, immunomodulatory, hematopoetic, anti-ageing, anxiolytic and also influences various neurotransmitter receptors in the central nervous system . In recent studies done on human breast, lung and colon cancer cell lines, plant extracts inhibited the growth of these cell lines (Sharma *et al*., 2011).

Its roots, leaves and seeds are used in Ayurvedic and Unani medicines, to combat diseases ranging from tuberculosis to arthritis. The pharmacological activity of the plant is attributed to the presence of several alkaloids and withaniols. Roots are prescribed in medicines for hiccup, several female disorders, bronchitis, rheumatism, dropsy, stomach and lung inflammations and skin diseases. Its roots and paste of green leaves are used to relieve joint pains and inflammation. It is also an ingredient of medicaments prescribed for curing disability and sexual weakness in male. Leaves are used in eye diseases. Seeds are diuretic. It is a constituent of the herbal drug '*Lactare*' which is a galactagogue (Joy *et al*., 1998).

Also have several medicinal properties such as sedative, hypotensive, aphrodisiac, bradycardiac, respiration stimulatory, antiperoxidative, cardiotonic, radiosensitizing and thyroregulatory effects (Chaurasia *et al*., 2000).

Beside its use as general tonic. And several recent reports have demonstrated immunomodulator (also known as an immunotherapy is a substance (e. g. a drug) which has an effect on the immune system) and antitumor effect of ashwagandha as well (Owais *et al.*, 2005).

2.1.1.4 *Lantana camara*

Figure 2.4 Leaves and flower of *L. camara*

Kingdom:	*Plantae*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Order:	*Lamiales*
Family:	*Verbenaceae*
Genus:	*Lantana*
Species:	*camara*

Table 2.4 Classification of *Lantana camara*

Lantana camara L. Belongs to family *Verbenaceae* (Table 4), commonly known as wild or red sage is the most widespread species of this genus and regarded both as a notorious weed and a popular ornamental garden plant (Ganjewala *et al*., 2009).

They are mostly cultivated for their ornamental purpose because of their flowers which can be pink, orange, yellow, white lilac depending on the variety. *L. camara* leaves have been reported to make animals ill after ingestion and its berries are toxic before they become ripe**.** They are mostly cultivated for their ornamental purpose because of their flowers which can be pink, orange, yellow, white lilac depending on the variety. *L. camara* leaves have been reported to make animals ill after ingestion and its berries are toxic before they become ripe (Sonibare and Effiong, 2008).

However, it is listed as one of the important medicinal plants of the world Many studies have revealed the presence of terpenoids, steroids, and alkaloids as major chemical constituents in *L. camara* (Ganjewala *et al*., 2009).

L. camara oil and extracts are used in herbal medicine for the treatment of various human diseases such as skin itches, leprosy, cancers, chicken pox, measles, asthma, ulcers, tumors, high blood pressure, tetanus, rheumatism, etc. Extracts from the Extracts from the leaves have been reported to have antimicrobial, fungicidal, insecticidal and nematicidal activity (Sonibare and Effiong, 2008).

L. camara essential oil containing ß-caryophyllene, geranyl acetate, terpinyl acetate, bornyl acetate and limonene remarkably inhibited the growth of many tested bacteria and fungi. *P.aeruginosa, A.niger, F.solani, C.albicans* appeared as the most sensitive ones (Deena and Thoppil, 2000).

A tea prepared from the leaves and flowers is taken against fever; influenza and stomach ache (Ghisberti, 2000). *Lantana camara* Linn flowers extract in coconut oil provides protection from Aedes mosquitoes (Kumar and Maneemegalai, 2008).

Different lantadenes (are poisons in *lantana camara*) show potent inhibitory effects on Epstein-Barr virus in Raji cells.

There are differences in activity depending from the molecular structures, like methyl- or dihydro groups (Inada *et al*., 1995).

2.1.1.5 *Ficus sycomorus*

The Sycamore Fig Belongs to family Moraceae (Table 5) is one of the old and historic plant species in the Palestine coastal valley and the study area as well. The trees have some medicinal values as the sap extracted from the trunk can cure some skin diseases (*Abd Rabou et al*., 2008).

Figure 2.5 Leaves of *Ficus sycomorus*.

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

Table2.5 Classification of *Ficus sycomorus*

The active principles of many drugs found in plants are secondary metabolites. These secondary metabolites which constitute an important source of the pharmaceutical drugs have been isolated from different parts of plants. Some of these compounds have been reported to be present in the *Ficus species* such as tannins, saponins, flavonoids, steroids, anthraquinone glycosides and reducing sugars. *Ficus sycomorus* have been suspected to possess anti-diarrhoeal activities and sedative and anticonvulsant (are a diverse group of pharmaceuticals used in the treatment of epileptic seizures) properties of this plant have also been reported (Olusesan *et al.,* 2010).

Reported different solvent extracts of some plants to have different pharmacological properties. Reported organic stem extracts of *F. sycomorus* with higher antifungal activity than aqueous extracts (Hassan *et al.,* 2007).

The fruit extracts of *Ficus sycomorus* L exhibited antitumor activity in the potato disc bioassay. it had significant antibacterial activity, but no antifungal activity (Mousa *et al.,* 1994).

2.1.6 *Allium sativum*

Allium sativum; commonly known as garlic, is a species of the onion family Alliaceae (Table 6) (Saravanan *et al*., 2010).

Figure 2.6 Bulbs of garlic.

Kingdom:	*Plantae*
Division:	*Magnoliophyta*
Class:	*Liliopsida*
Order:	*Liliales*
Family:	*Liliaceae*
Genus:	*Allium*
Species:	*sativum*

Table 2.6 Classification of *Allium sativum*

Allium sativum is a natural plant being used as a food as well as folk medicine for centuries in all over the world, In 1996, Reuter *et al.* described garlic a plant with various biological properties like antimicrobial, anti-cancer, antioxidant. As well as different properties such as antiviral, antifungal, expectorant, anti-septic, anti-histamine (Hanna *et al*., 2011).

And has a long folklore history as a treatment for cold, cough and asthma and is reported to strengthen the immune system. It has many medicinal effects such as lowering of blood cholesterol level , antiplatelet aggregation, anti-inflammatory activity and inhibition of cholesterol synthesis (Shobana, 2009).

Different garlic extracts demonstrated activity against Gram negative and Gram-positive bacteria including species of Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus, clostridium, *Helicobacter pylori* and even acid-fast bacilli (AFB) such as *Mycobacterium tuberculosis*. Allicin is thiosulfinate compound of garlic reported for its antibacterial activity. Allicin is proved to be anti-bacterial as it inhibits RNA synthesis (Hannan *et al*., 2011).

2.1.7 *Eucalyptus camaldulensis*

Eucalyptus camaldulensis is an important ethnomedicinal plant belonging to the family Myrtaceae (Table 7). There are more than 700 species that comprise this genus, most are native of Australia, though they are also widely cultivated throughout the tropics, especially in Asia and Central America as well as Africa (Brooker *et al.*, 2002).

Figure 2.7 *Eucalyptus camaldulensis* tree.

Kingdom:	*Plantae*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Order:	*Myrtales*
Family:	*Myrtaceae*
Genus:	*Eucalyptus*
Species:	*E. camaldulensis*

Table 2.7 Classification of *E. camaldulensis*

Are used in China folk medicine for a variety of medical conditions. For examples, hot water extracts of dried leaves used as analgesic, anti-inflammatory and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion. and also known to contain bioactive products that display

antibacterial, antifungal , analgesic and anti-inflammatory effects and antioxidative activities (Cheng *et al*., 2009).

It is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. Essential oils of the leaves are used in the treatment of lung diseases while the volatile oils are used as expectorant **(Table 2.8)**. Topical ointments containing eucalyptus oil have also been used in traditional Aboriginal medicines to heal wounds and fungal infections. Eucalyptus oil obtained by steam distillation and rectification of the fresh leaves has Eucalyptol (1, 8-cineole) as its active ingredient and this is responsible for its various pharmacological actions (Ayepola and Adeniyi, 2008).

Some studies have demonstrated that the oil and leaf extracts of Eucalyptus spp. have antifungal and repellent activity. Crude methanolic extract of *E. Camaldulensis* has been reported to inhibit the growth of Candida albicans. Also, it has been shown that ethanolic leaf extract of *Eucalyptus camaldulensis* had marked fungicidal effect against clinical dermatophytic fungal isolates; Microsporium gypseum and Trichophyton mentagrophytes (Falahati *et al*., 2005).

(Table 2.8) Ethnobotanical data of the investigated plants in literature.

Scientific name	Plant origin	Solvent	Antimicrobial activity	References
Withania somnifera	Root and leaves	Ethyl Acetate**,** Methanol, Water	*Escherichia coli, Staphylococcus aureus, Salmonella typhimurium*	Owais *et al,* 2003
	Root and leaves	Methanol**,** Hexane, Diethyl ether	*S. typhimurium and E. coli*	Arora *et al,* 2004
Nerium oleander	Flowers	Hexane	*E. coli ,Pseudomonas aeruginosa, S. aureus*	Derwich *et al,*2010
	Leaves	Chloroformic, ethnolic, methanolic.	*Bacillus pumillus, Bacillus subtilius, S. aureus, E.coli*	
		Chloroformic	*E.coli*	Hussain. M and Gorsi. M, 2004
	Roots bark	Ethnolic, methanolic.	*B. pumillus, B. subtilius, S. aureus, E.coli*	
Lantana camara	Leaf	Mixture of dichloromethane and methanol**.**	*P. aeruginosa***,** *E. coli*	Kumar *et al,* 2006
Ficus sycomorus	Leaves and Stem bark	70% aqueous ethanol	*S. aureus, Salmonella typhi*	Olusesan *et al,* 2010
Eucalyptus camaldulensis	Leaf	Methanol	*Klebsiella spp, S. typhi, Yersinia enterocolitica, P. aeruginosa, S. aureus, B. subtilis.*	Ayepola and Adeniyi, 2010.
	Leaf	Aqueous, acetone, chloramphenicol	*E. coli, K. pneumoniae, S. typhi, S. aureus*	El-Mahmood Muhammad Abubakar, 2010
Artemisia herba-alba	Leaf	Methanol	*S. aureus*	Seddik *et al,* 2010
Allium sativum	Bulbs	70% ethanol	*Mycobacterium tuberculosis.*	Hannan e*t al,* 2009.
	Bulbs	Water and methanol	*E.coli, K. Pneumoniae, S. typhi, B. cereus, S. mutans.*	Saravanan *et al,* 2010

2.2 The bacteria

Clinical isolated bacteria used in the study are *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus.*

2.2.1 *Escherichia coli*

2.2.1.1 Classification

Escherichia coli is the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections (Sherris, 1984). All members of the family Enterobacteriaceae are facultative, all ferment glucose and reduce nitrates to nitrites and all are oxidase negative (Sherris, 1984).

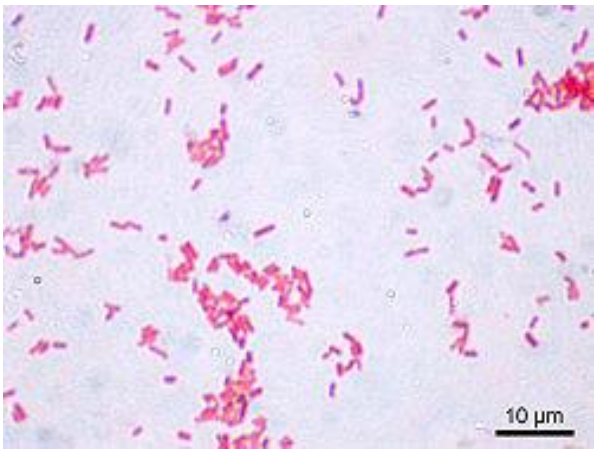


Figure 2.8 Gram stain of *E. coli*.

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

Table2.9 Classification of *E. coli*

2.2.1.2 Morphology and identification

Escherichia coli is gram-negative, non-sporing bacilli with most strains being motile and generally possessing both sex pili and adhesive fimbriae (Mahon and Manuselis, 1995). Because most strains rapidly ferment lactose, colonies grown on MacConkey media are smooth, glossy, and translucent and are rose-pink in colour. Some strains grown on on blood agar result in colonies being surrounded by zones of haemolysis. Colonies are smooth, circular, 1 – 1,5mm in diameter and yellow opaque if lactose fermenting (blue, if non-lactose fermenting) when grown on cystine-lactose-electrolyte deficient (CLED) medium (Mackie and McCartney, 1989).

2.2.1.3 Epidemiology

Strains of *Escherichia coli* predominate among the aerobic commensal bacteria present in the healthy gut (Mackie and McCartney, 1989).

2.2.1.4 *Escherichia coli* Infections

Escherichia coli was initially considered a non-harmful member of the colon flora, but is now associated with a wide range of diseases and infections including meningeal, gastrointestinal, urinary tract, wound and bacteremia infections in all age groups (Mahon and Manuselis, 1995).

Other infections caused by *Escherichia coli* include peritonitis, cholecystitis, septic wounds and bedsores. They may also infect the lower respiratory passages or cause bacteraemia and endotoxic shock especially in surgical or debilitated patients (Mackie and McCartney, 1989).

2.2.1.5 Antimicrobial Susceptibility

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

2.2.2 *Staphylococcus aureus*

2.2.2.1 Classification

Members of the genus Staphylococcus (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters (Ryan and Ray, 2004).

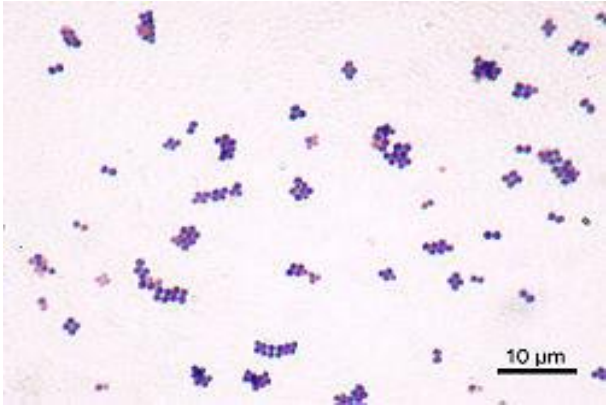


Figure 2.9 Gram stain of *S. aureus* cells

Domain	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	*Staphylococcus*
Species	*aureus*

Table2.10 Classification of *S. aureus*

2.2.2.2 Morphology and identification

Staphylococci are spherical cells about 1 m in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. Staphylococci are non-motile and do not form spores (Brooks *et al*, 2007).

Staphylococcus aureus is a facultative anaerobe that grows at an optimum temperature of 37ºC and an optimum pH of 7,5.

S. aureus produces white colonies that tend to turn a buff-golden color with time, which is the basis of the species epithet aureus (golden). Most, but not all, strains show a rim of clear β-hemolysis surrounding the colony (Ryan and Ray, 2004).

On nutrient agar, following aerobic incubation for 24 hours at 37ºC, colonies are 1 – 3mm in diameter, have a smooth glistening surface, an entire edge and an opaque pigmented appearance. In most strains, pigmentation is golden with orange, yellow and cream varieties. On MacConkey agar, colonies are small to medium in size and pink or pink-orange in colour (Mackie and McCartney, 1989).

2.2.2.3 Epidemiology

Staphylococci are highly successful colonizers of humans and animals. They reside mainly on the skin, particularly in moist areas such as the anterior nares (nose), axilla and groin. Between one-third and three-quarters of individuals carry these organisms at any one time. Staphylococcal infections occur worldwide, and newly emerging hypervirulent or multiresistant strains spread rapidly over wide geographical areas. The bacteria survive in the air, on objects or in dust for days, therefore they can contaminate environments (such as hospitals) and continue to be transmitted over long periods of time. Some individuals may shed the organism more heavily than others. Staphylococcal infections are acquired from either self (endogenous) or external (exogenous) sources (Irving *et al*., 2006).

2.2.2.4 Infections

S. aureus causes serious infections of the skin, soft tissues, bone, lung, heart, brain or blood (Irving *et al*., 2006). include pneumonia, bacteremia leading to secondary pneumonia and endocarditis, osteomyelitis secondary to bacteremia and septic arthritis, seen in children and in patients with a history of rheumatoid arthritis. Diseases caused

by Staphylococcal toxins include scalded skin syndrome and toxic shock syndrome (Sherris, 1984).

2.2.2.5 Antimicrobial Susceptibility

Resistance to penicillin G can be predicted by a positive test for β-lactamase; approximately 90% of S aureus produce β-lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% of S aureus and approximately 75% of S epidermidis isolates (Brooks *et al*., 2007).

Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin. Some strains become resistant to multiple antibiotics (Irving *et al*., 2006).

2.2.3 *Pseudomonas aeruginosa*

2.2.3.1 Classification

Pseudomonas aeruginosa is a classic opportunist pathogen belonging to the genus Pseudomonas (Mackie and McCartney, 1989).

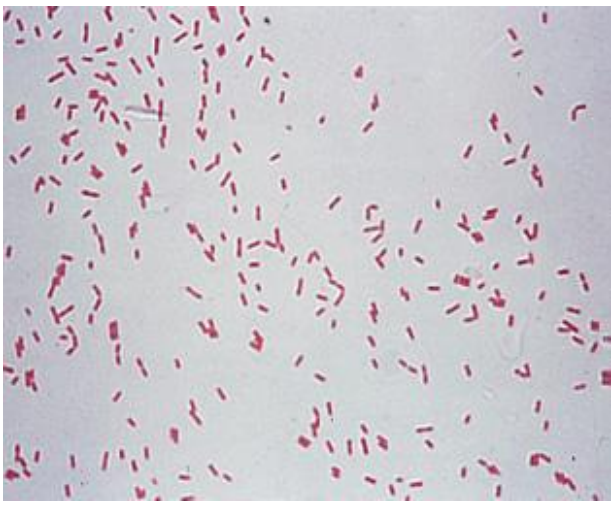


Figure 2.10 Gram stain of *P. aeruginosa* cells

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	*Pseudomonas*
Species	*aeruginosa*

Table 2.11 Classification of *P. aeruginosa*

2.2.3.2 Morphology and Identification

Is obligate aerobe, motile, rod-shaped, measuring about 0.6 x 2 µm. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains. sometimes producing a sweet or grape-like or corn taco-like odor (Brooks *et al*., 2007).

its production of blue, yellow, or rust-colored pigments differentiates it from most other Gram-negative bacteria. The blue pigment, **pyocyanin,** is produced only by *P.*

aeruginosa. **Fluorescin,** a yellow pigment that fluoresces under ultraviolet light, is by *P. aeruginosa* and other free-living less pathogenic *Pseudomonas* species. Pyocyanin produced and fluorescin combined produce a bright green color that diffuses throughout the medium (Ryan and Ray, 2004).

P aeruginosa grows well at 37–42 °C; its growth at 42 °C helps differentiate it from other *Pseudomonas* species. It does not ferment carbohydrates, but many strains oxidize glucose (Brooks *et al*., 2007).

2.2.3.3 Epidemiology

P. aeruginosa normally inhabit soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons. They often colonize hospital food, sinks, taps, mops, and respiratory equipment. Spread is from patient to patient via contact with fomites or by ingestion of contaminated food and water (Baron, 1996).

2.2.3.4 Infections

Pseudomonas aeruginosa causes infections in healthy individuals and those who are hospitalized or have a compromised immune system as a result of other diseases. A variety of human infections are commonly associated with this bacterium:

- Urinary tract infections
- Ventilator-associated pneumonia
- Surgical site infection
- Respiratory infections
- Ocular infections
- Ear infections (external otitis, malignant external otitis)
- Skin and soft tissue infections, including hot tub folliculitis, and osteomyelitis
- Burn sepsis

Individuals with compromising conditions, such as HIV/AIDS, cystic fibrosis, chemotherapy-related neutropenia, and diabetes have an increased risk of acquiring an infection and developing complications (Trautmann *et al*., 2008).

2.2.3.5 Antimicrobial Susceptibility

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

2.3 Antibiotic resistance

The discovery of antibiotics in the mid-twentieth century revolutionized the management and treatment of infectious disease caused by bacteria. Infections that would normally have been fatal were now curable. Since then, antimicrobial agents (antibiotics and related medicinal drugs acting on bacteria, viruses, fungi and parasites) have saved the lives and eased the suffering of millions of people. Today, antibiotics are crucial not only for the treatment of bacterial infections, but also for prophylactic coverage of high risk patients e.g. those in intensive care, organ transplants, cancer chemotherapy and prenatal care. However, these gains are now seriously jeopardised by the rapid emergence and spread of microbes that are resistant to antimicrobials (www.earto.eu **)**.

The mass production of penicillin in 1943 dramatically reduced illness and death from infectious diseases caused by bacteria. However, within four years, bacteria began appearing that could resist the action of penicillin. Pharmaceutical companies fought back by developing other types of antibiotics. After more than 50 years of widespread use of these "miracle drugs", antibiotics are no longer as effective as they once were. Virtually all important bacterial infections in throughout the world are becoming resistant (Johnson, 2006). And even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Nascimento *et al*., 2000).

From these microbes resistant to antibiotics:

2.3.1 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections. MRSA infections are very difficult to cure because MRSA strains are resistance against almost all clinically available antibiotics (Adwan and Mhanna, 2008).

MRSA infections that are acquired by persons who have not been recently hospitalized or had a medical procedure (such as dialysis, surgery and catheters) are known as Healthcare associated MRSA (HA MRSA) first appeared in the 1960s and has typically been linked to persons with health care associated risk factors such as hospitalization or nursing home care, chronic dialysis, antibiotic treatment, or exposure to invasive

devices or procedures. HA MRSA is a highly resistant and important nosocomial pathogen in both acute care and long term care settings and causes infections associated with increased morbidity, mortality, and cost when compared to infections due to susceptible strains of S. aureus (Cuaresma *et al*., 2008). Beginning in the 1990s community associated MRSA (CA MRSA) infections emerged in persons having none of the risk factors associated with MRSA in the past. .CA MRSA is currently defined as an infection with MRSA in a person who does not have any prior history of a health care exposure such as hospitalization, surgery, permanent intravenous lines or other indwelling devices,or hemodialysis (Davis and Fox, 2005).

CA-MRSA infections are usually manifested as skin infections, such as pimples and boils, and occur in otherwise healthy people. They are often misdiagnosed as "spider bites" and can cause serious infections if not treated early (www.bop.gov).

2.3.2 Multidrug-resistant *Pseudomonas aeruginosa*

Pseudomonas aeruginosa also causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments and resistance to many antibiotics and antiseptics. A main problem is the emergence of multidrug-resistant *P. aeruginosa* strains resistant to different antimicrobial agent classes. Perhaps, this high degree of multidrug resistance related to the presence of antibiotic efflux systems which provide resistance to multiple antimicrobial agents (Adwan *et al.*, 2009).

2.3.3 Multidrug-resistant Enterobacteriaceae

Multidrug-resistant Enterobacteriaceae, mostly *Escherichia coli*, produces extended-spectrum β lactamases (ESBLs) such as the CTX-M enzymes. These enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates such as ceftazidime, ceftriaxone, or cefepime have emerged within the community setting as an important cause of urinary tract infections (UTIs). Recent reports have also described ESBL-producing *E. coli* as a cause of bloodstream infections associated with these community-onsets of UTI (Darwish and Aburjai, 2010).

2.4 Non-antibiotic

A drug is a substance which may have medicinal, intoxicating, performance enhancing or other effects when taken or put into a human body or the body of another animal and is not considered a food. In pharmacology, a drug is "a chemical substance used in the treatment, cure, prevention, or diagnosis of disease or used to otherwise enhance physical or mental well-being." Drugs may be prescribed for a limited duration, or on a regular basis for chronic disorders (www.Drug. Dictionary.com).

'Non-antibiotic drugs' used in treatment of a variety of non-infectious human diseases such as diuretic drugs, antihistamines and sychotherapeutic drugs (Cederlund and Mårdh, 1993). Combinations of antibiotics are commonly used in medicine to broaden antimicrobial spectrum and generate synergistic effects. Alternatively, combination of non-antibiotic drugs with antibiotics offers an opportunity to sample a previously untapped expanse of bioactive chemical space (Ejim *et al*., 2011).

2.4.1 Loperamide hydrochloride

Loperamide HCl is widely used in adults for acute diarrhea. However, its use in children has been discouraged by the World Health Organization and the American Academy of Pediatrics owing to concerns over safety and efficacy in young children. (Li *et al.*, 2007). This tablets help reduce diarrhea by slowing down an overactive bowel, which helps the body to absorb more water and salts from this organ, making the stool more solid and less frequent. It is freely soluble in methanol, isopropyl alcohol, chloroform and slightly soluble in water (www.dailymed.nlm.nih.gov).

2.4.2 Vitamin C

Is a water-soluble vitamin that is used in many tissues throughout the body. The adrenal gland contains the highest concentration of vitamin C, and the vitamin plays a crucial role in both the adrenal cortex and adrenal medulla. Humans are one of the few species that cannot manufacture the vitamin in the body and must depend on diet or nutritional supplementation as a source of vitamin C. The best sources of vitamin C are fresh fruit (especially in the citrus family, including oranges, lemons, limes and tangerines), Vitamin C possesses immunostimulatory, anti-inflammatory and anti-allergic properties to a variety of illnesses. Many studies suggest that both the severity and duration of the

common cold may be reduced with moderately high doses of vitamin C (Walter Jessen, 2007).

2.4.3 Paracetamol

Paracetamol (acetaminophen) is one of the most popular over the counter analgesic and antipyretic drugs. Paracetamol is a safe medication for children when used appropriately. However, liver toxicity can occur with inappropriate use (www.bpac.org.nz).

Paracetamol has a very low solubility in nonpolar and chlorinated hydrocarbons such as toluene and carbon tetrachloride whereas the solubility is very high in solvents of medium polarity such as *N*,*N*-dimethylformamide, dimethyl sulfoxide, and diethylamine. Paracetamol is soluble in alcohols, but the solubility decreases with an increase in the length of the carbon chain in the *n*-alcohol homologous series (methanol to 1-octanol). The solubility of paracetamol in water is much lower than in other polar solvents such as the alcohols (Granberg and Rasmuson, 1999).

2.5 Previous Studies

In 2006, **Betoni *et al***. evaluated the synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus*. The in vitro anti-*Staphylococcus aureus* activities of the extracts were confirmed, and synergism was verified for all the extracts; clove, guava, and lemongrass presented the highest synergism rate with antimicrobial drugs, while ginger and garlic showed limited synergistic capacity.

In 2010, **Saravanan *et al***. evaluated the antibacterial activity of all sati on pathogenic bacterial. The results indicated the aqueous extract of garlic inhibited the growth of both Gram positive and gram negative tests bacterial cultures. The maximum activity was noted against *Klebsiella pneumoniae* (8mm), *Bacillus cereus* (7mm), *Escherichia coli* (6mm) and *Streptococcus mutans* (6mm) and minimum antibacterial activity against *salmonella typhi* (4mm). The methanol extract exhibited a zone of 3mm towards *E. coli* and *Klebsiella pneumoniae* and 2mm towards *salmonella typhi*, *Bacillus cereus* and *Streptococcus mutans*.

In 2010, **Mohamed *et al***. evaluated the chemical constituents and biological activities of *Artemisia herba-alba*. Only the essential oil was found to be active against some Gram-positive bacteria (*Streptococcus hemolyticus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Shigella sonnei* and *Salmonella typhosa*,). Also Artemisia shoots achived Anthelmintic activity against Enterobius vermicularis.

In 2008, **Ayepola and Adeniyi** evaluated the antibacterial activity of leaf extracts of *Eucalyptus camaldulensis***.** The methanol extracts showed greater activity against *Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis* (15 -16mm) than *Klebsiella spp, Yersinia enterocolitica* and *Pseudomonas aeruginosa* (14mm). The dichloromethane fraction exhibited higher activity against *Klebsiella spp, Salmonella typhi, Yersinia enterocolitica* and *Bacillus subtilis* (15–16mm) than *Staphylococcus aureus* and *Pseudomonas aeruginosa* (13-14mm). The methanol residue had a lower activity against all the test organisms except *Klebsiella spp* and *Salmonella typhi*.

In 2010, **Abubakar** evaluated the antibacterial potential of crude leaf extracts of *Eucalyptus camaldulensis* against some pathogenic bacteria. The least activity in terms

of zones of growth inhibition was shown by aqueous extract against *E. coli* (7 mm), *Klebsiella pneumoniae* (9 mm), *Proteus mirabilis* (13 mm), *S. typhi* (12 mm) and *S. aureus* (12 mm) while the highest was demonstrated by the acetone, with a recorded zone diameter for *E. coli (*12 mm), *K. pneumoniae* (13 mm), *Salmonella typhyi* (14 mm), *P. mirabilis* (15 mm) and *S. aureus* (14 mm).

In 2010, **Olusesan *et al***. evaluated the preliminary *in-vitro* antibacterial activities of ethanolic extracts of *Ficus sycomorus* Linn and *Ficus platyphylla* Del**...** Using the same concentration of the two test plants extracts, the zones of inhibition showed by *F. sycomorus* ranged between 11.5 - 21.5 mm while that of *F. platyphylla* was from 17.0 - 22.0 mm. The values of the M.I.C and M.B.C of *F. sycomorus* were 1.95, 31.3 and 3.91, 250 mg/ml, respectively. Similarly, *F. platyphylla* displayed 1.95 and 7.81 mg/ml M.I.C. values and 3.91 to 62.5 mg/ml M.B.C. values against the test organisms.

In 2009, **Ganjewala *et al***. evaluated the biochemical compositions and antibacterial activities of *Lantana camara* plants with yellow, lavender, red and white flowers. Shows that *L. camara* flower extracts have strong antibacterial activities more than the leaf extracts, only ethyl acetate extracts was found to be the most effective against all of the bacteria except *S. aureus*. Acetone and chloroform extracts did not show any significant inhibitory effects against the bacteria used. *L. camara* yellow and white flowers extracts showed the highest inhibitory effects against *B. subtillis*. Leaf extracts compared to the flower extracts, displayed less inhibitory effects against all the bacteria tested . *E. coli* was found to be the most sensitive bacteria to all *L. camara* flowers and leaf extracts. *P. aeruginosa* and *B. subtillis* was also found to be highly susceptible to all *L. camara* flower and leaf extracts.

In 2008, **Sonibare and Effiong** evaluated the antibacterial activity and cytotoxicity of essential oil of *Lantana camara* L. leaves from nigeria. The essential oil shows activity against *P. mirabilis* and *B. subtilis* at minimum inhibitory concentration (MIC) value of 1000 ppm. It shows activity against *P. aeruginosa*, *C. albican*, *S. typhi*, and *B. aureus* at MIC value of 10000 ppm. The antimicrobial activities of the essential oil suggest its usefulness in the treatment of various infectious diseases cause by bacteria.

In 2010, **Derwich *et al***. evaluated the antibacterial activity and chemical composition of the essential oil from flowers of *Nerium oleander*. The data indicated that *Escherichia coli* were the most sensitive strain tested to the oil of Nerium oleander with the strongest inhibition zone (28.89mm).The *Pseudomonas aeruginosa* was, in general, found to be more sensitive among bacteria with inhibition zone of 18.22mm. Modest activities were observed against *Staphylococcus aureus*, with inhibition zones of 6.32mm.The component of this oil, 1.8- cineole, has been known to exhibit antimicrobial activity against the bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus typhi, Staphylococcus aureus, Staphylococcus intermedius, and Bacillus subtilis*).

In 2004**, Hussain and Gorsi** evaluated the antimicrobial activity of *Nerium oleander* Linn. The result in this study show that the ethanolic extract of leaves of *Nerium oleander* high antimicrobial activity against all the tested microorganism except *Aspergillus niger*. The results obtained show that the ethanolic extract of the root of *Nerium oleander* exhibited moderate activity against *Bacillus pumillus* and *Staphylococcus aureus* while with *Escherichia coli* it was high whereas against Bacillus subtilis low activity was observed. While methanolic extract of *Nerium oleander* roots revealed marked activity against all the bacteria used. None of the crude extracts showed activity against *Aspergillus niger*. And chloroformic extracts of leaves and roots of *Nerium oleander* did not show any appreciable activity against any of the microbes used.

In 2003**, Owais *et al***. evaluated the antibacterial efficacy of *Withania somnifera* (ashwagandha) an indigenous medicinal plant against experimental murine Salmonellosis. The results indicate that both alcoholic as well as aqueous extracts possessed strong antibacterial activity while hexane fraction was not effective at all against any strain of bacteria.

In 2004, **Arora *et al***. evaluated the in vitro antibacterial/synergistic activities of *Withania somnifera* extracts**.** The results show that Methanol extract of leaves show high activity against *S. typhimurtum* than *E. coli.* While in roots *E. coli* more activity than *S. typhimurtum* while hexane extract of both leaves and roots low activity against *S. typhimurtum* than *E. coli.*

In 2011, **Elbashiti *et al***. evaluated the antibacterial and synergistic effect of some Palestinian plant extracts on *Escherichia coli* and *Staphylococcus aureus*. The result show that extracted by water reflux on *E. coli* show there was no synergistic effect of any plant extract against *E. coli*. While ethanol extract for 8 h. for all plant show synergistic effect against *E. coli* (*Marrubium vulgare* steam and leaves had the most synergistic effect against *E. coli*). All extracts by methanol reflux had no synergistic effect against *E. coli*, and all extracts by ethanol reflux had no synergistic effect against *E. coli* except *M. vulgare*(leaves) extracts, with a synergistic effect with amikacine and kanamycin only, While extracted by water reflux on *S. areues* show each *M. vulgare* (stems and leaves) and *Mesembryanthemum crystallinum* (whole plant) had the most synergistic inhibitory effect against S. *aureus*. While ethanol extract for 8 h. show synergistic effect of all plant extracts against *S. aureus*. The highest synergistic effect was observed with tetracycline and minocyclin.

In 2011, **Ejim *et al***. evaluated the combinations of antibiotics and non-antibiotic drugs enhance antimicrobial efficacy. The result show that the loperamide- minocycline pair had little activity against a panel of Gram-positive bacteria but retained synergistic growth inhibition for several other important Gram-negative pathogens Loperamide synergy was observed with eight different tetracycline antibiotics tested suggesting the effect is a general property of the antibiotic class.

In 2005**, Cursino *et al***. evaluated the synergic interaction between ascorbic acid and antibiotics against *Pseudomonas aeruginosa*. Ampicillin and tobramycin with ascorbic acid did not show synergy against any of the 12 isolates of *Pseudomonas aeruginosa*. But ampicillin or tobramycin with ascorbic acid were given in combination to all the isolates**.**

In 2009, **Shobana *et al***. evaluated Antibacterial Activity of Garlic Varieties (Ophioscordon and Sativum) on Enteric Pathogens. The result show Aqueous extract of both the garlic varieties inhibited the growth of enteric pathogens at the concentrations of 200,300,400 and 500mg. However *Enterobacter aerogenes* was not susceptible to the aqueous extract of both the garlic varieties. Ethanolic extract of sativum was found to be highly effective against all the bacteria tested.

in 2008, **Sonibare and Effiong** evaluated Antibacterial activity and cytotoxicity of essential oil of *Lantana Camara* L. leaves from Nigeria. The oil showed moderate activity against *Candida albican*s, *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus aureus*. These activities support its potential use as a remedy for bacterial infectious diseases.

In 2011, **Abdul Hanna *et al***. evaluated Antimicrobial Activity of Garlic (*Allium sativum*) Against Multi-Drug Resistance and Multi-Drug Resistance *Mycobacterium tuberculosis*. The result showed That MIC of garlic extract was ranged from 1 to 3 mg/ml; showing inhibitory effects of garlic against both non-MDR and MDR M. tuberculosis isolates.

In 2012, **Obeidat *et al***. evaluated *Antimicrobial Activity of Crude Extracts of Some Plant Leaves*. The result show that water extract of *Arum discoridis* leaves exerted significant effect and recorded the lowest MIC and MMC. Ethanol leaf extraction method is the best. It produced broad-spectrum of antimicrobial activity followed by methanol leaf extraction. Interestingly, methanol extraction method was found to be the most effective extraction method of anti candidal agents. Among the pathogenic bacteria tested, *S. pneumonia* was the least sensitive. Nevertheless, the anticandidal MIC and MMC values are higher than antibacterial values suggesting that *C. albicans* is less sensitive.

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Plant Sample Collection

The plant materials used in this study consisted of *Nerium oleander, Artemisia herba alba, Withania somnifera, Lantana camara, Ficus sycomorus, Allium sativum, Eucalyptus camaldulensis* which are growing in Palestine. These plants collected from different area in Gaza strip (Table 3.1).

3.1.2 Bacteria

Pathogenic strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* were obtained from microbiology department at Al-Shifa hospital, and were maintained on Brain Heart Infusion (BHI) agar medium (HiMedia) at 4 ºC for further experiments.

3.1.3 Culture Media and Chemicals

Types of media was required for carrying out this study, Brain Heart Infusion broth, Nutrient agar (biolife) and Mueller-Hinton agar (HiMedia). Also ethanol and methanol was used for extraction process. These media and the solvent were purchased from some company in Gaza.

3.1.4 Antibiotics & Non-Antibiotic drugs

Antibiotics used include: Vancomycin, Cefotaxime, Ofloxacin, Ceftriaxone Ceftazidime, Tetracyclines, Amikacin**,** Chloramphenicol**,** Gentamicin**,** Ampicillin**,** Neomycin, Cefazolin, Cefalexin, Nalidixic acid, Co-trimoxazole, Erythromycin, Pencillin G and Rifampicin. Table (3.2) shows antibiotics potency.

Non-Antibiotics drugs include: Loperamide, Paracetamol and Vitamin C were purchased from pharmacies in Gaza city (Table 3.3).

Table 3.1 Plant materials used in this study

Plant/Part used	Place	Time of collection
N. oleander / leaves	Balsam hospital garden - North gaza	Afternoon / March
A. herba alba / leaves	Market	-
W. somnifera / leaves	Islamic university garden- Gaza	Morning/ March
L. camara / leaves	Agricultural land near AL-Karama towers - Gaza	Afternoon / March
F. sycomorus / leaves & bark	Al-Nasr Street - Gaza	Afternoon / March & April
A. sativum / bulbs	Market/ china	-
E. camaldulensis / leaves	Psychiatric Hospital garden - Gaza	Afternoon / March & April

Table 3.2 list of antibiotic potency

Antibiotics	Antibiotics potency	Manufactured by
Vancomycin	30 µg	Himedia, Indian
Cefotaxime	30 µg	Bioanalyse, Turkey
Ofloxacin	5 µg	Himedia, Indian
Ceftriaxone	30 µg	Himedia, Indian
Ceftazidime	30 µg	Himedia, Indian
Tetracyclines	30 µg	Bioanalyse, Turkey
Amikacin	30 µg	Bioanalyse, Turkey
Chloramphenicol	30 µg	Bioanalyse, Turkey
Gentamicin	10 µg	Bioanalyse, Turkey
Ampicillin	10 µg	Bioanalyse, Turkey
Erythromycin	15 µg	Liofilchem, Italy
Rifampicin	30 µg	Liofilchem, Italy
Neomycin	30 µg	Himedia, Indian
Co-trimoxazole	25 µg	Liofilchem, Italy
Pencillin G	10 IU	Liofilchem, Italy
Cefazolin	30 µg	Liofilchem, Italy
Ceflexin	30 µg	Himedia, Indian
Nalidixic acid	30 µg	Liofilchem, Italy

Table 3.3 list of Non-antibiotic drug that used in this study

Non-antibiotics drugs	Drugs dose	Trade name/Manufactured by
Loperamide HCl	2mg	Loperamid-Ratiopharm akut/ Ratiopharm-Germany
Paracetamol	500mg	Tailol/ Pharmacare-Palestine
Vitamin C	Pure - Powder	Vitamin C pur/ Amosvital-Germany

3.2 Methods

3.2.1 Preparation of plant extract

3.2.1.1 Water reflux

For aqueous extraction, 20 g of air-dried powder was added to150 ml of distilled water and boiled on slow heat for 2 hours. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min and the supernatant was collected. This procedure was repeated twice; after 6 hours, the supernatant was collected at an interval of 2 hours, pooled together and concentrated to make the final volume one-fourth of the original volume (Parekh, and Chanda, 2006).

3.2.1.2 Methanol

Twenty g of air-dried plant extracts powder was taken in 150 ml of 96% methanol for 8 hours in Soxhlet apparatus and then the extract was filtered and allowed to evaporate in oven at45 ºC. The dried extract is dissolved in Dimethyl sulfoxide (DMSO) and stored in refrigerator for further use (Shihabudeen *et al.*, 2010).

3.2.1.3 Ethanol

The method of **Jameela *et al***. (2011), was used to obtain plant extracts in which 20 gram of aerial plant parts were extracted separately with 150 ml of 80 % ethanol as a solvent for 8 hours, using soxhlet equipment. Then the extract was filtered and allowed to evaporate in oven (45 ºC). The dried extract was dissolved in Dimethyl sulfoxide (DMSO) and stored in refrigerator for further use.

3.2.2 Preparation of of plant extracts standard concentrations

One g of each aqueous extract and alcohol pre-prepared (each separately) was taken and the aqueous extract was dissolved in 5 grams sterile distilled water, while alcoholic

extracts were dissolved in 5 ml of DiMethyl Sulphoxide (DMSO). Thus 200 mg / ml of stock was obtained as a standard concentration of aqueous and alcoholic extracts. Aqueous extracts were sterilized using 0.22 µm membrane filters and alcoholic extracts were pasteurization for 15 minutes at temperature 62 ºC (Almola, 2010).

3.2.3 Preparation of stock solution of the Non-Antibiotic drugs

Different concentraions of Non-antibiotic drugs were prepared using water as solvent for Vitamin C and methanol for Loperamide HCl and Paracetamol solutions. Different working concentrations (100µM, 50µM and 10µM) were prepared using serial dilution of th preperd stock solution of 1mM concentration.

3.2.4 Preparation of inocula

According to **Jayaraman *et al*.**, stock cultures were maintained at 4^{o}C on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring a loopful of culture to 5 ml of Brain Heart Infusion broth and incubated at 37 ^{o}C for 24 hours.

3.2.5 Antibiotics activity assay

Antibiotic discs were placed on the surface of a Mueller-Hinton agar that has been inoculated with test microorganisms. During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient. After 18-24 hours, the zone diameter of inhibition is measured and reference tables are used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antimicrobial drugs (Sockett, 2006).

3.2.6 Plant extracts activity assay

3.2.6.1 Paper Disk Diffusion Assay

A suspension of testing microorganisms were spread on Muller Hinton Agar (MHA) medium. The filter paper discs (5mm in diameter) was placed on the agar plates which was inoculated with the tested microorganisms and then impregnating with 20µl of plant extract (concentration 200 mg/ml). The plates were subsequently incubated at 37^{o} C for 24 Hrs. After incubation the growth inhibition zone were quantified by measuring the diameter of the zone of inhibition in mm (Kumar *et al*., 2009).

3.2.6.2 Well diffusion method assay

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

3.2.6.3 Determination of MIC of plant extract by Microdilution Method

The 96-well plates were prepared by dispensing 50 µl of Mueller–Hinton broth for bacteria, into each well. A 50 µl from the stock solution of tested extracts (concentration of 200 mg/ml) was added into the first row of the plate. Then, twofold, serial dilutions were performed by using a micropipette. The obtained concentration range was from 100 to 0.1953 mg/ml, and then added 10 µl of inocula to each well except a positive control (inocula were adjusted to contain approximately 1.5X10^{8} CFU/mL, **Table 3.2**). Plant extract with media was used as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37 ^{o}C for 18 h. After 18 h 50 µl of a 0.01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the wells and the plate was incubated for another hour. Since the colorless tetrazolium salt is reduced to red colored product by biological active bacteria, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth (Abu-Shanab *et al*., 2004 and Abou Elkhair *et al*., 2010 Radojević *et al*., 2012).

Table 3.4 McFarland Nephelometer Standards

McFarland Standard No.	0.5	1	2	3	4
1.0% Barium chloride (ml)	0.05	0.1	0.2	0.3	0.4
1.0% Sulfuric acid (ml)	9.95	9.9	9.8	9.7	9.6
Approx. cell density (1X10^8 CFU/mL)	1.5	3.0	6.0	9.0	12.0
% Transmittance*	74.3	55.6	35.6	26.4	21.5
Absorbance*	0.132	0.257	0.451	0.582	0.669

*at wavelength of 600 nm

3.2.7 Synergism between plant extract, antibiotics and Non-antibiotics

The bacterial cultures were grown in BHI broth at 37º C. After 4 h of growth, each bacteria was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, the antibiotic disk (diameter=5mm) was placed on the surface of each inoculated plate and then added 20 µl of plant extract (at a concentration of 200mg/ml), to identify synergies effect between the plant extract and antibiotics, and in the same way 20 µl was taken from each dilution of the Non-antibiotic drugs and put on antibiotic disk, to identify synergies between the Non- antibiotics and antibiotic.While to identify synergies between the plant extract & Non- antibiotics, 20 µl of Non- antibiotics and 20 µl of plant extracts were mixed and put together on a filter paper disk which was left for one hour to dry.

The plates were incubated at 37º C for 24 h. The diameters of clearing zones was measured.

Chapter 4
Results

4.1 Evaluation of antibiotics activity

4.1.1 Against *Escherichia coli*

By disc plate method **(section 3.2.5)** the effectiveness of a range of antibiotics was determined against *E. coli* (Table 4.1 and Figure 4.1). chloramphenicol was showed the highest inhibition zone against *E. coli* (24 mm). While it was resistance tetracyclines, ofloxacin, ampicillin, cefazolin, nalidixic acid and co-trimoxazole.

4.1.2 Against *Staphylococcus aureus*

As shown in **Table 4.1**, Gentamicin, Chloramphenicol and Tetracyclines had the highest inhibition zone (21 mm) followed by Ofloxacin, Amikacin and Neomycin (20 mm). While there was no effect of Ceftazidime, Ampicillin, Penicillin G and Cefazolin against *S.aureus*.

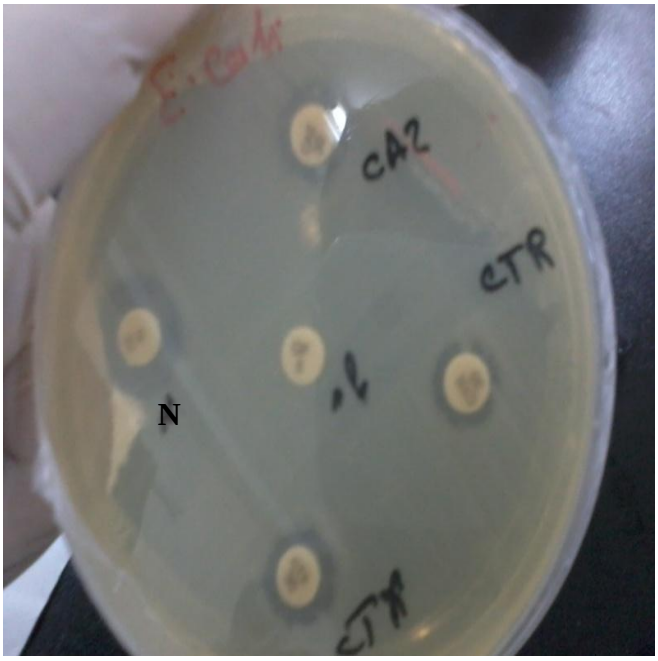
4.1.3 Against *Pseudomonas aeruginosa*

Amikacin, Ceftazidime and Gentamicin were showed the strongest activity against *P. aeruginosa* while the rest antibiotics had no effect as shown in Table 4.1 and Figure 4.3

Table 4.1 Evaluation of antibiotics activity against *S. aureus, E. coli* and *P. aeruginosa*

Microorganism / Antibiotics	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
	Inhibition zone (mm)		
Vancomycin	15mm	*	*
Cefotaxime	11mm	8 mm	0mm
Ofloxacin	20mm	0 mm	0mm
Ceftriaxone	12mm	9 mm	0mm
Ceftazidime	0 mm	11 mm	9 mm
Tetracyclines	21mm	0 mm	*
Amikacin	20mm	10 mm	17 mm
Chloramphenicol	21mm	24 mm	*
Gentamicin	21mm	7 mm	8mm
Ampicillin	0 mm	0 mm	*
Erythromycin	17 mm	*	*
Rifampicin	19 mm	*	*
Neomycin	20 mm	14 mm	0mm
Co-trimoxazole	10 mm	0 mm	*
Pencillin G	0 mm	*	*
Cefazolin	0 mm	0 mm	*
Ceflexin	10 mm	7 mm	0 mm
Nalidixic acid	*	0 mm	*

*= Have not been tested.
mm= millimeter.



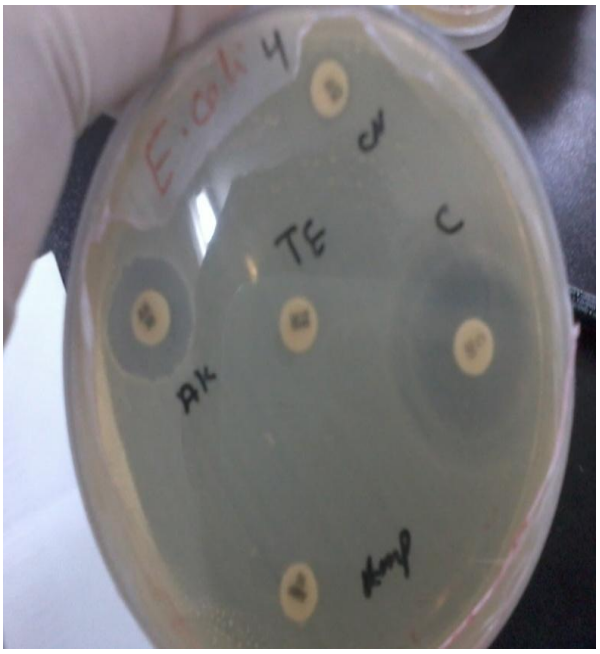


Figure (4.1): Inhibition zone (mm) of some antibiotics against *E coli*

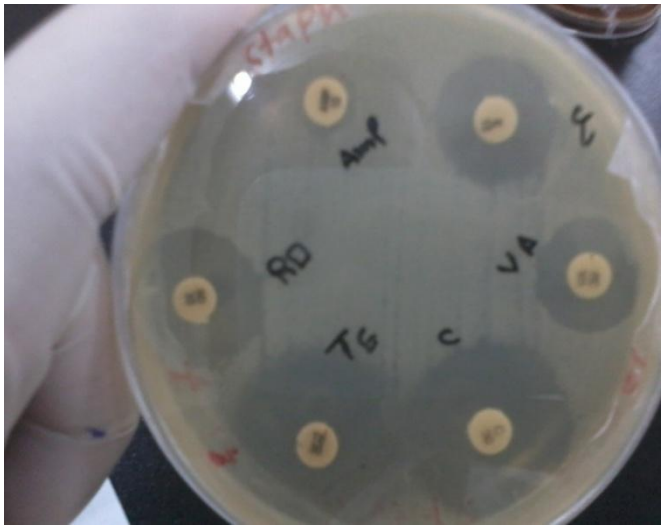


Figure 4.2: Inhibition zone (mm) of some antibiotics against *S. aureus*

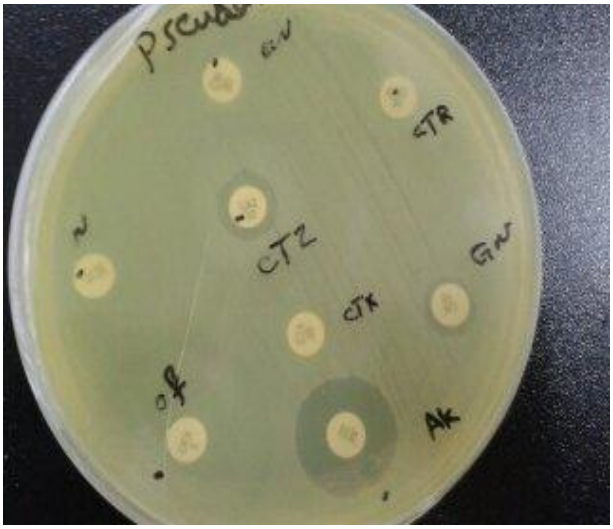


Figure 4. 3: Inhibition zone (mm) of some antibiotics against *P. aeruginosa*

VA: Vancomycin; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime TE: Tetracycline; AK: Amikacin; C: Chloramphenicol, E: Erythromycin; AMP: Ampicillin; N: Neomycin; CN: Ceflexin.

4.2 Evaluation of plant extracts bioactivity

4.2.1 Against *Escherichia coli*

The result in Table 4.2 revealed that, the disc diffusion method evaluated the antimicrobial activity of plant extracts better than the well diffusion method against *E. coli*. *Artemisia herba-alba* (leaves) and *Ficus sycomorus* (bark) (extracted by methanol for 8 h) were showed the highest effect against *E. coli* with a zone of inhibition = 9 mm. No antimicrobial activity was observed by *Allium sativum*, *Ficus sycomorus* and *Lantana camara* at a concentration of 200 mg/ml (extracted by methanol for 8 h) as shown in Table 4.2.

Ficus sycomorus (Leaves and Bark), *Eucalyptus camaldulensis* and *Withania somnifera* (extracted by ethanol for 8 h) were showed the highest activity against *E. coli* with a zone of inhibition = 8 mm and then *Artemisia herba-alba* and *Nerium oleander* showed a zone of inhibition = 7 mm. No antimicrobial activity was found by *Lantana camara* and *Allium sativum* (extracted by ethanol for 8 h) against *E.coli*.

No antimicrobial activity of most plant extracts (extracted by water for 2 h) was found against *E. coli* except with *Artemisia herba-alba* which showed low antimicrobial activity with a zone of inhibition = 6 mm (as shown in Table 4.2 and Figure 4.6).

Table 4.2 Antimicrobial Activity of Plant extracts on *Escherichia coli* by well diffusion method and disc diffusion method

A.A.A* / Plant extract		Well diffusion method				Disc diffusion method			
		M	**E**	**W**	**Control [o]**	**M**	**E**	**W**	**Control [o]**
Nerium oleander		-	-	-	-	**7**	**7**	-	-
Artemisia herba-alba		-	-	-	-	**9**	**7**	**6**	-
Withania somnifera		-	-	-	-	**7**	**8**	-	-
Lantana camara		-	-	-	-	-	-	-	-
Ficus sycomorus	L	-	-	-	-	-	**8**	-	-
	B	-	-	-	-	**9**	**8**	-	-
Allium sativum		-	-	-	-	-	-	-	-
Eucalyptus camaldulensis		-	-	-	-	**7**	**8**	-	-

***** Antimicrobial Activity Assays.

[o] Control= DMSO

Method of extraction: M= methanol, E= ethanol, W= water

L= leaves, B= bark.

(-) No inhibition zone.

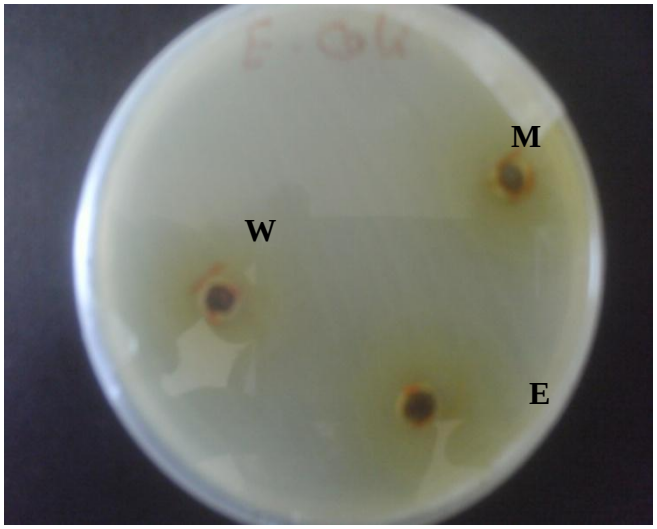


Figure (4.4): The effect of *A. sativum* extract (By Well diffusion method) against *E. coli*

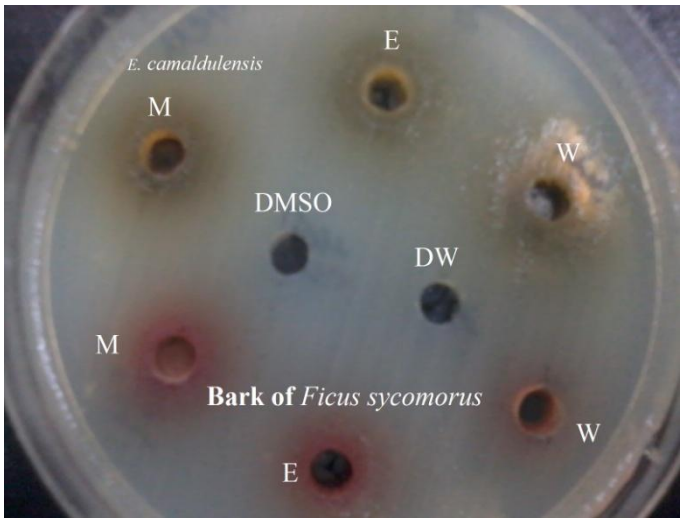


Figure (4.5): The effect of *E.camaldulensis* and *F. sycomorus* (Bark) extract (By Well diffusion method) against *E. coli*

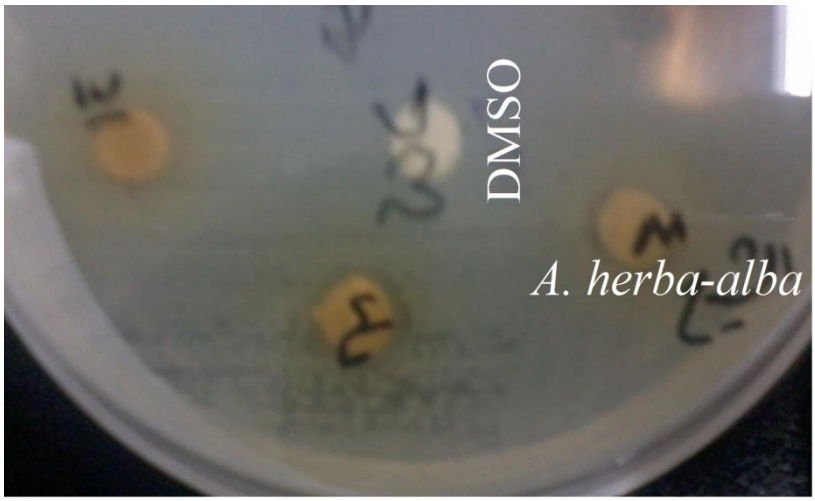


Figure (4.6): The effect of *Artemisia herba-alba* extract (disc diffusion method) against *E. coli*

4.2.2 Against *Staphylococcus aureus*

4.2.2.1 Well Diffusion Method

The results of the effects of methanolic, ethanolic and aqueous extracts of the plants using 20µl from the extracts (200 mg/ml crude extract) against the tested *S. aureus* are presented in table (4.3). It is shown that methanolic and ethanolic extract of *Artemisia herba-alba* have the highest effect on *S. aureus*, with a zone of inhibition (19 mm) and (20 mm) respectively.

In aqueous extracts, *Ficus sycomorus* was showed the highest effect against *S. aureus*. In which *Ficus sycomorus* leaves had a zone of inhibition (14 mm) which was more effective than the bark that had a zone of inhibition (12 mm). *Lantana camara* was less effective against *S. aureus*, and each of *Nerium oleander, Withania somnifera* and *Allium sativum* do not have any effect against *S. aureus* as shown in Table 4.3 and Figure 4.10.

4.2.2.2 Disc Diffusion Method

The methanol and ethanol extracts of *Ficus sycomorus* bark showed the highest effect towards *S. aureus* (with a 15 mm zone of inhibition) followed by *Lantana camara* (with a 14 mm zone of inhibition) (by methanol extract). *Eucalyptus camaldulensis* leaves (extracted by ethanol) with a zone of inhibition (13 mm). *Nerium oleander, Artemisia herba-alba, Allium sativum and Withania somnifera* extracted by methanol and ethanol showed little activity.

The aqueous extracts for 2 h of *Ficus sycomorus* (leaves and bark) showed the highest activity against *S. aureus* with 15 and 12 mm zone of inhibition, respectively. *Lantana camara* and *Eucalyptus camaldulensis* showed little activity against *S. aureus* (with 8 mm inhibition zone). *Nerium oleander, Artemisia herba-alba, Withania somnifera* and *Allium sativum* did not show antimicrobial activity against *S. aureus* as shown in Table 4.3 and Figure 4.10.

Table 4.3 Antimicrobial Activity of Plant extracts on *Staphylococcus aureus* by well diffusion method and disc diffusion method

A.A.A* / Plant extract		Well diffusion method				Disc diffusion method			
		M	**E**	**W**	**Contral [o]**	**M**	**E**	**W**	**Control [o]**
Nerium oleander		**-**	**-**	**-**	**-**	**7**	**6**	**-**	**-**
Artemisia herba-alba		**19**	**20**	**-**	**-**	**8**	**9**	**-**	**-**
Withania somnifera		**-**	**-**	**-**	**-**	**7**	**8**	**-**	**-**
Lantana camara		**9**	**11**	**7**	**-**	**14**	**10**	**8**	**-**
Ficus sycomorus	L	**14**	**15**	**-**		**11**	**12**	**-**	
	B	**13**	**14**	**-**		**15**	**15**	**-**	
Allium sativum		**-**	**-**	**-**	**-**	**7**	**7**	**-**	**-**
Eucalyptus camaldulensis		**13**	**14**	**7**	**-**	**11**	**13**	**8**	**-**

***** Antimicrobial Activity Assays.

[o] Control= DMSO

Method of extraction: M= methanol, E= ethanol, W= water

L= leaves, B= bark.

(-) No inhibition zone

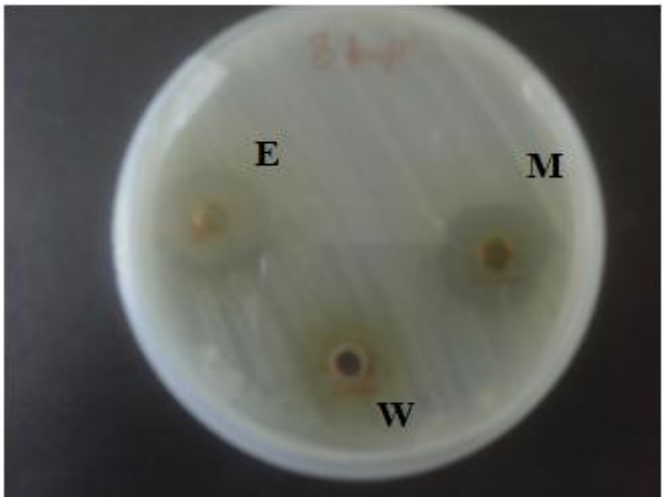


Figure (4.7): The effect of *Eucalyptus camaldulensis* extract (By Well diffusion method) against *S. aureus*

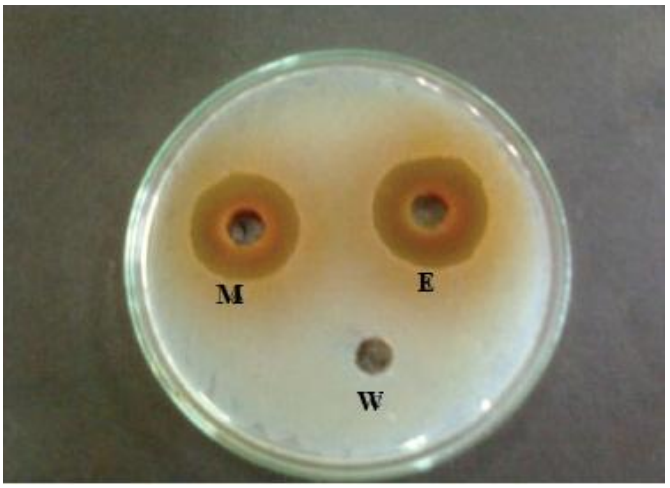


Figure (4.8): The effect of *A. herba-alba* extract (By Well diffusion method) against *S. aureus*

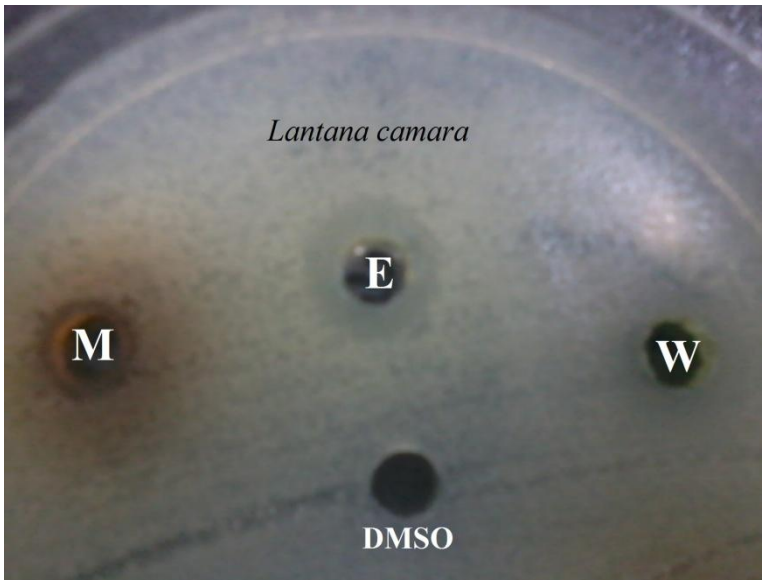


Figure (4.9): The effect of *Lantana camara* extract (By Well diffusion method) against *S. aureus*

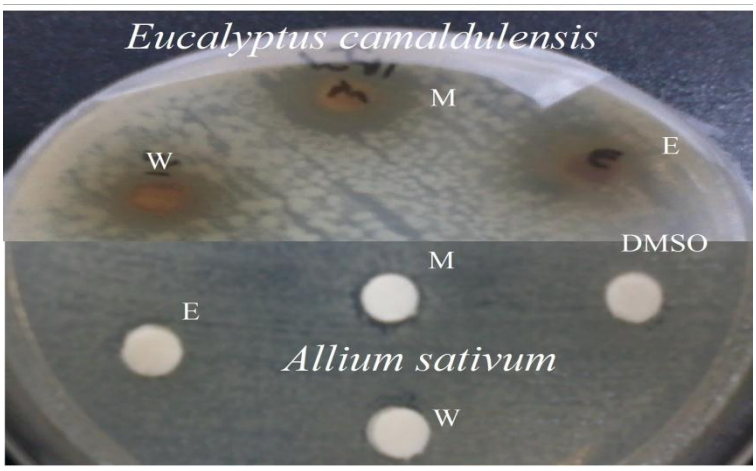


Figure (4.10): The effect of *E. camaldulensis* and *A. sativum* extract (By Disk diffusion method) against *S. aureus*

4.2.3 Against *Pseudomonas aeruginosa*

4.2.3.1 Well Diffusion Method

The results of the antibacterial activity revealed that only two of the eight plants extracted have demonstrated antibacterial activity against *P. aeruginosa*. The activity of methanol and ethanol extracts of *Ficus sycomorus* bark with a zone of inhibition 12 and 11mm, respectively and *Eucalyptus camaldulensis* leaves with a zone of inhibition 11mm and 10mm, respectively were recorded against *P. aeruginosa*.

Whereas only of aquatic extracts of the *Eucalyptus camaldulensis* was showed activity against *P. aeruginosa*.

4.2.3.2 Disc diffusion method

The largest zone of inhibition against *P. aeruginosa* was observed with the methanolic extracts of *Ficus sycomorus* bark and *Eucalyptus camaldulensis* with a zone of inhibition (10 mm) as shown in Table 4.4 and Figure 4.13 and 4.14, respectively.

The ethanolic extract of *Ficus sycomorus* bark was showed the highest activity against *P. aeruginosa* (10mm) followed by *Artemisia herba-alba* and *Eucalyptus camaldulensis* with a zone of inhibition 8mm, for each of them. But the aqueous extract of Eucalyptus *camaldulensis* and *Artemisia herba-alba* had weak antibacterial activity against *P. aeruginosa* with a zone of inhibition 8 and 7 mm respectively. While the extracts of *Nerium oleander, Withania somnifera, Lantana camara and Allium sativum* did not show antimicrobial activity against *P. aeruginosa*, whether by Well diffusion method nor disc diffusion method.

Table 4.4 Antimicrobial Activity of Plant extracts on *Pseudomonas aeruginosa* by Well Diffusion Method and Disc Diffusion Method

A.A.A* / Plant extract		Well diffusion method				Disc diffusion method			
		M	**E**	**W**	**Control [o]**	**M**	**E**	**W**	**Control [o]**
Nerium oleander		-	-	-	-	-	-	-	-
Artemisia herba-alba		-	-	-	-	-	**8**	**7**	-
Withania somnifera		-	-	-	-	-	-	-	-
Lantana camara		-	-	-	-	-	-	-	-
Ficus sycomorus	L	-	-	-	-	-	**7**	-	-
	B	**12**	**11**	-	-	**10**	**10**	-	-
Allium sativum		-	-	-	-	-	-	-	-
Eucalyptus camaldulensis		**11**	**10**	**10**	-	**10**	**8**	**8**	-

* Antimicrobial Activity Assays.

[o] Control= DMSO.

Method of extraction: M= methanol, E= ethanol, W= water, L= leaves, B= bark.

(-) No inhibition.

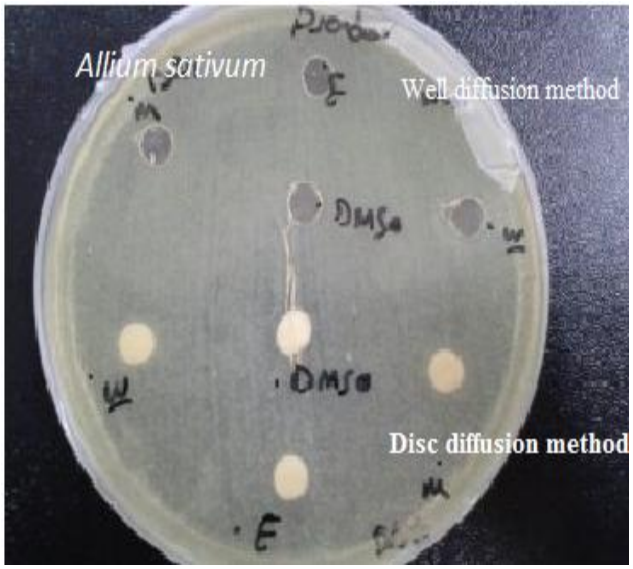


Figure (4.11): The effect of *Allium sativum* extract against *P. aeruginosa*

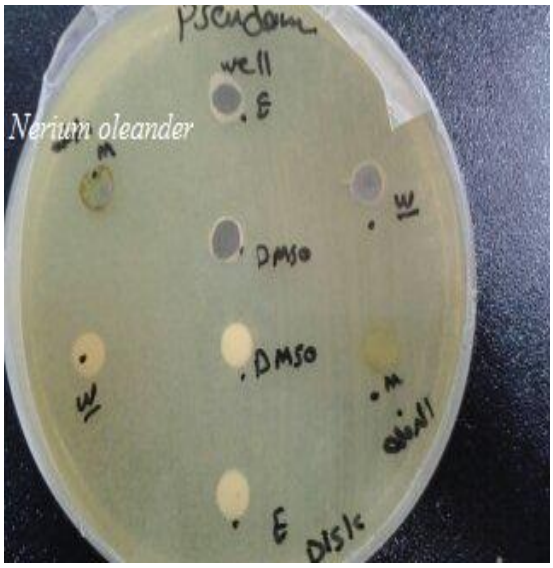


Figure (4.12): The effect of *Nerium oleander* extract against *P. aeruginosa*

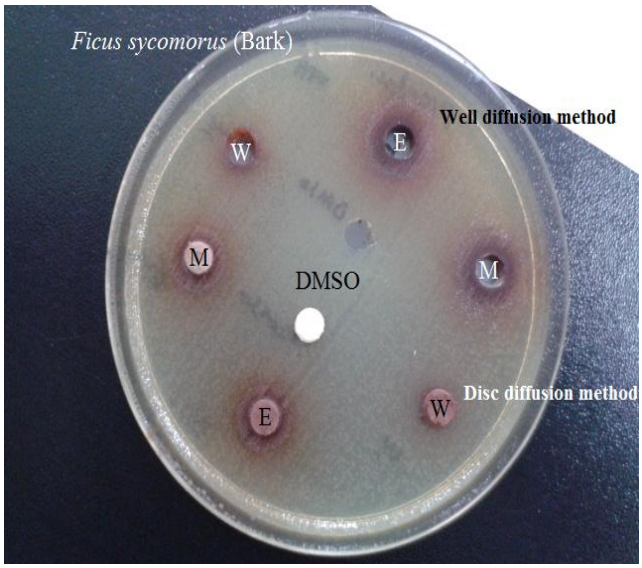


Figure (4.13): The effect of *Ficus sycomorus* (bark) extract against *P. aeruginosa*

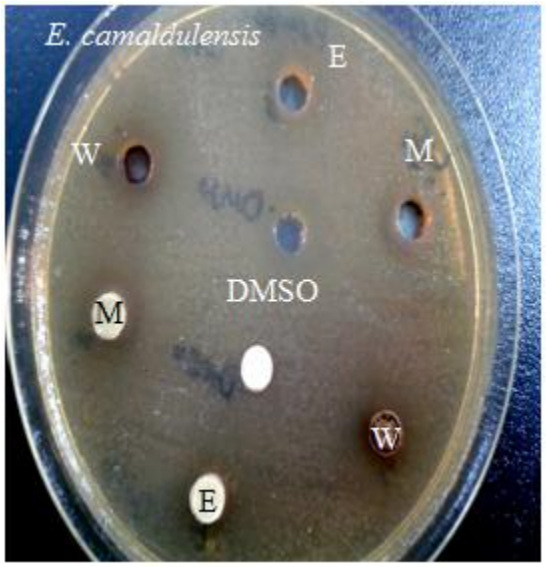


Figure (4.14): The effect of *Eucalyptus camaldulensis* extract against *P. aeruginosa*

4.3 Minimum inhibitory concentration of plant extracts alone using Microdilution method

The minimum inhibitory concentration (MIC) results showed that all tested plant extracts were showed antibacterial activity against *E. coli*, *S. aureus* and *P. aeruginosa* with MIC values ranging from 0.19 to 100 mg/ml. The tested extracts showed different levels of antimicrobial activity depending on tested species as shown in Table 4.5.

4.3.1 Against *Escherichia coli*

MIC values of all tested plant extracts against *E. coli* are summarized in Table 4.5. The MIC of the methanol extract of *N. oleander* and *F. sycomorus* (leaves) was from 6.25-12.5 mg/ml. While *W. somnifera* and *L. camara* was 25 mg/ml; *F. sycomorus* (Bark) and *A. sativum* was from 12.5-25 mg/ml, and *A. herba-alba* was 25 mg/ml. The MIC for *E. camaldulensis* against *E. coli* was the least effect (3.125 mg/ml).

The MIC values of the ethanolic extracts of *N. oleander*, *F. sycomorus* (leaves and Bark) and *W. somnifera* was 12.5 mg/ml; for each of *L. camara* and *A. sativum* was 25 mg/ml; *A. herba-alba* was from 6.25-12.5mg/ml, and the MIC value of *E. camaldulensis* was 6.25 mg/ml. The MIC results of the aquatic extracts of *N. oleander*, *A. herba-alba*, *W. somnifera* and *F. sycomorus* was 25 mg/ml; for *L. camara* and *A. sativum* was 12.5 and 50 mg/ml respectively and for *E. camaldulensis* was from 12.5-6.25 mg/ml as shown in Table 4.5 and Figures 4.15.

Table 4.5 Minimal inhibitory concentrations (MIC) of the plants extracts against *E. coli*.

MIC (mg/ml)								
Plant / Solvent	***N. oleander***	***A. herba -alba***	***W. somnifera***	***L. camara***	***F. sycomorus* (leaves)**	***F. sycomorus* (Bark)**	***A. sativum***	***E. camaldule-nsis***
Methanol	**12.5-6.25**	**12.5**	**25**	**25**	**12.5-6.25**	**25-12.5**	**25-12.5**	**3.125**
Ethanol	**12.5**	**12.5-6.25**	**12.5**	**25**	**12.5**	**12.5**	**25**	**6.25**
Water	**25**	**25**	**25**	**12.5**	**25**	**25**	**50**	**12.5-6.25**

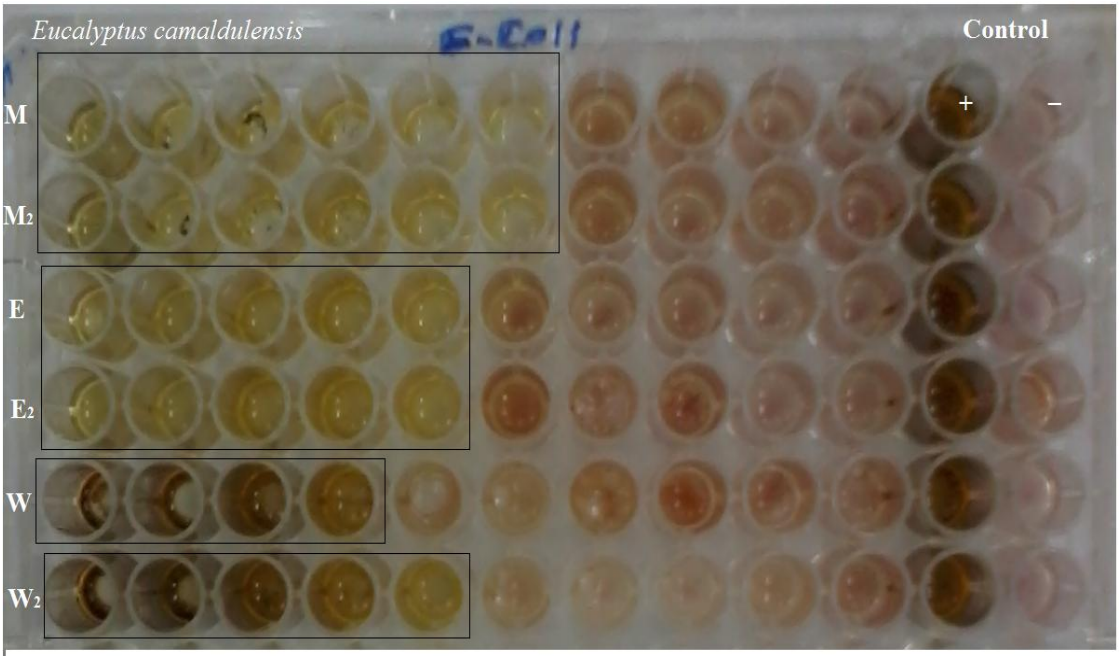


Figure (4.15): The MIC of *E. camaldulensis* extract against *E. coli*

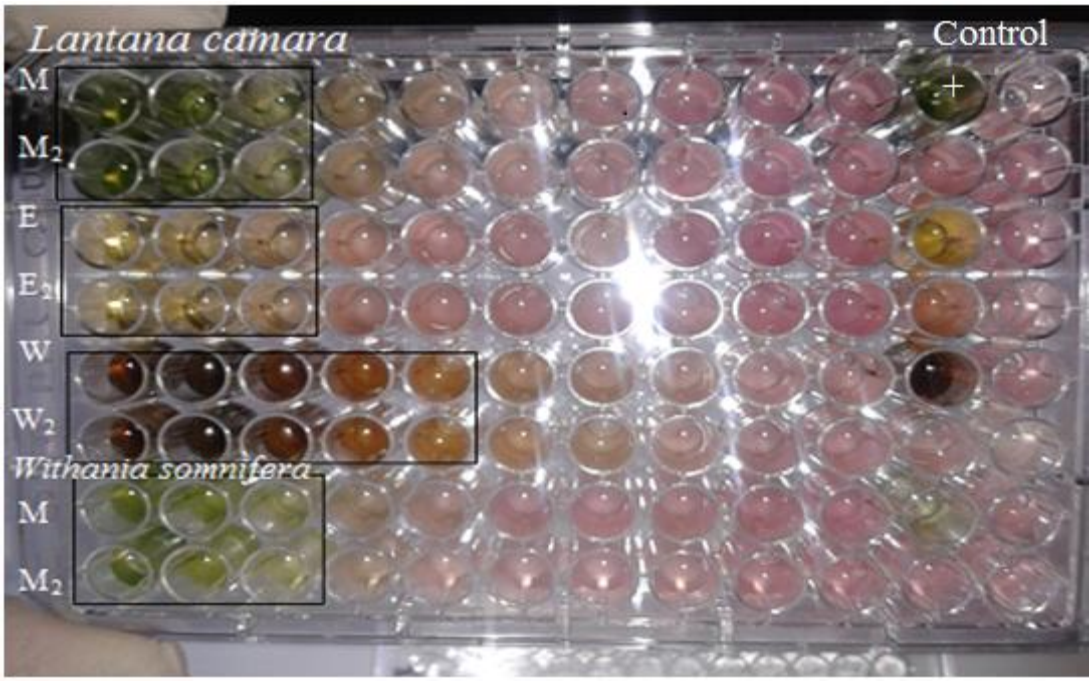


Figure (4.16): The MIC of *Lantana camara* and *Withania somnifera* extract against *E. coli*

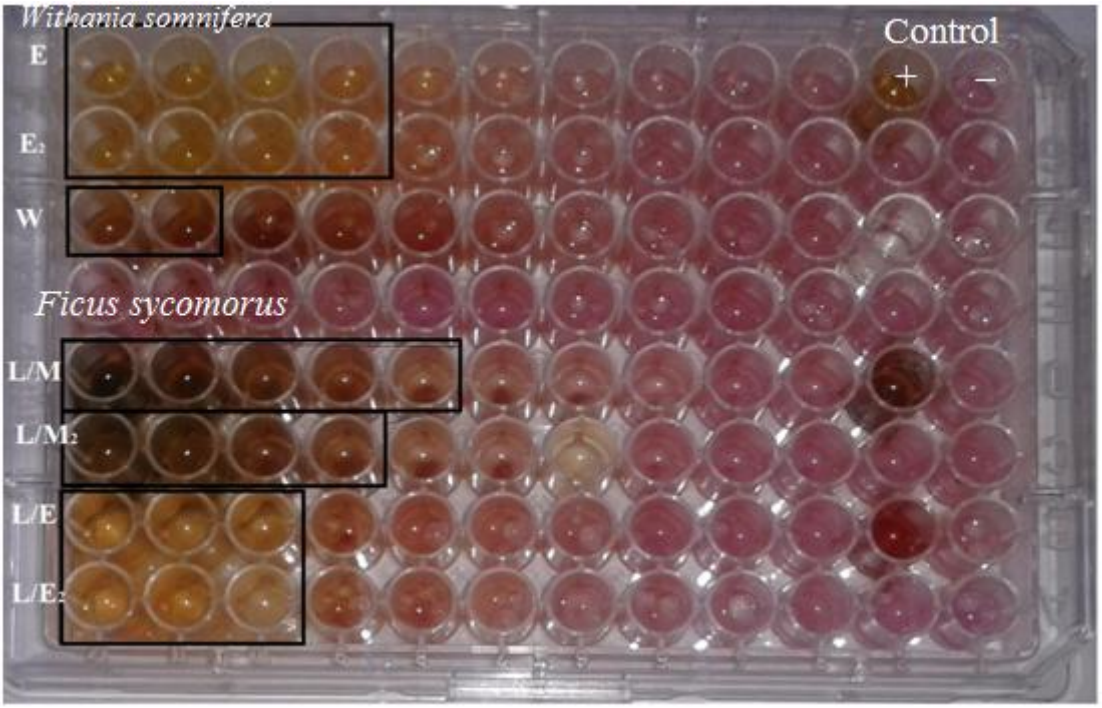


Figure (4.17): The MIC of *Withania somnifera* and *Ficus sycomorus* (leaves) extract against *E. coli*

4.3.2 Against *Staphylococcus aureus*

Table 4.6. Represented results of the MIC of plant extracts against *S.aureus.* The MIC of the methanol extracts against *S. aureu*s for each of *N. oleander*, *A. herba-alba*, *W. somnifera*, *L. camara* 12.5 mg/ml and *F. sycomorus* (Bark) and *A. sativum* were 6.25 and 50 mg/ml respectively. Meanwhile *F. sycomorus* (leaves) was from 3.125 to 6.25 mg/ml and *E. camaldulensis* was from 12.5- 6.25mg/ml.

The MIC of the ethanol extract for each of *N. oleander*, *A. herba-alba*, *L. camara*, *F. sycomorus* (leaves), *A. sativum* and *E. camaldulensis* against *S. aureu*s were 25, 6.25, 12. 5, 25, 50 and 6.25 mg/ml, respectively. But for *W. somnifera* and *F. sycomorus* (Bark) was from 6.25 to 12.5 mg/ml.

The MIC of the aquatic extracts of *N. oleander*, *A. herba-alba* and *A. sativum* against *S.aureus* was 50 mg/ml and of *W. somnifera* and *L. camara* was 25mg/ml; *F. sycomorus* (leaves) MIC was from 3.125 to 6.25 mg/ml and the MIC of *E. camaldulensis* was 12.5 mg/ml.

Table 4.6 Minimal inhibitory concentrations (MIC) of the plants extracts against *S. aureus*

MIC (mg/ml)								
Plant / Solv.	***N. oleander***	***A. herba-alba***	***W. somnifera***	***L. camara***	***F. sycomorus* (leaves)**	***F. sycomorus* (Bark)**	***A. sativum***	***E. camaldule-nsis***
M	**12.5**	**12.5**	**12.5**	**12.5**	**6.25-3.125**	**6.25**	**50**	**12.5- 6.25**
E	**25**	**6.25**	**12.5-6.25**	**12. 5**	**25**	**12.5-6.25**	**50**	**6.25**
W	**50**	**50**	**25**	**25**	**6.25-3.125**	**25**	**50**	**12.5**

Figure (4.18): The MIC of *Artemisia herba-alba and Withania somnifera* extract against *S. aureus*

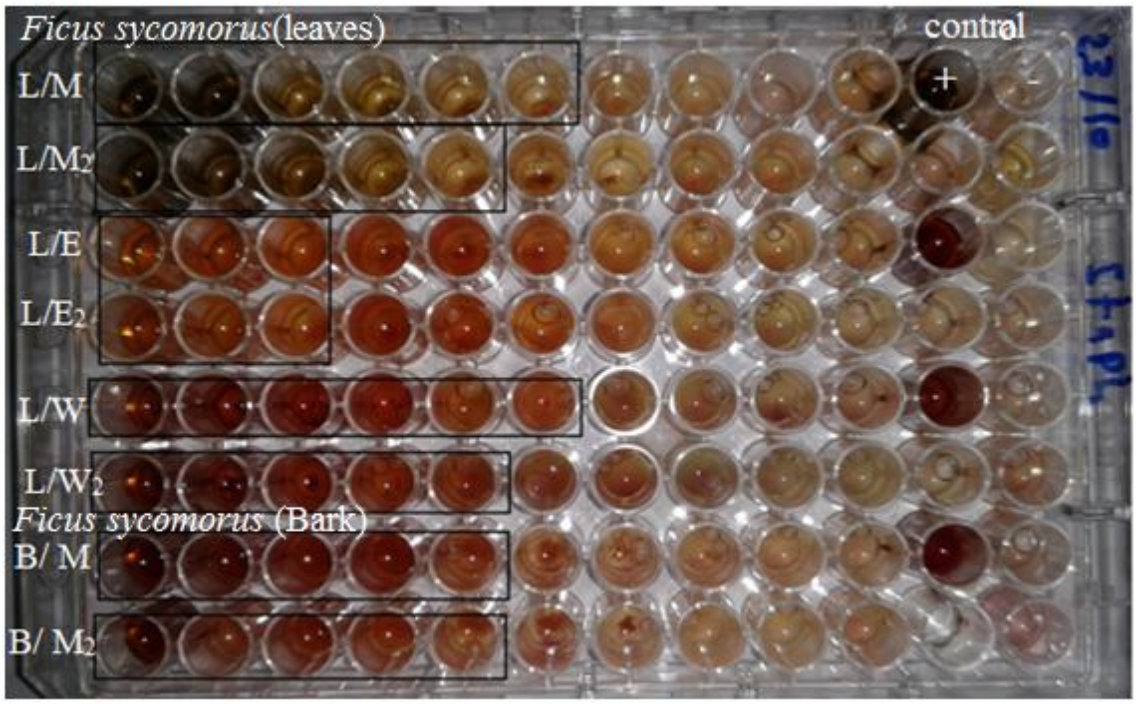


Figure (4.19): The MIC of *Ficus sycomorus* (leaves and bark) extract against *S. aureus*

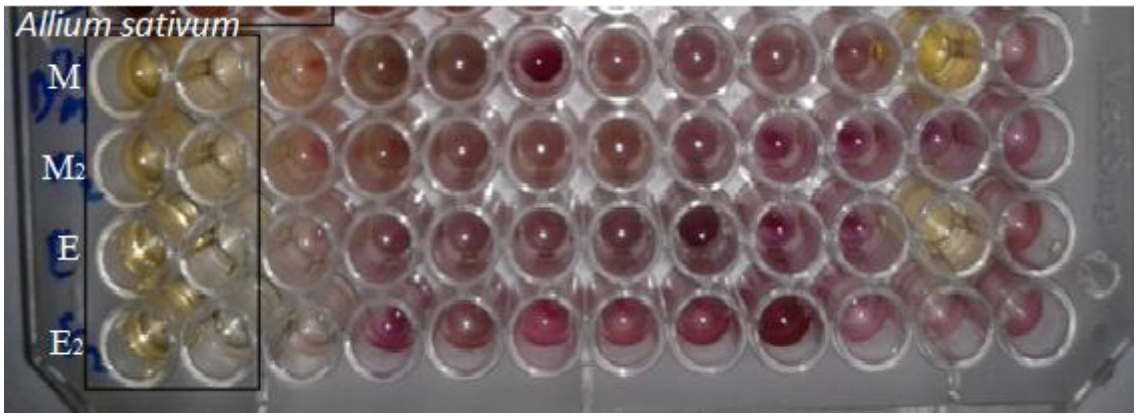


Figure (4.20): The MIC of *Allium sativum* extract against *S. aureus*

4.3.3 Against of *Pseudomonas aeruginosa*

The MIC of the methanol extract for each of *L. camara, W. somnifera and F. sycomorus* (Leaves and bark) against *P. aeruginosa* was 25 mg/ml and 12.5 mg/ml for each of *A. Sativum* and *E. Camaldulensis*, while it was from 25-50 mg/ml for *N. oleander* and 50 mg/ml for *A. herba-alba*.

The MIC of the ethanol extract for each of *A. herba-alba, W. somnifera, L. camara, F. sycomorus* (Leaves) and *A. Sativum* was 25 mg/ml. whereas it was 50 mg/ml for each of *N. oleander* and *F. sycomorus* (Bark). But for *E. Camaldulensis* was 6.25 mg/ml.

The MIC of the aquatic extract for each of *N. oleander, A. herba-alba W. somnifera and F. sycomorus* (Leaves and bark) and *A. Sativum* was 50 mg/ml. And was 12.5 mg/ml for each of *L. camara* and *E. Camaldulensis*.

Table 4.7 Minimal inhibitory concentrations (MIC) of the plants extracts against *P. aeruginosa*

MIC (mg/ml)								
Plant / Solv.	*N. oleander*	*A. herba-alba*	*W. somnifera*	*L. camara*	*F. sycomorus* (leaves)	*F. sycomorus* (Bark)	*A. sativum*	*E. camaldule-nsis*
M	**50-25**	**50**	**25**	**25**	**25**	**25**	**12.5**	**12.5**
E	**50**	**25**	**25**	**25**	**25**	**50**	**25**	**6.25**
W	**50**	**50**	**50**	**12.5**	**50**	**50**	**50**	**12.5**

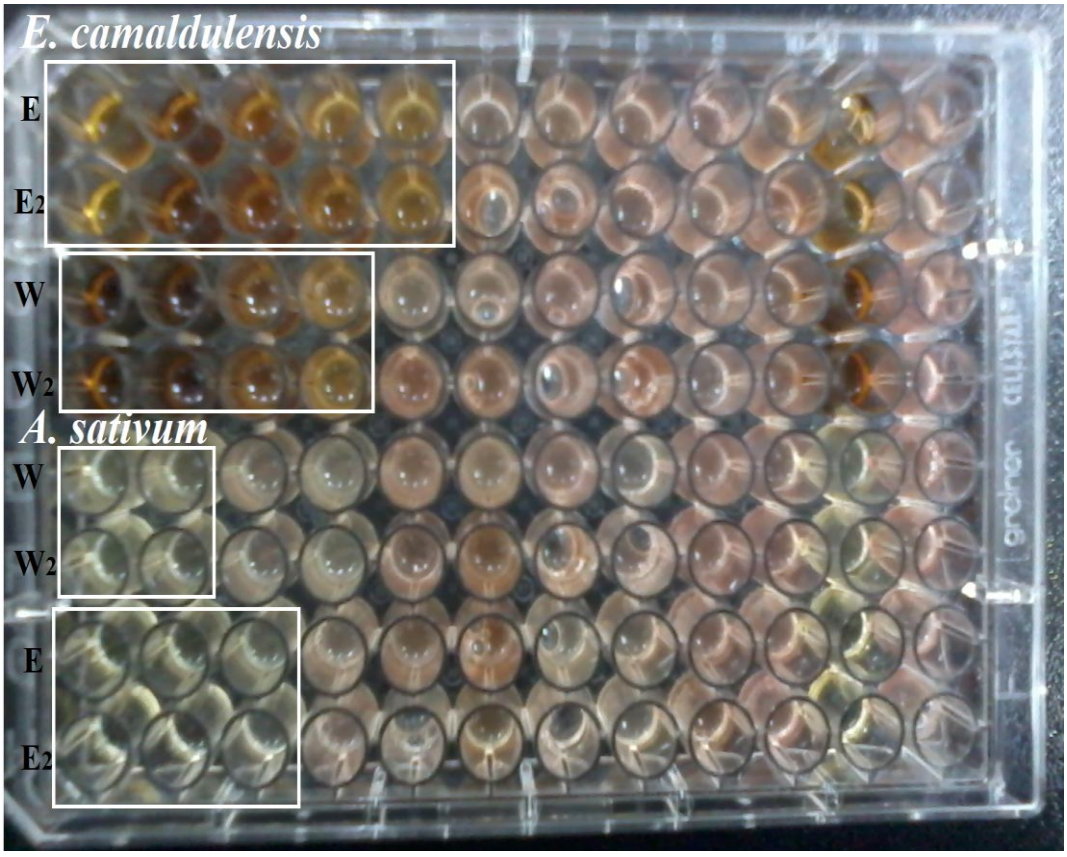


Figure (4.21): The MIC of *Allium sativum* and Eucalyptus camaldulensis extract against *P. aeruginosa*

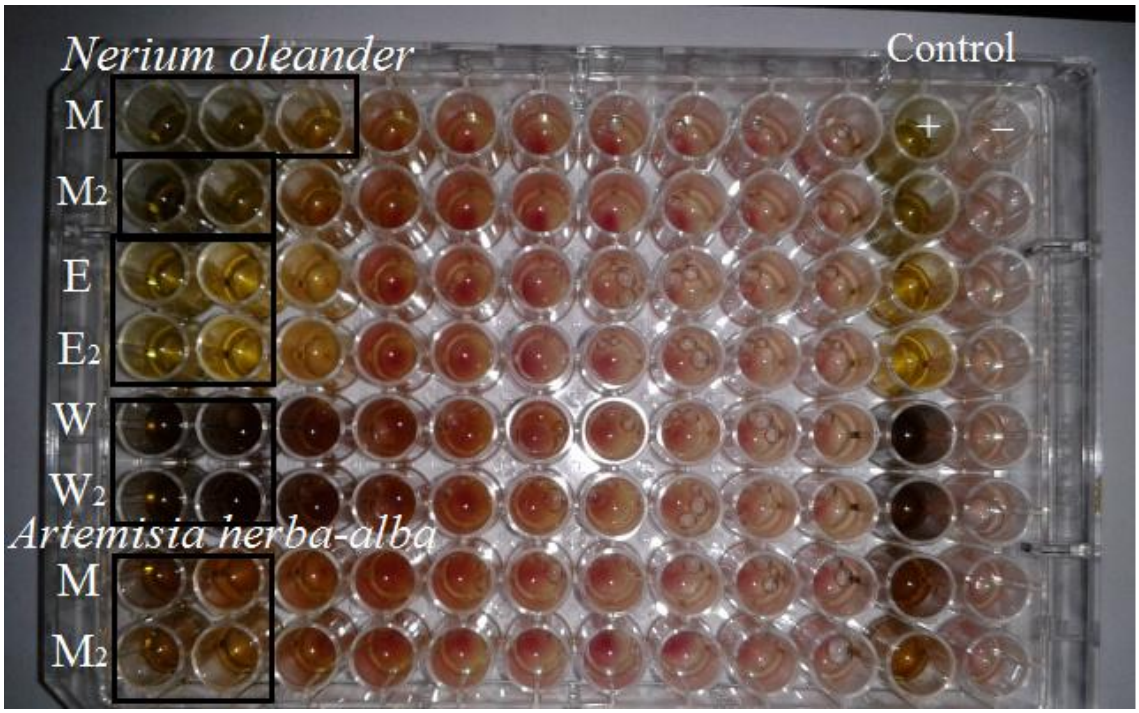


Figure (4.22): The MIC of *N. oleander* and *A. herba-alba* extract against *P. aeruginosa*

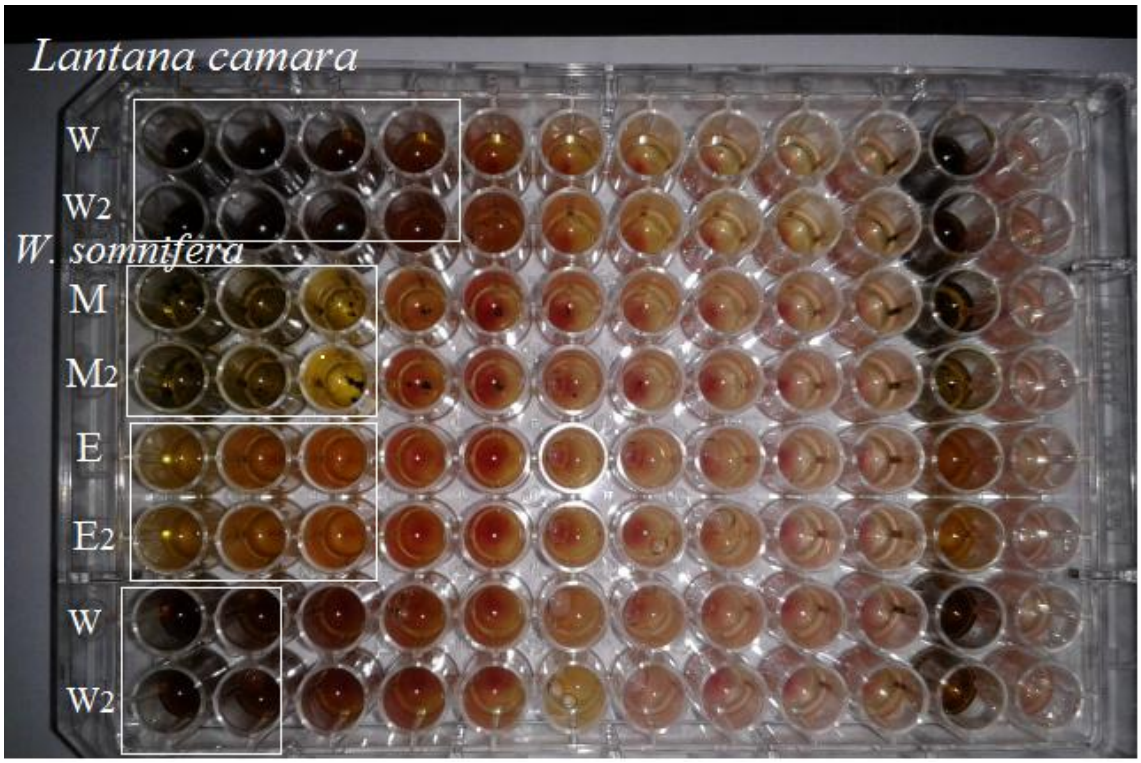


Figure (4.23): The MIC of *L. camara* and *W.somnifera* extract against *P. aeruginosa*

4.4 Evaluation of Non-Antibiotics activity

Loperamide HCl (with all concentrations) was exhibited distinct antibacterial activity against *E. coli*, but Paracetamol and Vitamin C did not show this antibacterial activity as shown in Table 4.8.

Paracetamol and Loperamide Hcl were showed antibacterial activity against *P. aeruginosa* as shown in Table 4.8. While paracetamol and Loperamide Hcl showed the best antibacterial activity against *S. aureus* at a concentration 100 µM and 10 µM respectively. Vitamin C did not show any antibacterial activity at any of the concentrations used against *S. aureus* and *P. aeruginosa* as shown in Table 4.8.

Table 4.8 Non-antibiotic activity assay

Non-Antib. / Microorg.	**Paracetamol**				**Loperamide Hcl**				**Vitamin C**			
	100 µM	**50 µM**	**10 µM**	**C***	**100 µM**	**50 µM**	**10 µM**	**C***	**100 µM**	**50 µM**	**10 µM**	**C****
Escherichia coli	**9**	**9**	**9**	**9**	**10**	**11**	**13**	**9**	**0**	**0**	**0**	**0**
Pseudomonas aeruginosa	**10**	**10**	**11**	**9**	**11**	**11**	**12**	**9**	**0**	**0**	**0**	**0**
Staphylococcus aureus	**10**	**8**	**11**	**9**	**12**	**8**	**10**	**9**	**0**	**0**	**0**	**0**

*** Control = methanol**

**** Control = Distilled water**

Figure 4.24: Diameter of inhibition zone (mm) of Vit.C on *P. aeruginosa*

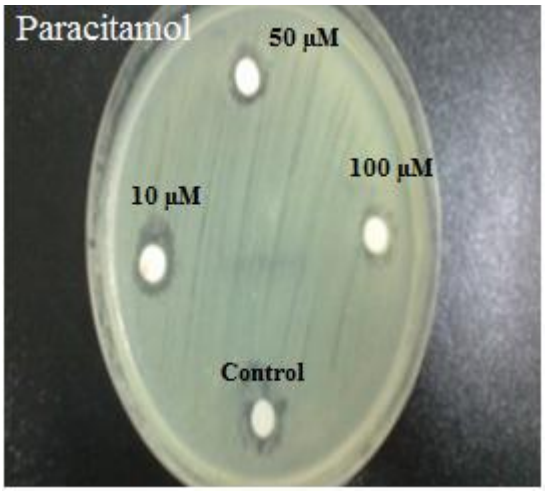


Figure 4.25: Diameter of inhibition zone (mm) of Paracitamol on *P. aeruginosa*

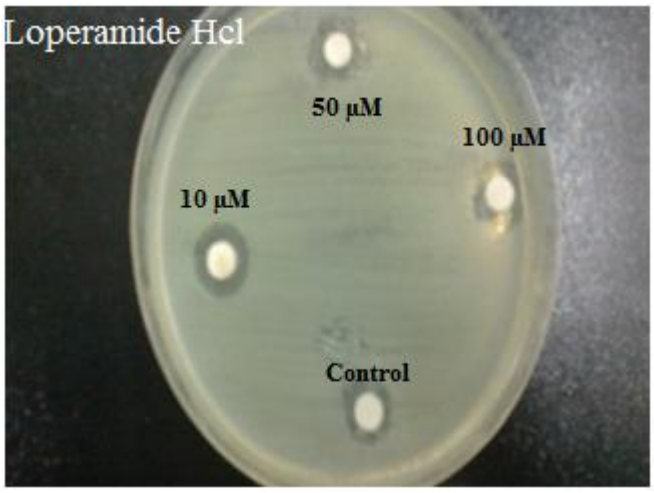


Figure 4.26: Diameter of inhibition zone (mm) Loperamide Hcl on *P. aeruginosa*

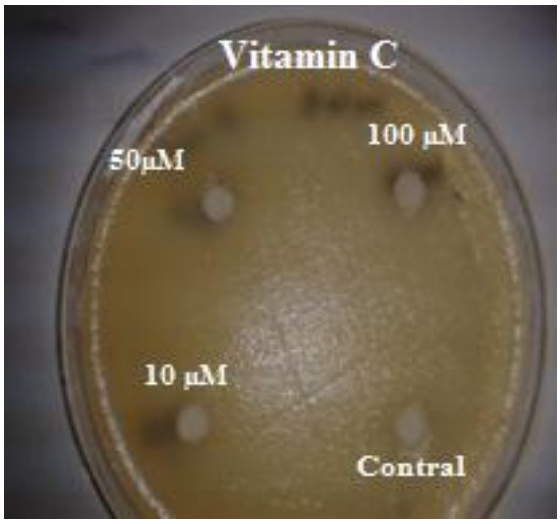


Figure 4.27: Diameter of inhibition zone (mm) to Vit.C on *E. coli*

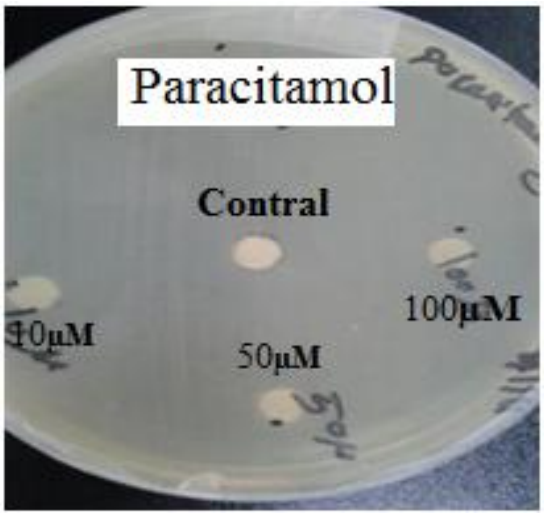


Figure 4.28: Diameter of inhibition zone (mm) to Paracitamol on *E. coli*

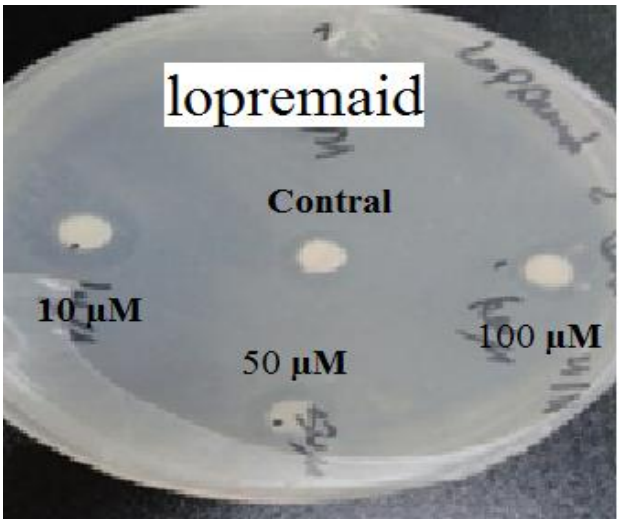


Figure 4.29: Diameter of inhibition zone (mm) of Loperamide Hcl on *E. coli*

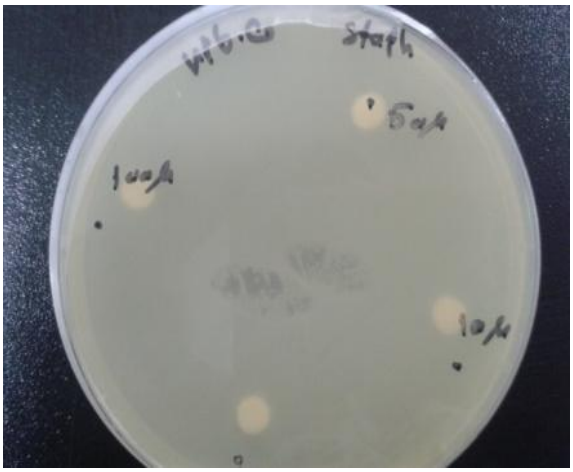


Figure 4.30: Diameter of inhibition zone (mm) of Vit.C on *S. aureus*

Figure 4.31: Diameter of inhibition zone (mm) to Paracitamol on *S. aureus*

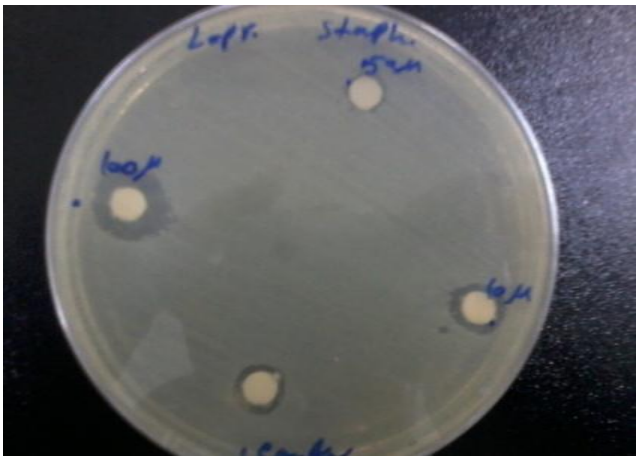


Figure 4.32: Diameter of inhibition zone (mm) of Loperamide Hcl on *S. aureus*

4.5 Evaluation the Synergistic Effect

4.5.1 The Synergistic Effect between Plant Extract and Antibiotics

We evaluated *in vitro* synergism between extracts of (i.e. *Nerium oleander, Artemisia herba-alba, Withania somnifera, Lantana camara, Ficus sycomorus* (leaves and bark), *Allium sativum and Eucalyptus camaldulensis*) and antimicrobial drugs utilized against *S. aureus*, *E.coli* and *P. aeruginosa* using disk diffusion method as mentioned in (section 3.2.7.1).

4.5.1.1 Against *Escherichia coli*

4.5.1.1.1 Methanolic Extraction and Antibiotics

As shown **in Table 4.9**. *N. oleander* extract has the best synergistic effect on *E. coli* when added on amikacin disk (19mm) followed by neomycin (17mm) and chloramphenicol (26mm). As for tetracycline and ampicillin, their influence (on *E. coli*) with the oleander extract indifference. With the rest antibiotics there was either no effect or there was antagonism.

The *A. herba-alba* extract showed the best synergism with amikacin (19mm) followed by ceftriaxone (13mm), and as in influence the oleander extract with Tetracycline and Ampicillin, It was their influence with *A. herba-alba* extract indifference. The cefotaxime, ceftazidime, cefazolin and co- trimoxazole have antagonism effect.

The best synergy of *W. somnifera* extracts with amikacin and then ceftriaxone and neomycin. *L. camara* extract, has the best synergistic effect with tetracycline (8mm) while there was no effect of the extract alone or with tetracycline. Also as in previous extracts *L. camara* leaf extracts had synergistic effect with amikacin (18mm).

A. sativum had a synergistic effect with many tested antibiotics. The highest synergistic effect to this extract was with amikacin, ofloxacin, gentamicin and tetracycline (20mm, 9mm and 8mm, Respectively). The rest of the antibiotics have shown a synergistic effect to varying degrees; while Ceflexin showed antagonism effect.

Finally the synergistic effect of *E. camaldulensis* extract with amikacin (19mm) was the highest synergistic effect on this bacteria followed by chloramphenicol (28mm). It had the same synergistic effect with both of tetracycline and nalidixic acid (9mm) against *E. coli*. With regard to ofloxacin, ceftriaxone and gentamicin there was no any synergistic effect with *E. camaldulensis* extract. On the other hand, there was antagonism with ceftazidime, neomycin, cefazolin and ceflexin.

Table 4.9 Synergism Between Antibiotics and Methanolic Extracts of Plant against *E.coli*

Antib.	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
CTX	8		-		8		10		9		12		14		8		10
OF	0		-		8		-		-		20		18		9		7
CTR	9		-		13		16		14		-		-		9		9
CTZ	11		-		-		8		7		7		-		7		7
AK	10		19		18		18		18		19		20		20		19
GN	6		-		9		9		9		-		-		13		7
TE	0		7		9		-		8		-		-		8		9
AMP	0	7	7	9	9	7	-	0	-	0	-	9	-	0	7	7	8
CL	24		26		25		23		26		28		28		26		28
N	14		17		15		19		17		15		15		18		13
N.A	0		-		7		-		7		-		-		7		9
KZ	0		-		-		-		-		-		-		7		-
CN	7		7		7		-		-		-		-		-		-
STX	0		-		-		-		7		-		-		7		8

(-) No synergism; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime**;** AK: Amikacin; GN: Gentamicin; TE: Tetracycline; AMP: Ampicillin; CL: Chloramphenicol; N: Neomycin; N.A: Nalidixic acid; STX: Co- trimoxazole; KZ: Cefazolin; CN: Ceflexin (Cephalexin).

4.5.1.1.2 Ethanolic Extraction and Antibiotics

In **Table 4.10**. Oleander extract had the highest synergistic effect with amikacin (19 mm) followed by ceftriaxone (14mm). ampicillin, ofloxacin, nalidixic acid and cefazolin had weak or negligible synergistic effect (8, 7, 7 and 7mm, Respectively) and tetracycline with ethanolic extract of oleander was indifference. And in *A. herba-alba* extract. Showed it with both of amikacin and ceftriaxone has the best synergy (17 and 16mm, Respectively). And each of ampicillin, ceftazidime, Cefotaxime and nalidixic acid are shown with it the same effect on *E. coli* (Inhibition zone 9mm). As for effect each of tetracycline, cefazolin and co- trimoxazole were indifference. The reason is that effect of ethanolic extract of *A. herba-alba* alone is (7mm). And these antibiotics have no effect on *E. coli*, whether alone or with this extract.

And also showed table 4.10 that highest effect of synergic for *W. somnifera* are with amikacin and ceftriaxone (19 and 17 mm, Respectively).

And also amikacin and ceftriaxone with *L. camara* they showed the highest synergistic effect (18 and 14mm, Respectively). As for each of cefotaxime, ofloxacin, ceftazidime, ampicillin, cefazolin and cephalexin they showed with *L. camara* of the antagonist effect.

As for the ethanolic extract each of *A. sativum* and *E. camaldulensis* have had the highest synergistic effect with amikacin (20 and 21mm, Respectively). And their influence with ceftriaxone, ceftazidime, cefazolin and cephalexin are antagonistic.

Table 4.10 Synergism Between Antibiotics and Ethanolic Extracts of Plant against *E.coli*

Antib.	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
CTX	8		10		9		8		-		15		13		10		10
OF	0		7		8		-		-		20		18		7		8
CTR	9		14		16		17		14		15		-		7		7
CTZ	11		10		9		9		-		9		7		7		7
AK	10		19		17		19		18		19		20		20		21
GN	6		8		8		8		9		-		-		12		9
TE	0		6		7		7		8		-		-		8		9
AMP	0	6	8	7	9	7	8	0	-	8	-	8	-	0	8	7	9
CL	24		26		25		26		23		29		29		26		28
N	14		16		15		19		19		14		13		18		14
N.A	0		7		9		8		9		-		-		7		7
KZ	0		7		7		7		-		-		-		-		-
CN	7		8		10		-		-		7		-		-		-
STX	0		8		7		8		8		-		-		-		8

(-) No synergism; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime**;** AK: Amikacin; GN: Gentamicin; TE: Tetracycline; AMP: Ampicillin; CL: Chloramphenicol; N: Neomycin; N.A: Nalidixic acid; STX: Co- trimoxazole; KZ: Cefazolin; CN: Ceflexin (Cephalexin).

4.5.1.1.3 Aquatic Extraction and Antibiotics

The best synergistic effect was with cefotaxime with aqueous extracts of the bark of sycamore (inhibitory zone 14 mm). As well ofloxacin had better synergistic effect with sycamore leaf extract and then extract the bark extract the of sycamore (19 and 17 mm, respectively) and with the rest of the extracts, there was no any effect or influence of the antagonistic.

The ceftriaxone has increased effectiveness with presence a extract of the sycamore leaves (16 mm) and followed by *W. Somnnifera* (15 mm) and *L. camara* extract (14mm). And each of *N. oleander* and *A. herba-alba* was their influence with ceftriaxone are similar (13mm). But ceftriaxone with the extract each from the bark of sycamore, garlic and eucalyptus was their influence is antagonistic.

As for amikacin has increased its effectiveness with all extracts. But the best effect with both of the extract of leaves and bark of sycamore and with garlic extract (Inhibition zone for each them 18 mm).

As for gentamicin was his best effect with aqueous extract of garlic (10mm). While each of tetracycline and co- trimoxazole there has not been any synergistic effect to them except with *A. herba-alba* though its little effect (8mm).

As for ampicillin there has not been any effect but only with both extract of *N. oleander*, *A. herba-alba* and *A. sativum*. It was their best with extract of the *A. herba-alba*.

While chloramphenicol has had a synergistic effect with all extracts except extract *L. camara* (22mm), leaves extract of *F. sycomorus* (24mm), *A. sativum* (24mm) and also *E. camaldulensis*. It was his best effect with extract of the *N. oleander* (28mm). And while neomycin has a synergistic effect with all extracts except extract of the *E. camaldulensis* (13mm).

Finally, there was no significant effect for each of cephalexin, nalidixic acid and cefazolin with all extracts.

Table 4.11 Synergism Between Antibiotics and Aquatic Extracts of Plant against *E.coli*

Antib.	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
CTX	8		9		-		9		10		12		14		9		-
OF	0		-		-		-		-		19		17		-		-
CTR	9		13		13		15		14		16		-		-		-
CTZ	11		-		7		10		7		-		7		-		-
AK	10		17		17		17		15		18		18		18		17
GN	6		-		-		-		-		-		-		10		-
TE	0		-		8		-		-		-		-		-		-
AMP	0	6	7	7	9	7	-	0	-	8	-	8	-	0	8	7	-
CL	24		28		25		25		22		24		26		24		-
N	14		15		17		17		19		16		15		17		13
N.A	0		-		7		-		-		-		-		-		-
KZ	0		-		-		-		-		-		-		-		-
CN	7		7		-		-		-		-		-		-		-
STX	0		-		8		-		-		-		-		-		-

(-) No synergism; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime**;** AK: Amikacin; GN: Gentamicin; TE: Tetracycline; AMP: Ampicillin; CL: Chloramphenicol; N: Neomycin; N.A: Nalidixic acid; STX: Co- trimoxazole; KZ: Cefazolin; CN: Ceflexin (Cephalexin).

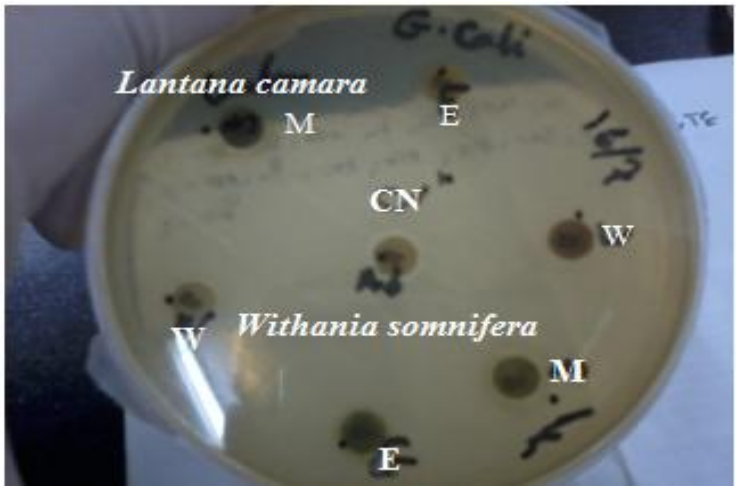


Figure (4.33): Effect of Cephalexin alone and in combination with *Withania somnifera* and *Lantana camara* on growth of *E.coli*

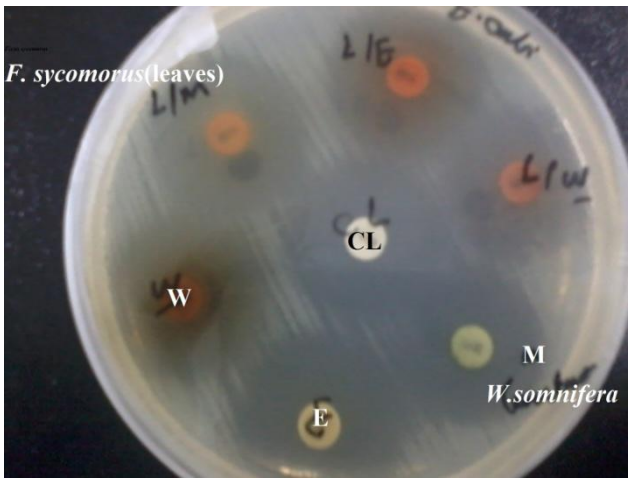


Figure (4.34): Effect of Chloramphenicol alone and in combination with *Ficus sycomorus*(leaves) and *Withania somnifera* on growth of *E.coli*

4.5.1.2 Against *Staphylococcus aureu*s

4.5.1.2.1 Methanolic Extraction and Antibiotics

Table 4.12. Showed that the oleander have increased the effectiveness of most antibiotics tested on *S. aureus*. The highest effect was with tetracycline (27mm). As for ceftriaxone, ceftazidime and co-trimoxazole was their influence with oleander are antagonism.

The *A. herba-alba* has had an effect with all antibiotics tested except co-trimoxazole has had an effect indifferent (10mm) and ceftriaxone was antagonism. The best synergistic effect it was with the tetracycline (30mm).

As for *W. somnifera* has had increased inhibition zone for all antibiotics tested. the highest of inhibition zone it was with ofloxacin, amikacin and neomycin (24, 24 and 23mm, respectively). But its effect with vancomycin and chloramphenicol are indifferent. And it's not an antagonist effect with any antibiotics.

And around extracted of the *L. camara*. Showed **Table 4.12** that extract has a synergistic effect with all antibiotics tested except rifampicin and co-trimoxazole were to have the effect of indifferent. The highest effect was with ofloxacin (25mm) and followed by tetracycline and chloramphenicol (24mm, To each one of them).

The extract from the leaves and bark of sycamore. **Table 4.12** showed that the highest synergistic effect was between sycamore leaf extract with ofloxacin (28 mm) and gentamicin with sycamore bark extract (28 mm). It is then followed by the sycamore leaf extract and tetracycline (inhibition zone 27 mm) and sycamore bark extract with cephalexin (inhibition zone 27 mm). The penicillin G has had an effect antagonism with sycamore leaf extract. But with the bark of sycamore has had a synergistic effect (Inhibition zone 17mm).

As for the garlic extract has increased inhibition zone for most of the antibiotics tested. It was the highest synergistic effect with ofloxacin (29 mm) and then tetracycline (27 mm).But both chloramphenicol and co-trimoxazole was their indifferent effect with this extract. And antagonistic effect with neomycin and ceftriaxone.

For eucalyptus leaf extract. Has had a synergistic effect with all antibiotics tested except gentamicin was its influence antagonistic and neomycin was indifferent. The highest synergistic effect was between it and each of ofloxacin, tetracycline and ceflexin

Table 4.12 Synergism Between Antibiotics and Methanolic Extracts of Plant against *S. aureus*

Antib.	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
VA	15		17		16		15		16		16		17		18		21
CTX	11		14		16		15		16		17		18		18		22
OF	20		20		23		24		25		28		22		29		26
CTR	12		8		9		16		14		15		16		11		15
CTZ	0		-		8		10		9		13		14		7		14
TE	21		27		30		22		24		27		25		27		26
AK	20		24		24		24		22		22		24		25		25
CL	21		22		22		21		24		22		22		21		25
RE	19	7	21	8	22	7	20	14	19	11	18	15	20	7	21	11	23
GN	21		24		23		22		23		24		28		22		19
AMP	0		11		12		10		8		12		14		11		13
ER	17		20		18		21		22		16		17		21		19
N	20		23		21		23		23		18		20		18		20
STX	10		9		10		11		10		14		18		10		14
P	0		9		10		11		8		-		17		10		17
KZ	0		11		12		12		10		14		16		10		16
CN	10		15		17		15		14		22		27		15		26

(-) No synergism; VA: Vancomycin; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime TE: Tetracycline; AK: Amikacin; CL: Chloramphenicol, ER: Erythromycin; GN: Gentamicin; AMP: Ampicillin; RF: Rifampicin; N: Neomycin; STX: Co-trimoxazole; P: Pencillin G; KZ: Cefazolin; CN: Ceflexin.

4.5.1.2.2 Ethanolic Extraction and Antibiotics

In Table 4.13. Showed vancomycin highest synergistic effect has on *S. aureus* (By increase in the zone of inhibition) after 18 hours of adding 20 µ from the leaf extract of the *E. camaldulensis* (19 mm) and then followed with extract each of garlic and leaves Sycamore (18 mm for each them). But with the rest of the extracts was its effect on the bacteria among the indifferent effect when added each of sycamore bark extract, *W. somnifera* and *N. oleander*. And antagonistic effect only when added *L. camara* on the disk. And also each of pencillin and ceflexin were the highest effect for them on these bacteria by adding of eucalyptus leaf extract on the disk saturated of the antibiotics.

For each of the cefotaxime, ceftriaxone, ceftazidime, ampicillin, co-trimoxazole and cefazolin was the highest synergistic effect to them when added sycamore bark extract on each disc of these antibiotics (20, 19, 16, 16, 17 and 17mm respectively). But ceftriaxone has shown the effect of antagonistic after 18 hours of add each of extract (both separately) *N. oleander* and *A. herba-alba*. While co-trimoxazole also has antagonistic effect with oleander leaf extract.

The each of ofloxacin, tetracycline, amikacin and rifampicin it has been the highest synergistic effect to them with garlic extract (29, 30, 27 and 24 mm, Respectively).

As for chloramphenicol it has been the highest effect on bacteria when add oleander leaf extract and also when add sycamore leaf extract (24 mm, For each them) on the disk. And it was not has antagonistic influence with any of the other extracts.

The erythromycin. it has been the highest effect on bacteria with each of garlic extract and *W. Somnnifera* (separately) (23 mm for each them). And also there was no any hostile influence with any of the other extracts.

As for neomycin was the best effect on bacteria with the presence of the extract *A. herba-alba* (23 mm).

Table 4.13 Synergism Between Antibiotics and Ethanolic Extracts of Plant against *S. aureus*

Antibiotics	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
VA	15		15		17		15		14		18		15		18		19
CTX	11		15		12		15		15		15		20		18		17
OF	20		23		21		22		24		25		19		29		24
CTR	12		9		11		15		17		13		19		8		16
CTZ	0		7		9		11		12		14		16		7		13
TE	21		27		27		25		25		29		25		30		24
AK	20		25		24		22		24		24		24		27		24
CL	21		24		23		23		21		24		21		23		23
RE	19	6	20	9	19	8	22	10	21	12	21	15	18	7	24	13	23
GN	21		22		23		24		22		28		25		24		21
AMP	0		9		11		11		11		12		16		9		14
ER	17		18		18		23		22		17		15		23		21
N	20		22		23		21		22		19		20		21		20
STX	10		9		11		13		10		12		17		12		13
P	0		9		11		13		11		11		13		10		15
KZ	0		8		10		12		8		15		17		9		13
CN	10		12		22		25		15		23		24		12		26

(-) No synergism; VA: Vancomycin; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime TE: Tetracycline; AK: Amikacin; CL: Chloramphenicol, ER: Erythromycin; GN: Gentamicin; AMP: Ampicillin; RF: Rifampicin; N: Neomycin; STX: Co-trimoxazole; P: Pencillin G; KZ: Cefazolin; CN: Ceflexin.

4.5.1.2.3 Aquatic Extraction and Antibiotics

Table 4.14. Shows synergistic effect between the aqueous extract and antibiotics tested.
Although the effect aqueous extract of these plants alone on the bacteria was little. However, has led to increased the impact of antibiotics on the bacteria when it is added on them .

Where the extract of oleander increased the effectiveness each of tetracycline and amikacin and ampicillin against *S. aureus* (26, 25 and 8 mm, Respectively). As for each of cefotaxime, cefotaxime, ofloxacin, gentamicin, erythromycin, rifampicin, neomycin and chloramphenicol have been them little effect on the bacteria. While has influence antagonistic with each of the vancomycin, ceftriaxone, ceftazidime, cefazolin and ceflexin. And indifferent effect when it added on the disk to each of co-trimoxazole and erythromycin.

As for the *A. herba-alba* extract was the results (when added to antibiotics) almost similar to oleander extract, where it also has increased the impact of each of tetracycline, amikacin and ampicillin as well as gentmisin on the bacteria (26, 25 and 8, 24 mm, Respectively).

The extract *W. Somnnifera* has increased the effectiveness of most antibiotics on bacteria. And has been the best with Tetracycline (24 mm), followed by each of ofloxacin and amikacin (23 mm for each them).And has had an effect antagonistic with both vancomycin and gentamicin.

As for the *L. camara* extract it has been antagonistic effect with the majority of antibiotics tested. And although it has had a synergistic effect with each of tetracycline and erythromycin (23 and 21 mm).

And association of antibiotics and leaves extract of *F. sycomorus* showed synergistic antibacterial activity especially with tetracycline, ofloxacin and amikacin on *S. aureus* (26, 25 and 24mm,Respectively). but its showed antagonistic activity with ceftriaxone, gentamicin and neomycin.

As for bark extract of *F. sycomorus* synergistic activity it was with amikacin *against S. aureus* (26 mm). And followed by each of tetracycline, gentamicin and ceflexin (inhibition zone: 24mm, for each them). And its showed antagonistic activity with vancomycin, co-trimoxazole, pencillin G and cefazolin.

But garlic extract showed synergistic activity with most antibiotics tested especially with ofloxacin and tetracycline on *S. aureus* (28 and 27 mm, Respectively), However with ceftazidime, neomycin, ceftriaxone and ampicillin its showed antagonistic activity on the bacteria.

Finally; showed *E. camaldulensis* extract highest synergistic activity with amikacin and Cephalexin on S. aureus. and antagonistic activity with ceftriaxone, neomycin, gentamicin, pencillin G and tetracycline. And had its activity with ofloxacin and chloramphenicol are indifferent.

Table 4.14 Synergistic effects of Antibacterial drugs with aqueous plant extracts on *S. aureus*

Antibiotics	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
VA	15		13		13		10		11		15		14		16		16
CTX	11		12		15		14		13		15		19		15		10
OF	20		22		21		23		20		25		20		28		20
CTR	12		8		8		12		13		11		14		7		13
CTZ	0		-		-		7		7		13		14		-		13
TE	21		26		26		24		23		26		24		27		12
AK	20		25		25		23		21		24		26		25		24
CL	21		22		21		21		21		22		21		24		21
RE	19	-	20	-	18	-	20	8	20	15	19	12	19	-	21	8	21
GN	21		22		24		19		19		20		24		24		15
AMP	0		8		8		7		-		10		7		-		11
ER	17		17		18		21		21		17		18		20		20
N	20		22		22		20		19		18		16		18		19
STX	10		10		8		-		7		16		9		16		11
P	0		7		-		7		-		13		-		9		-
KZ	0		-		8		8		-		10		-		-		7
CN	10		9		11		12		10		15		24		12		24

(-) No synergism; VA: Vancomycin; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime TE: Tetracycline; AK: Amikacin; CL: Chloramphenicol, ER: Erythromycin; GN: Gentamicin; AMP: Ampicillin; RF: Rifampicin; N: Neomycin; SXT: Co-trimoxazole; P: Pencillin G; KZ: Cefazolin; CN: Ceflexin (Cephalexin).

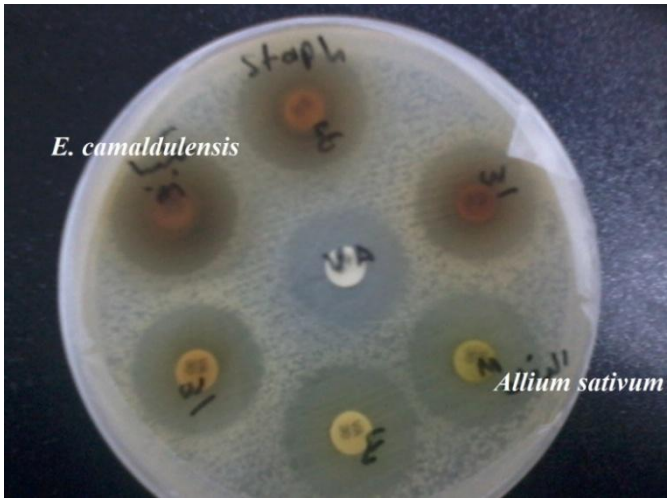


Figure (4.35): Effect of Vancomycin alone and in combination with *Eucalyptus camaldulensis* and *Allium sativum* on *S. aureu*s

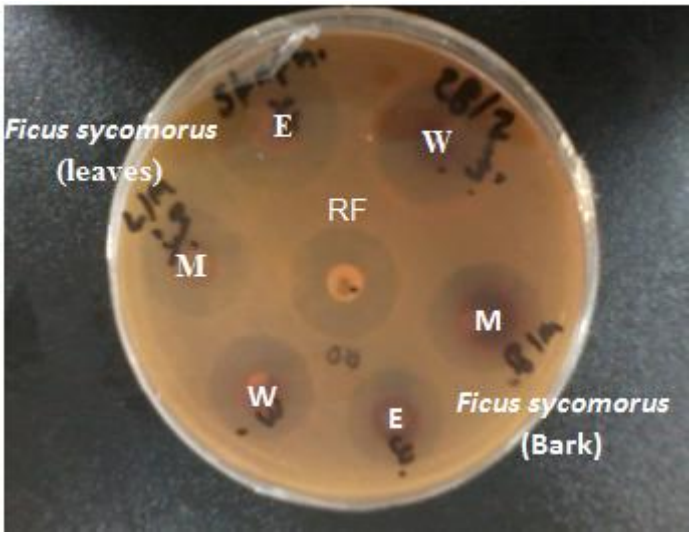


Figure (4.36): Effect of Rifampicin alone and in combination with *Ficus sycomorus* (Leaves and Bark) on *S. aureu*s

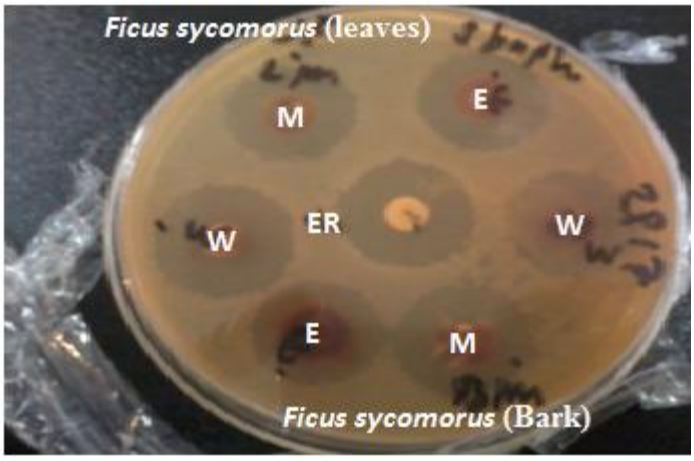


Figure (4.37): Effect of Erythromycin alone and in combination with *Ficus sycomorus* (Leaves and Bark) on *S. aureu*s

4.5.1.3 Against *Pseudomonas aeruginosa*

4.5.1.3.1 Methanolic Extraction and Antibiotics

Table 4.15. Shows synergistic effect between the methanol extract and antibiotics tested.

The association between antibiotics and leaves extracts of *N. oleander* and *A. herba-alba* showed synergistic antibacterial activity with all antibiotics tested on *P. aeruginosa*, except ceftriaxone and cefotaxime were showed antagonistic activity with them.

But for the extract of *W. somnifera* has shown synergistic activity with ofloxacin**,** amikacin**,** neomycin and gentamicin on *P. aeruginosa*. And its showed antagonistic activity with neomycin, ceftriaxone and cefotaxime. And indifferent activity with ceftazidime.

And also association of antibiotics and leaves extract of *L. camara* showed synergistic antibacterial activity with the most antibiotic tested especially neomycin and amikacin(inhibition zone: 20 and 23 mm, Respectively). But antagonistic activity with ceftriaxone, cefotaxime and cefalexin.

The activity to each of amikacin, ceftazidime, gentamicin and neomycin was increased after mixing with the leaves extract of *F. sycomorus* (20, 14, 11 and 10mm, Respectivly). But the bark extract of *F. sycomorus* was increased activity of amikacin and ceftazidime (20 and 13 mm, Respectivly).

The activity of amikacin, ofloxacin and neomycin was increased after mixing with the extract of *A. sativum*. But showed antagonistic activity when added extract of *A. sativum* on each of cephalexin, gentamicin, ceftriaxone and cefotaxime. And indifferent activity with ceftazidime.

But *E. camaldulensis* was showed synergistic activity with all antibiotics tested, Except ceftriaxone showed antagonistic activity with it.

Table 4.15 Synergistic effects of Antibacterial drugs with methanolic plant extracts on *P. aeruginosa*

Antibiotics	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex*	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
CTX	0		-		-		-		-		-		-		-		13
OF	0		11		10		10		10		-		-		11		10
CTR	0		-		-		-		-		-		-		-		-
CTZ	9	-	11	-	13	-	10	-	12	-	14	7	13	-	10	10	12
AK	17		26		25		25		23		20		20		22		22
GN	8		10		12		13		10		11		-		-		15
N	0		8		8		20		20		10		-		11		10
CN	0		9		10		-		-		-		-		-		8

* Ex= Extract alone.
Ex+Anti= Extract with antibiotic

(-) No synergism; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime; AK: Amikacin; GN: Gentamicin; N: Neomycin; CN: Ceflexin (Cephalexin).

4.5.1.3.2 Ethanolic Extraction and Antibiotics

Synergistic activity of ethanolic extract of plants with different antibiotics against bacteria is shown in **Table 4.16**.

The synergistic effect was found against *P. aeruginosa* for all antibiotics tested, when Ethanolic extract of *N. oleander* was combined with this antibiotics, Except cefotaxime showed antagonism activity. And the of the extract of *A. herba-alba* was showed synergistic effect with all antibiotics tested.

As for the extract of *W. somnifera* was showed synergistic activity with almost all the antibiotics tested, Except ceftriaxone and cephalexin was antagonistic activity with it.

And also the extract of *L. camara* was showed synergistic activity with most the antibiotics tested, Except cefotaxime and ceftriaxone were antagonistic activity with it.

And when leaves extract of *F. sycomorus* was combined with amikacin, ceftazidime and neomycin (18, 14 and 8 mm, respectively) the synergistic effect was showed against *P. aeruginosa*. But with the rest of antibiotics was showed antagonistic activity.

But bark extract of *F. sycomorus* was showed antagonistic activity with most the antibiotics tested . And only amikacin and ceftazidime were showed synergistic activity when combined with the extract.

The extract of Garlic was showed synergistic effect when combined with amikacin, ceftazidime, ofloxacin and neomycin (21, 13, 12 and 10 mm, respectively). But with the rest antibiotics was showed antagonistic activity.

And the extract of *E. camaldulensis* was showed synergistic activity with all antibiotics tested, Except ceftriaxone and gentamicin showed antagonistic activity with it.

Table 4.16 Synergistic effects of Antibacterial drugs with Ethanolic plant extracts on *P. aeruginosa*

Antibiotics	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex.*	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
CTX	0		-		8		7		-		-		-		-		12
OF	0		9		13		11		15		-		-		12		10
CTR	0		7		7		-		-		-		-		-		-
CTZ	9	-	13	8	13	-	14	-	13	7	14	10	14	-	13	8	13
AK	17		25		25		27		21		18		20		21		23
GN	8		11		10		10		11		-		-		9		9
N	0		9		9		19		22		8		-		10		10
CN	0		8		13		-		12		-		-		-		10

* Ex= Extract alone.
Ex+Anti= Extract with antibiotic

(-) No synergism; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime; AK: Amikacin; GN: Gentamicin; N: Neomycin; CN: Ceflexin (Cephalexin).

4.5.1.3.3 Aquatic Extraction and Antibiotics

The results of the synergistic activity to aquatic extracts with antibiotics determined by diameters of inhibition zones are presented in **Table 4.17**.

The extract of

The synergistic effect was found against *P. aeruginosa*, when Aquatic extract of *N. oleander* was combined with amikacin, ceftazidime and gentamicin. Similar synergistic effect of Aquatic extract of *A. herba-alba*. And they showed antagonistic effect with the rest of antibiotics.

As for *W. somnifera* and *L. camara* were showed synergistic activity when they were added on amikacin, ofloxacin and neomycin. Antagonistic effect was observed when extract *L. camara* was combined with ceftazidime.

And extract of *F. sycomorus* (leaves) was showed synergistic activity when was combined with amikacin and ceftazidime (inhibition zone =20 and13mm, Respectively). But extract of *F. sycomorus* (Bark) was showed synergistic activity with amikacin, ceftazidime, ofloxacin and neomycin (19,14, 9 and 8mm, Respectively). And its showed antagonistic effect with the rest of antibiotics.

And also association of antibiotics and leaves extract of *A. sativum* showed synergistic antibacterial activity especially amikacin, ceftazidime, ofloxacin and neomycin.

But extract of *E. camaldulensis* was showed synergistic activity with most antibiotics tested; Except ceftriaxone and gentamicin was showed antagonistic activity

Table 4.17 Synergistic effects of Antibacterial drugs with Aquatic plant extracts on *P. aeruginosa*

Antibiotics	Antibiotics alone	*Nerium oleander*		*Artemisia herba-alba*		*Withania somnifera*		*Lantana camara*		*Ficus sycomorus* (leaves)		*Ficus sycomorus* (bark)		*Allium sativum*		*Eucalyptus camaldulensis*	
		Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.	Ex.	Ex+ Anti.
CTX	0		-		-		-		-		-		-		-		10
OF	0		-		-		11		9		-		9		11		12
CTR	0		-		-		-		-		-		-		-		-
CTZ	9		10		11		11		8		13		14		13		11
AK	17	-	23	7	24	-	19	-	22	-	20	10	19	-	20	8	20
GN	8		10		9		8		9		-		7		7		-
N	0		-		-		15		16		-		8		7		8
CN	0		-		-		-		-		-		-		-		7

* Ex= Extract alone.
Ex+Anti= Extract with antibiotic

(-) No synergism; CTX: Cefotaxime; OF: Ofloxacin; CTR: Ceftriaxone; CTZ: Ceftazidime; AK: Amikacin; GN: Gentamicin; N: Neomycin; CN: Ceflexin (Cephalexin).

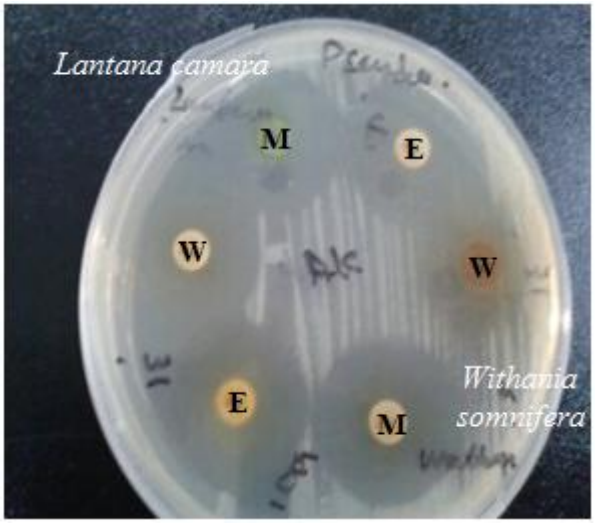


Figure (4.38): The combination effect of *Lantana camara* and *Withania somnifera* with Amikacin on *P. aeruginosa*

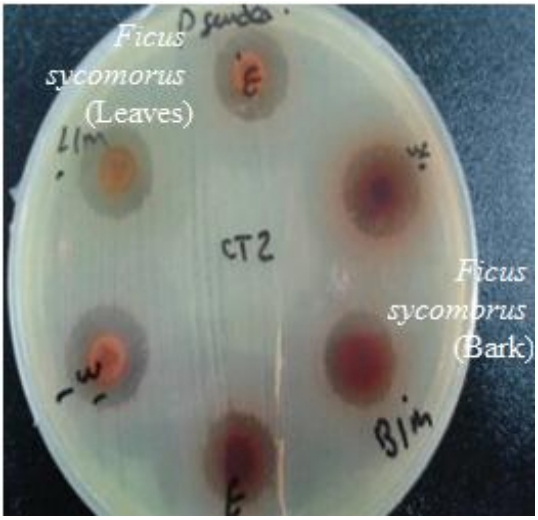


Figure (4.39): The combination effect of *Ficus sycomorus* (Leaves and Bark) with Ceftazidime on *P. aeruginosa*

Figure (4.40): The combination effect of *Nerium oleander* and *Artemisia herba-alba* with Gentamicin on *P. aeruginosa*

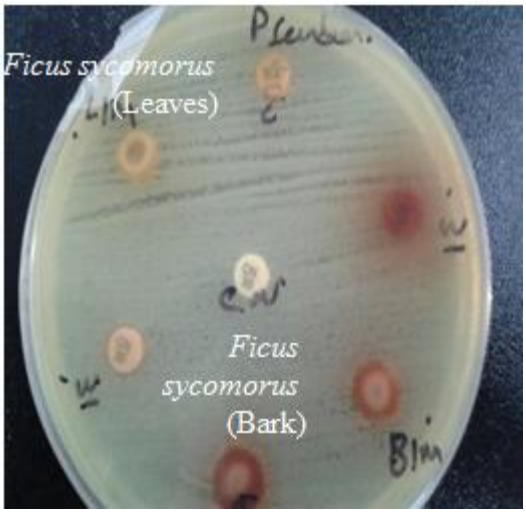


Figure (4.41): The combination effect of *Ficus sycomorus* (Leaves and Bark) with Cephalexin on *P. aeruginosa*

4.5.2 The Synergistic Effect between Plant Extract and Non-Antibiotic drugs

4.5.2.1 Against *Staphylococcus aureu*s

In methanolic extraction, *N. oleander* showed synergyism with Paracetamol (at all concentrations) (Table 4.18&4.19) and with Loperamide Hcl at a concentration of 50 and 10 µM. *W. somnifera* showed synergyism with Paracetamol at a concentration of 50 µM, and with Loperamide Hcl at a concentration of 10 µM.

L. camara showed synergy with Paracetamol at a concentration of 50 and 10 µM, and with Loperamide Hcl at a concentration of 100 and 10 µM.

It was observed that there was antagonism by the combination of Paracetamol or Loperamide Hcl with *A. herba-alba, F. sycomorus* (Leaves and Bark), *A. sativum* or *E. camaldulensis*.

In ethanolic extraction, *L. camara* was showed synergy with Paracetamol and Loperamide Hcl at a concentration of 100, 10 µM, respectively (Table 18&19). While the *N. oleander* has shown synergy with paracetamol at a concentration of 10 µM, as well as with Loperamide Hcl at all concentrations. While the *N. oleander* has shown synergyism with paracetamol at a concentration of 10 µM, the synergyism with Loperamide Hcl observed at all concentrations. *A. herba-alba* was showed synergyism with Loperamide Hcl only at a concentration of 100 and 50 µM, and *A. sativum* showed synergyism with paracetamol at a concentration of 10 µM only.

The antagonistic and/ or indifferent effect was found in Paracetamol or Loperamide Hcl, with *F. sycomorus* (Leaves and Bark), *W. somnifera* and *E.camaldulensis*.

In water extraction, the combinations of Paracetamol (at a concentration of 100 µM) with each of *N. oleander* , *A. herba-alba, F. sycomorus* (Leaves and Bark) and *A. sativum* had a synergistic effect against *S. aureu*s, which was resistant to these extracts**.**

However, the concentration of 50 µM of Paracetamol, showed synergistic activity with *N. oleander* , *A. herba-alba* and *L. camara*. While the concentration of 10 µM of Paracetamol, showed synergistic activity with *A. herba-alba, L. camara* and *F. sycomorus* (Bark). *N. oleander* , *A. herba-alba* and *W. somnifera* with Loperamide Hcl were showed synergy against *S. aureus*, at all concentrations100 µM .

As for *L. camara* was showed synergistic activity with Loperamide Hcl at a concentration of 50 and 10 µM.

While each of *E.camaldulensis*, *A. sativum* and *F. sycomorus* (Leaves) were showed synergistic activity with Loperamide Hcl at a concentration of 50, 50 and 10 µM, respectively.

But when Vitamin C is combination with all extracts, the interaction observed was Antagonism. Except *W. somnifera* showed synergistic activity with vitamin C at a concentration of 100 µM.

Table 4.18and 4.19. Shows synergistic activity between plant extracts and non-antibiotics used.

Table 4.18 Synergistic activity between plant extracts and each of paracetamol and Loperamide Hcl against *S. aureus*

Non-Antib. / Antibiotics	Methanol extracts								Ethanol extracts								Water extracts							
			Paracetamol			Loperamide Hcl					Paracetamol			Loperamide Hcl					Paracetamol			Loperamide Hcl		
	*	**	100 µM	50 µM	10 µM	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	100µM	50 µM	10 µM
N. oleander	7	8	13	10	10	7	10	10	6	9	8	8	10	10	10	11	0	0	9	10	0	8	8	11
A. herba-alba	8	8	7	7	7	7	7	7	9	8	7	7	8	11	10	8	0	0	7	8	9	8	10	13
W. somnifera	7	8	8	9	7	8	8	10	8	10	9	9	9	10	7	9	0	10	7	8	8	12	8	0
L. camara	14	16	0	17	18	17	12	17	10	11	12	11	10	10	10	12	8	7	0	12	9	0	10	8
F. sycomorus (Leaves)	11	12	8	0	0	0	0	10	12	13	13	13	12	12	13	12	0	0	10	0	0	0	0	9
F. sycomorus (Bark)	15	16	9	10	9	8	8	8	15	17	12	9	10	10	10	9	0	0	8	0	9	0	0	0
A. sativum	7	0	0	9	8	9	8	7	7	9	0	0	10	8	7	7	0	0	8	0	0	0	12	0
E. camaldulensis	11	8	8	9	8	10	9	10	13	9	9	10	10	9	8	9	8	7	7	8	0	0	10	0

Table 4.19 Synergistic activity between plant extracts Vitamin C against *S. aureus*

Non-Antib. / Antibiotics	Vit. C+ Methanol ext.					Vit. C+ Ethanol ext.					Vit. C+ Water ext.				
	*	**	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM
N. oleander	7	0	0	0	0	6	0	0	0	0	-	0	0	0	0
A. herba-alba	8	0	0	0	0	9	0	0	0	0	-	0	0	0	0
W. somnifera	7	8	10	8	8	8	11	9	9	9	-	0	0	0	0
L. camara	14	7	9	9	7	10	8	10	0	7	8	0	0	0	0
F. sycomorus (Leaves)	11	9	0	0	0	12	9	9	8	0	-	0	0	0	0
F. sycomorus (Bark)	15	0	0	0	0	15	0	0	0	0	-	0	0	0	0
A. sativum	7	0	0	0	0	7	0	0	0	0	-	0	0	0	0
E. camaldulensis	11	7	9	0	9	13	12	10	9	10	8	0	0	0	0

* Extraction alone

** Control= Extraction + solvent

Figure (4.42): The effect of *W. somnifera* (water extract) with Paracetamol and Loperamide Hcl on *S. aureus*

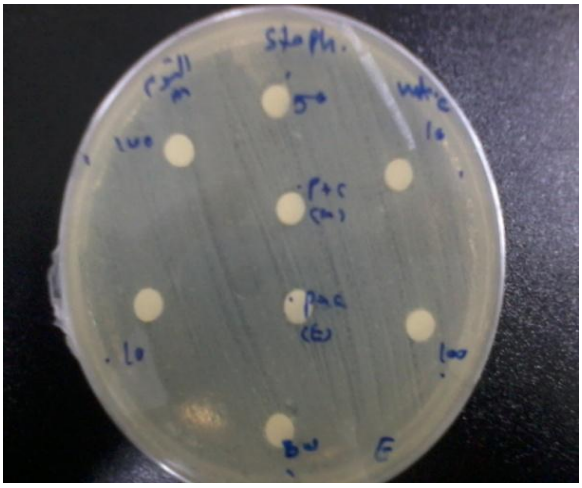


Figure (4.43): The effect of *A. sativum* (methanol extract) with Vitamin C on *S. aureus*

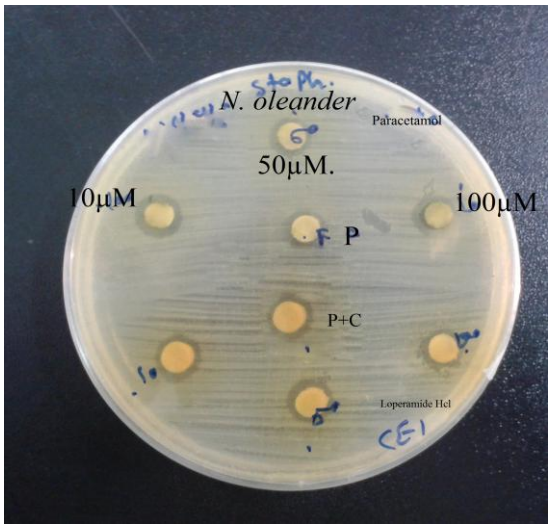


Figure (4.44): The effect of *N. oleander* (ethanol extract) with Paracetamol and Loperamide Hcl on *S. aureus*

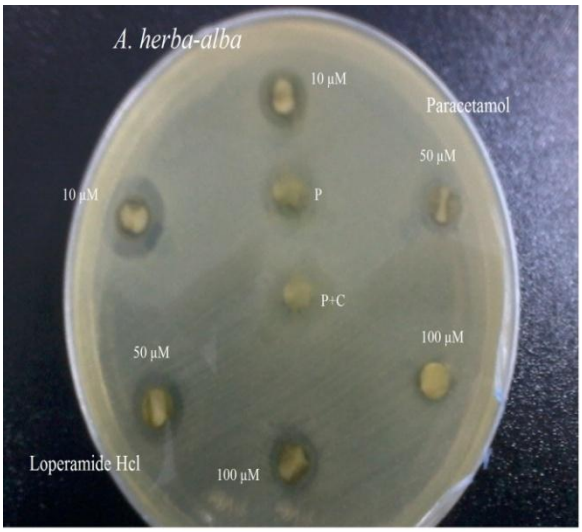


Figure (4.45): The effect of *A. herba-alba* (water extract) with Paracetamol and Loperamide Hcl on *S. aureus*

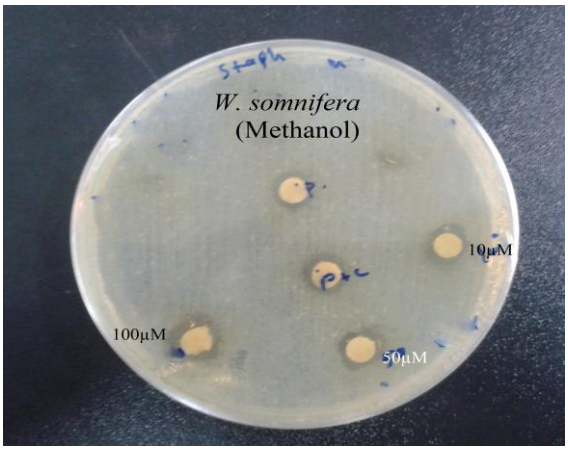


Figure (4.46): The effect of *W. somnifera* with Vitamin C on *S. aureus*

P = Plant
P+C= plant extract with control (solvent)

4.5.2.2 Against *Escherichia coli*

In methanolic extraction, The synergistic effect was found against *E. coli*, when methanolic extract of *N. oleander* was combined with Paracetamol at a concentration of 10 µM (inhibition zone=10mm). While *A. herba-alba* was showed synergistic effect with Paracetamol and Loperamide Hcl at a concentration of 50 and 10 µM, Respectively (inhibition zone=13 and 12mm, Respectively). And also *W. somnifera* was showed synergistic effect with Paracetamol (at concentration of 100 µM) and Loperamide Hcl (at concentration of 100 and 50 µM). Where the combination of *L.*

camara with each of Paracetamol (at a concentration of 10 µM) and Loperamide Hcl (at all concentrations) was showed synergistic activity against *E. coli*. As for *Ficus sycomorus* (leaves) was showed synergistic activity only with Loperamide Hcl (at a concentration of 10 µM). The associations of the extracts of *Ficus sycomorus* (bark), *A. sativum* and *E. camaldulensis* with each of Paracetamol and Loperamide Hcl were showed case of antagonism and/or indifference**.**

In methanolic extraction, The Combinations of Paracetamol (at a concentration of 100 µM) with each of *N. oleander*, *L. camara* and *E. camaldulensis* were showed synergistic effect against *E. coli*. While it's at a concentration of 50 and 10 µM was showed synergistic effect with *E. camaldulensis* and *A. sativum*, respectively. And the Combinations of Loperamide Hcl (at a concentration of 100 µM) with each of *A. herba-alba* and *W. somnifera* were showed synergistic effect against *E. coli*. But it at a concentration of 50 µM was showed antagonism with all extracts. While at a concentration of 10 µM of paracetamol has shown synergistic activity with each of *N. oleander* and *W. somnifera*.

In water extraction, Each of *A. herba-alba*, *L. camara* and *E. camaldulensis* were showed synergy with Paracetamol (at a concentration 100 µM). While *L. camara* only was showed synergy with Paracetamol (at a concentration 50 µM). But at a concentration of 10 µM, was observed synergistic activity between each of *A. herba-alba*, *L. camara* and *F. sycomorus* (Leaves and Bark). The Combinations of Loperamide Hcl (at a concentration of 100 µM) with each of *L. camara* and *F. sycomorus* (Leaves) were showed synergistic effect against *E. coli*. While it's at a concentration of 50 and 10 µM was showed synergistic effect with *L. camara* only.

But when Vitamin C is combination with all extracts, the interaction observed was synergism, especially with methanolic extract for each of *N. oleander, W. somnifera*, *L. camara* and *E. camaldulensis* (at a concentration 100 µM). While at a concentration of 50 and 10 µM, was observed synergy between Vitamin C and methanol extract *E. camaldulensis* only. And the combination of Vit. C with *A. herba-alba* and *L. camara* (at a concentration 100 µM) was showed synergistic effect. While it's at a concentration of 50 and 10 µM was showed synergistic effect with *L. camara* only.

Table 4.20 and 4.21. Shows synergistic activity between plant extracts and non-antibiotics used against *E. coli*.

Table 4.20 Synergistic activity between plant extracts and each of paracetamol and Loperamide Hcl against *E. coli*

Non-Antib. / Antibiotics	Methanol extracts								Ethanol extracts								Water extracts							
			Paracetamol			Loperamide Hcl					Paracetamol			Loperamide Hcl					Paracetamol			Loperamide Hcl		
	*	**	100 µM	50 µM	10 µM	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	100µM	50 µM	10 µM
N. oleander	7	0	0	0	10	0	0	0	7	0	8	0	0	7	7	8	0	0	0	0	0	0	0	0
A. herba-alba	9	10	0	13	9	0	0	12	7	9	9	0	8	11	8	9	6	10	12	9	12	8	8	9
W. somnifera	7	8	9	8	0	9	10	8	8	9	9	8	7	12	9	10	0	7	0	0	0	0	0	0
L. camara	0	8	8	0	9	10	10	9	0	7	12	8	0	0	0	9	0	7	13	13	11	10	9	12
F. sycomorus (Leaves)	0	7	7	0	7	8	7	7	8	0	7	8	7	8	7	8	0	7	0	0	8	9	7	0
F. sycomorus (Bark)	9	0	0	0	0	0	0	0	8	7	0	0	0	7	0	0	0	0	0	0	10	0	9	0
A. sativum	0	8	0	0	0	0	0	7	0	7	0	0	9	7	0	0	0	0	0	0	0	0	0	0
E. camaldulensis	7	8	8	8	7	7	7	8	8	9	10	10	9	9	9	9	0	8	9	8	0	9	7	8

Table 4.21 Synergistic activity between plant extracts Vitamin C against *E. coli*

Non-Antib. / Antibiotics	Vit. C+ Methanol ext.					Vit. C+ Ethanol ext.					Vit. C+ Water ext.				
	*	**	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM
N. oleander	7	0	8	0	0	7	0	0	0	0	0	0	0	0	0
A. herba-alba	9	0	8	0	0	7	0	9	9	8	6	0	0	0	0
W. somnifera	7	0	9	0	0	8	0	0	0	0	0	0	0	0	0
L. camara	0	0	9	0	0	0	0	9	0	0	0	0	9	9	8
F. sycomorus (Leaves)	0	0	0	0	0	8	0	8	8	0	0	0	0	0	0
F. sycomorus (Bark)	9	0	0	0	0	8	0	0	0	0	0	0	0	0	0
A. sativum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. camaldulensis	7	7	9	8	9	8	8	8	8	8	0	0	0	0	0

* Extraction alone
** Control= Extraction + solvent

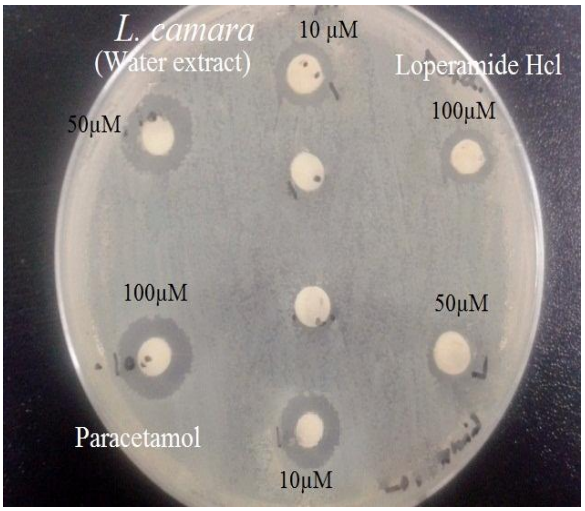


Figure (4.47): The effect of *L. camara* (water extract) with Paracetamol and Loperamide Hcl on *E. coli*

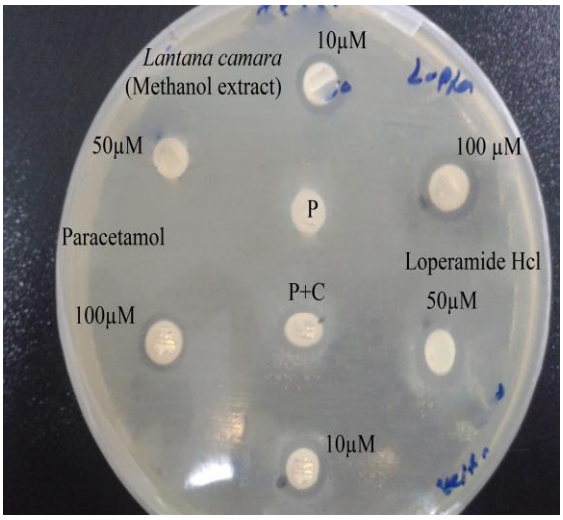


Figure (4.48): The effect of *L. camara* (methanol extract) with Paracetamol and Loperamide Hcl on *E. coli*

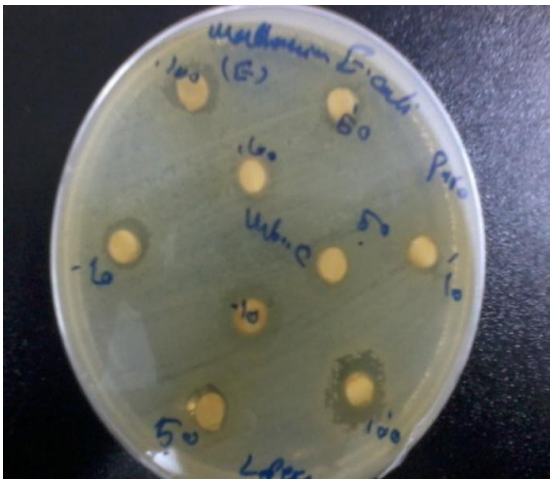


Figure (4.49): The effect of *W. somnifera* (ethanol extract) with Paracetamol, Loperamide Hcl and Vitamin C on *E. coli*

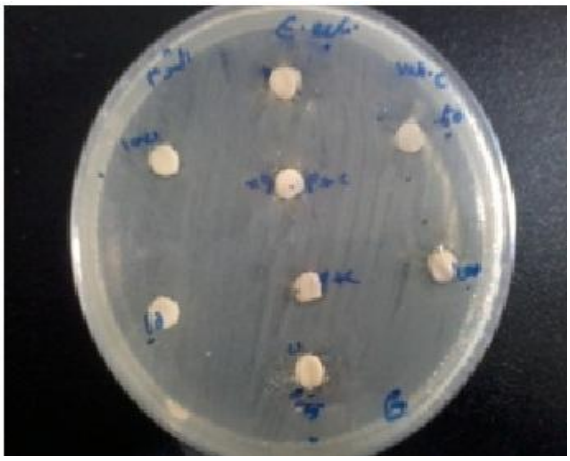


Figure (4.50): The effect of *A. sativum* (ethanol extract) with Vitamin C on *E. coli*

P = Plant
P+C= plant extract with control (solvent)

4.5.2.3 *Pseudomonas aeruginosa*

The Combinations of Non- antibiotics with all plant extracts was showed antagonism effect against *P. aeruginosa*. Except ethanol extract of *N. oleander* with Paracetamol (at a concentration 50 and 10 µM), In addition to methanol extract of *A. herba-alba* with Loperamide Hcl (at a concentration 10 µM) were showed synergistic effect against *P. aeruginosa*.

Table 4.22 Synergistic activity between plant extracts and each of paracetamol and Loperamide Hcl against *P. aeruginosa*

Microorganism / Non-Antib. / Antibiotics	Methanol extracts								Ethanol extracts								Water extracts							
			Paracetamol			Loperamide Hcl					Paracetamol			Loperamide Hcl					Paracetamol			Loperamide Hcl		
	*	**	100 µM	50 µM.	10 µM	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	100µM	50 µM	10 µM
N. oleander	0	0	0	0	0	0	0	0	0	8	0	10	9	8	8	0	0	0	0	0	0	0	0	0
A. herba-alba	0	0	0	0	7	0	0	10	8	9	7	7	8	7	7	7	7	10	0	8	8	8	10	7
W. somnifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L. camara	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. sycomorus (Leaves)	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. sycomorus (Bark)	10	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. sativum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. camaldulensis	10	9	10	8	8	0	9	10	8	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0

* Extraction alone
** Control= Extraction + solvent

Table 4.23 Synergistic activity between plant extracts Vitamin C against *P. aeruginosa*

Non-Antib. / Antibiotics	Vit. C+ Methanol ext.					Vit. C+ Ethanol ext.					Vit. C+ Water ext.				
	*	**	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM
N. oleander	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. herba-alba	0	0	0	0	0	8	0	0	0	0	7	0	0	0	0
W. somnifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L. camara	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F. sycomorus (Leaves)	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0
F. sycomorus (Bark)	10	0	0	0	0	10	0	0	0	0	0	0	0	0	0
A. sativum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. camaldulensis	10	0	0	0	0	8	0	0	0	0	8	0	0	0	0

* Extraction alone
** Control= Extraction + solvent

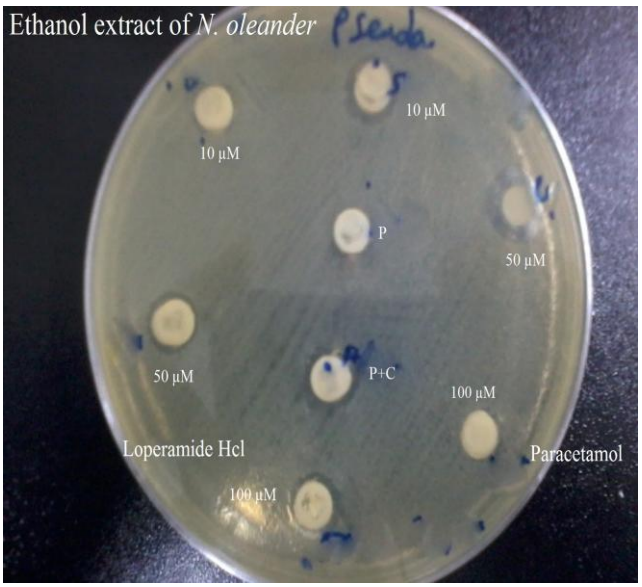


Figure (4.51): The effect of *N. oleander* (ethanol extract) with Paracetamol and Loperamide Hcl on *P. aeruginosa*

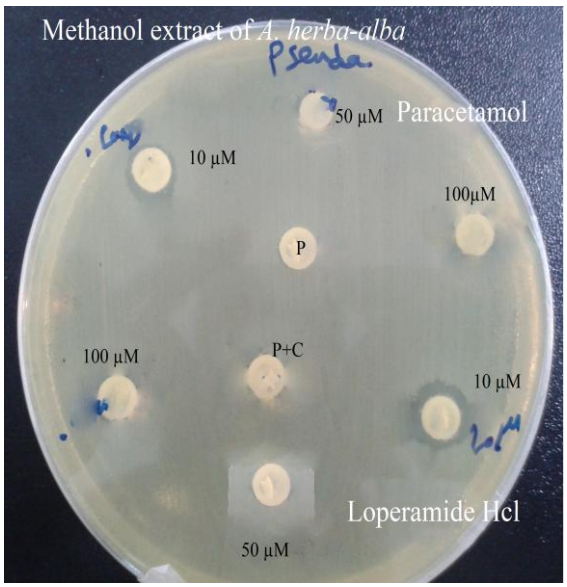


Figure (4.52): The effect of *A. herba-alba* (methanol extract) with Paracetamol and Loperamide Hcl on *P. aeruginosa*

P = Plant
P+C= plant extract with control (solvent)

4.5.3 The Synergistic Effect of Non-Antibiotics with Antibiotics

Antibiotic resistance is an ever-growing clinical problem. Compounding the issue is the fact that as bacteria are learning to tolerate and even circumvent existing classes of antibiotics, not enough work is being done to discover new ones. Combinations or cocktails of antibiotics are often used to broaden the antimicrobial spectrum of each and to achieve synergistic effects. In the current study, researchers systematically examined combinations of 1,057 compounds previously approved as drugs to find those that exhibited synergy with the antibiotic minocycline. Their work is reported in the April 24, 2011 issue of the journal Nature Chemical Biology (Gitig, 2013). In our study we used a group of antibiotics with some of non-antibiotics for this purpose.

4.5.3.1 Against *Staphylococcus aureus*

The combinations of antibiotics and Non-antibiotics was showed a weak synergistic activity. As Non-antibiotics drugs showed antagonistic effect with most antibiotics, the best synergistic activity was between Ampicillin and Paracetamol (at concentration of 100 µM), and between Pencillin G and also Paracetamol(at concentration of 10 µM). Loperamide Hcl had the best synergistic activity wih Ceftazidime and Ampicillin (at concentration of 100 µM), and with Vancomycin (at concentration of 50 µM), and with Ampicillin and Pencillin G (at concentration of 50 and 10 µM).

While the combination of antibiotics with Vitamin C has shown antagonistic effect with all antibiotics, except Ceftazidime.

4.5.3.2 Against *Escherichia coli*

Also synergy between antibiotics and Non- antibiotics was weak. Significant synergism was observed when Paracetamol was combined with Co-trimoxazole following the disc diffusion assay system (at a concentration of 100, 50 and 10 µM).

The combination of Loperamide Hcl (at a concentration of 100 µM) and each of Ceftazidime, Co-trimoxazole and Nalidixic acid produced significant synergistic activity against *E. coli* . While at a concentration 50 µM, the synergistic activity was showed between Loperamide Hcl and each of Chloramphenicol, Co-trimoxazole and Nalidixic acid. But at a concentration 10 µM ,the best synergistic activity was showed between Loperamide Hcl and each of Co-trimoxazole, Nalidixic acid and Ceftriaxone (15, 11 and 12 mm, Respectively).

When antibiotics and ascorbic acid were given in combination, the interaction observed was Antagonism.

4.5.3.3 Against *Pseudomonas aeruginosa*

While synergy between antibiotics and each of Paracetamol and Loperamide Hcl against *P. aeruginosa* was weak with all concentrations. But the best synergistic activity was between Amikacin and Paracetamol at a concentration 100 µM (inhibition zone= 23 mm). And also between Ceftriaxone and Loperamide Hcl at a concentration 50 and 10 µM (inhibition zone= 12 mm).

The synergistic effect was found in Vitamin C combination, with Ceftriaxone, Amikacin and Neomycin.

Table 4.24 Synergistic effects of Antibiotics with Paracetamol and Loperamide Hcl

Microorganism / Non-Antib. / Antibiotics	*Staphylococcus aureus*								*Escherichia coli*								*Pseudomonas aeruginosa*							
			Paracetamol			Loperamide Hcl					Paracetamol			Loperamide Hcl					Paracetamol			Loperamide Hcl		
	+	++	100 µM	50 µM	10 µM	100 µM	50 µM	10 µM	+	++	100 µM	50 µM	10 µM	100 µM	50 µM	10 µM	+	++	100 µM	50 µM	10 µM	100µM	50 µM	10 µM
Vancomycin	15	0	0	0	0	9	17	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cefotaxime	11	10	11	12	11	11	10	9	8	10	9	0	8	10	9	11	0	7	7	7	8	9	8	8
Ofloxacin	20	14	12	10	8	8	9	10	0	10	8	12	12	10	10	9	0	9	8	7	9	10	10	7
Ceftriaxone	12	12	12	11	12	8	8	9	9	10	11	0	8	9	0	15	0	10	10	11	9	12	12	11
Ceftazidime	0	12	12	10	8	13	12	7	11	12	11	9	8	13	11	8	9	0	8	0	9	10	10	10
Tetracyclines	21	7	9	0	11	0	8	0	0	10	11	8	11	7	8	10	–	–	–	–	–	–	–	–
Amikacin	20	17	11	10	12	12	10	12	10	19	12	17	17	17	18	17	17	20	23	22	21	22	22	20
Chloramphenicol	21	21	16	15	17	20	19	20	24	24	25	25	22	23	25	24	–	–	–	–	–	–	–	–
Gentamicin	21	8	14	10	10	10	9	13	7	0	0	0	0	0	0	0	8	0	8	8	9	9	9	10
Ampicillin	0	0	8	0	0	12	10	10	0	10	8	7	10	0	0	0	–	–	–	–	–	–	–	–
Erythromycin	17	0	9	8	10	9	8	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Rifampicin	19	17	16	17	9	10	9	17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Neomycin	20	15	11	10	11	9	9	10	14	12	10	8	10	11	8	10	0	9	9	10	10	10	10	9
Co-trimoxazole	10	0	0	0	10	0	0	0	0	0	13	9	15	10	9	11	–	–	–	–	–	–	–	–
Pencillin G	0	7	0	8	10	0	9	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cefazolin	8	10	9	0	9	0	0	9	7	7	0	0	0	0	0	0	–	–	–	–	–	–	–	–
Ceflexin	10	0	9	0	9	0	0	9	0	7	0	0	0	0	0	0	0	9	8	0	9	10	10	10
Nalidixic acid	–	–	–	–	–	–	–	–	0	8	10	10	9	10	9	12	–	–	–	–	–	–	–	–

***** antibiotics alone

****** antibiotics with methanol (as a control)

– Have not been tested.

Table 4.25 Synergistic effects between Antibiotics and Vitamin C

Microorganism / Non-Antib. / Antibiotics	*S. aureus* Vitamin C					*E. coli* Vitamin C					*P. aeruginosa* Vitamin C				
	*	**	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM	*	**	100 µM	50 µM	10 µM
Vancomycin	15	0	0	0	0	–	–	–	–	–	–	–	–	–	–
Cefotaxime	11	9	9	9	9	8	0	0	0	0	0	0	0	0	0
Ofloxacin	20	17	8	8	8	0	0	8	10	9	0	0	0	0	0
Ceftriaxone	12	7	0	0	0	9	7	7	8	7	0	0	7	7	7
Ceftazidime	0	0	12	9	10	11	9	0	0	0	9	7	0	0	0
Tetracyclines	21	0	0	0	0	0	0	0	0	0	–	–	–	–	–
Amikacin	20	17	18	17	18	10	8	9	8	10	17	15	21	20	22
Chloramphenicol	21	21	21	21	21	24	19	21	23	24	–	–	–	–	–
Gentamicin	21	0	0	0	0	7	0	0	0	0	8	7	0	0	0
Ampicillin	0	0	0	0	0	0	0	0	0	0	–	–	–	–	–
Erythromycin	17	0	0	0	0	–	–	–	–	–	–	–	–	–	–
Rifampicin	19	21	16	16	18	–	–	–	–	–	–	–	–	–	–
Neomycin	20	15	0	0	0	14	11	0	0	0	0	7	10	8	10
Co-trimoxazole	10	0	0	0	0	0	0	0	0	0	–	–	–	–	–
Pencillin G	0	0	0	0	0	–	–	–	–	–	–	–	–	–	–
Cefazolin	0	0	0	0	0	7	0	0	0	0	–	–	–	–	–
Ceflexin	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nalidixic acid	–	–	–	–	–	0	0	0	0	0	–	–	–	–	–

***** antibiotics alone
****** antibiotics with methanol (as a control)
– Have not been tested.

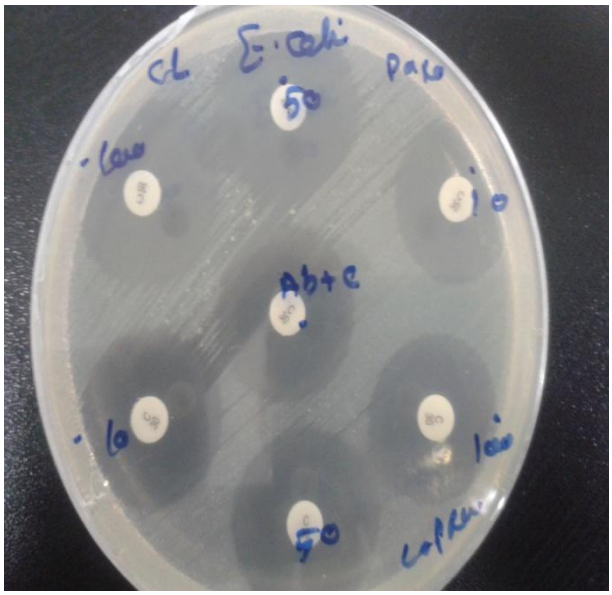


Figure (4.53): The combination effect of Chloramphenicol with Paracetamol and Loperamide Hcl on *E. coli*

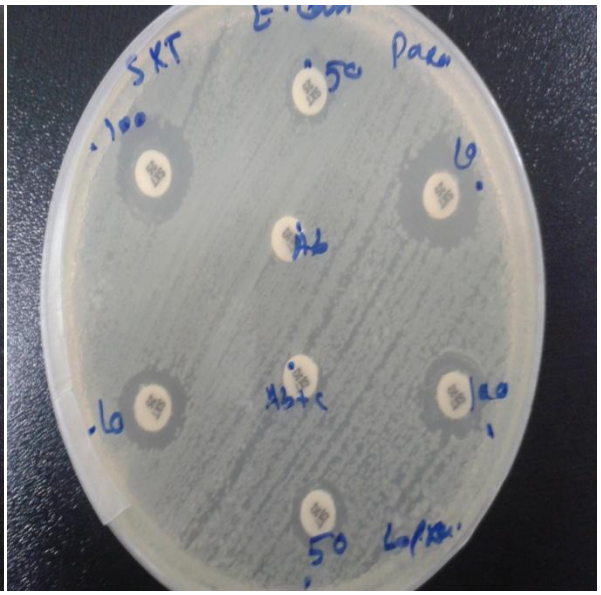


Figure (4.54): The combination effect of Co-trimoxazole with Paracetamol and Loperamide Hcl on *E. coli*

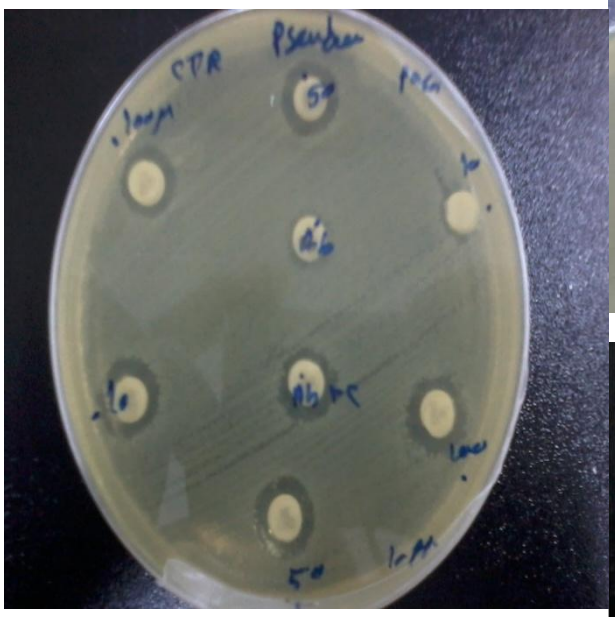


Figure (4.55): The combination effect of Ceftriaxone with Paracetamol and Loperamide Hcl on *P. aeruginosa*

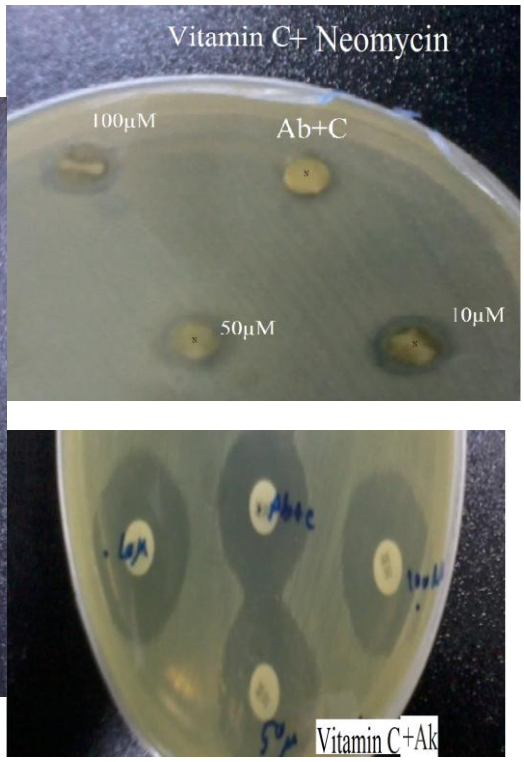


Figure (4.56): The combination effect of Neomycin and Amikacin with Vitamin C on *P. aeruginosa*

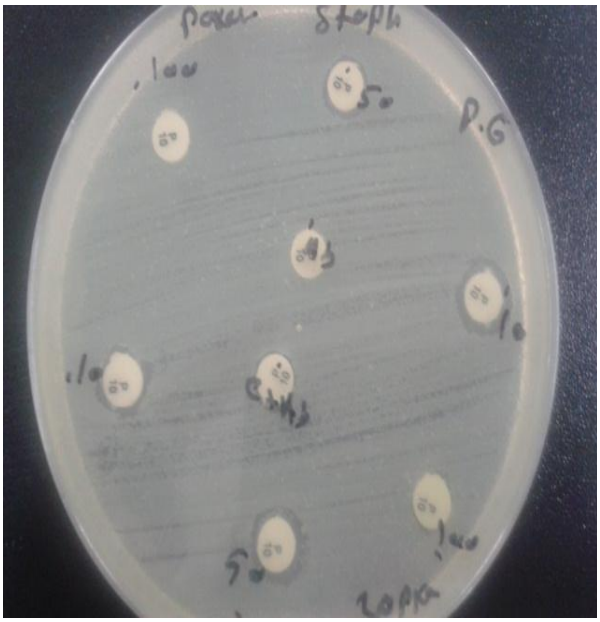


Figure (4.57): The combination effect of Pencillin G with Paracetamol and Loperamide Hcl on *S. aureus*

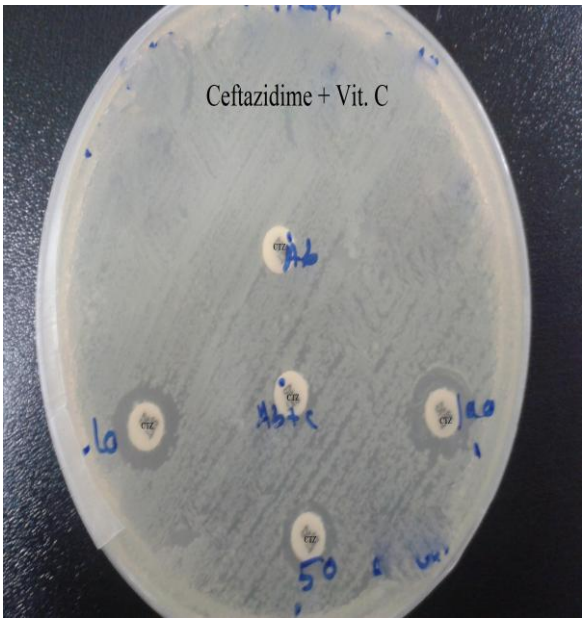


Figure (4.58): The combination effect of Ceftazidime with Vitamin C Hcl on *S. aureus*

P = Plant
P+C= plant extract with control (solvent

Chapter 5

Discussion and Conclusions

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens (Deans and Ritchie 1987; Kumar *et al*., 2006). Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antimicrobial agents for the treatment of bacterial infections is of increasing interest. The main objective of the present study was to evaluate the ability of the plants extract to inhibit the growth of pathogenic bacteria with and without antibiotics and non-antibiotics drugs and to determine their ability to enhance the activity of antibiotics or non-antibiotics drugs. Antimicrobial activity was recorded when the zone of inhibition is greater than 5 mm.

5.1. Antibacterial Activity of the Plant Extracts

Most tested plant extracts showed antibacterial activity against *E.coli*, *S.aureus* and *P.aurgenosa* which may reflect the antibacterial activity of plant active ingredients that inhibit bacterial growth.

In our experiments, disc diffusion method was used to asses the activity of plant extracts was showed activity against *E. coli*, while well diffusion method do not show any activity against *E. coli*, and may due to the plant extract added was diffusion in the bottom of the plate and thus be far from bacteria grown on the surface, while against *P.aeruginosa*, both of these methods have comparable results, but against *S.aureus* each of two methods was showed activity against it, where disc diffusion method was showed higher activity than well diffusion method, despite the *Artemisia herba-alba* extract showed the highest inhibition zone by Well diffusion method in comparison with another method, probably because the paper disc retains the active component and does not allow it to diffuse into the Muller Hinton Agar, because some compounds does not diffuse in the agar especially non polar compounds.

It was also noted that alcoholic extract has greater effect in the inhibition from aqueous extract, which may be due to the fact that alcohol is the best solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts. The difference in antibacterial activity of a plant extract might

be attributable to the age of the plant used, freshness of plant materials, physical factors (temperature, light water), time of harvesting of plant materials and drying method used before the extraction process.

As for absence of effectiveness to *A. sativum* on *E.coli* and *P. areuginosa,*even they have a very strong synregestic effect which may probably due to overuse of garlic by human that may lead to increasebacterial resistant to it even it has an effective antibacterial ingradiants. In addition, the therapeutic effect of garlic was very weakly when it was exposed to heat (during drying), which may be explained by the fact that heat is working to break down the enzyme alliinase, thus preventing the conversion of a compound alliin to allicin (active compound) (Ilić *et al*.,). Only the aqueous extracts of *A. herba-alba* had a weak antibacterial activity against *E.coli*, compared with the other plant extracts used against *E. coli* with no antibacterial effect; this result is in agreement with **Seddik *et al*., 2010** and **Mohamed *et al*., 2010** results which demonstrated that *A. herba-alba* aqueous extracts had a weak antibacterial activity against *E.coli*.

5.2 Antibacterial activity of nonantibiotic drugs

Vitamin C alone did not show antibacterial activity, which may be due to the need for high concentration of Vitamin C until have an antibiotic effect; this is in conformity with what was said by **Klenner *et al***.

Loperamid Hcl alone was able to inhibit the growth of all tested bacteria and Paracetamol which was observed to inhibit the activity of *P. aeruginosa* and *S. aureus*, this may be due to the solvent (methanol) which was used in dissolved.

5.3 MIC of plant extracts

Microdilution method was used to determine the lowest plant extracts concentration that inhibiting the growth of the bacteria and found effective in the evaluation of MIC.

The MIC value of *E. camaldulensis* was found as the lowest (3.125mg/ml) against *E. coli* and the methanol extracts of *E. camaldulensis* gave the best antibacterial activity against *E. coli*.

The methanol and aqoutic extract of *F. sycomorus* (leaves) was significantly active exhibiting the highest potency with MIC from 6.25-3.125 mg/mL against *S.aureu*s. This activity may be attributed to the rich plant contents of active components such as tannins, saponins, alkaloids and flavone aglycones. The MIC for *A. sativum* extracts against *S.aureus* particularly was found to be significantly active exhibiting the little

potency with all solvents used (50 mg/ml), and this confirms of the need for a high concentration of garlic until affect of the bacteria.

The MIC values obtained showed that ethanol extract of *E. camaldulensis* has the most potent effect against *P. aeruginosa*

5.4 Synergistic activity of Plants Extracts and Antibiotics

In our study, the plant extracts had different synergistic ability to inhibit the growth of microorganism depending on the method of extraction. Plants antimicrobials have been found to be synergistic enhancers in that though they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs they enhance the effect of that drug (Rakholiya and Chanda, 2012).

It has been known that one of the effective approaches to overcome bacterial resistance is restoration of antibiotic activity through the synergistic action of antibacterial materials from natural and synthesized agents (Stefanovic *et al*., 2011).

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) (Adwan and Mhanna, 2008).

Despite the abundant literature about the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been used for clinical use as antibiotics (Adwan and Mhanna, 2008).

5.4.1Against *Escherichia coli*

The protein synthesis inhibitors such as (Amikacin and Chloramphenicol) were showed the strongest synergistic effect with most of methanol plan extracts t. The better synergistic effect was found with *Artemisia herba-alba* and *Allium sativum*. Only, amikacin was showed synergistic effect with all methanol plant extracts.Whereas folic acid, bacterial cell wall synthesis and nucleic acid synthesis inhibitors (such as Co-trimoxazole, Cefotaxime and Nalidixic acid, respectively) were showed weak synergism with methanol extracts. The ethanolic extract of *Nerium oleander* and *Artemisia herba-alba* were showed synergistic effect with all tested antibiotics except Ceftazidime that showed antagonistic effect with all ethanolic plant extractsand also protein synthesis inhibitors were showed stronger synergistic effect with most ethanol plant extracts compared with the rest of the antibiotics used.

For the aqueous extract, a combination between most plant extracts and the antibiotics protein synthesis inhibitors showed synergistic activity against *E. coli* better than other antibiotics that works as inhibitors of cell wall synthesis (such as Cefazolin, Cefotaxime and Ampicillin). However,folic acid and nucleic acid synthesis inhibitors of antibiotics have a weak or no synergistically activity against *E.coli*.

For the aqueous extract, a combination between most plant extracts and the antibiotics protein synthesis inhibitors showed synergistic activity against *E. coli* better than other antibiotics that works as inhibitors of cell wall synthesis (such as Cefazolin, Cefotaxime and Ampicillin). However,folic acid and nucleic acid synthesis inhibitors of antibiotics have a weak or no synergistically activity against *E.coli*.

5.4.2 Against *Staphylococcus aureus*

The protein synthesis inhibitors were showed synergistic effect with most plant extracts better than cell wall synthesis inhibitors. The strongest synergistic effect was with methanolic extract of *Artemisia herba-alba* and ethanolic extracts of *Ficus sycomorus* (leaves) and *Allium sativum* with Tetracycline.

Ofloxacin which exhibit nucleic acid synthesis inhibitor showed stronger synergistic effect with *Allium sativum*.

Whereas folic acid synthesis inhibitors (Co-trimoxazole) showed stronger synergistic activity with methanolic and ethanolic extracts of *Ficus sycomorus* (Bark).

5.4.3 Against *Pseudomonas aeruginosa*

Protein synthesis inhibitors (such as Amikacin and Gentamicin) were showed strong synergistic effect with most plant extract using methanol, ethanol andwater as a solvent ,followed by nucleic acid synthesis inhibitors such as Ofloxacin.

Cell wall synthesis inhibitors such as Ceftriaxone showed weak or no synergistic activity against *P. aeruginosa*, except Ceftazidime which showed significant synergistic activity.

5.5 Synergistic activity of Plant Extracts and Non-Antibiotic drugs

L. camara was more responsive to Paracetamol and loperamid Hcl against *E. coli* and the combinations of Vit. C and with each of *E. camaldulensis* (methanol extract), *A. herba-alba* (ethanol extract) and *L. camara* (water extract) were showed a synergistic effect against *E. coli*.

While water extract of *A. herba-alba* was more effectivein combination with Paracetamol and loperamid Hcl against *S. aureus*,but when Vitamin C was combined with most plant extracts, antagonistic interaction were observed, except with *W. somnifera*, which showed a synergistic activity against *S. aureus,* when combined with vitamin C at a concentration of 100 µM.

The combinationof ethanol extracts of *N. oleander* with Paracetamol (at a concentration of 50 and 10 µM), as well as methanol extracts of *A. herba-alba* with Loperamide Hcl (at a concentration 10 µM) were showed asynregistic activity against *P. aeruginosa*.

5.6 Synergistic activity of Antibiotics and Non-Antibiotic drugs

The highest synergistic activity against *S. aureus* were observed when a combination of loperamid Hcl and Ampicillin were used.. As well a combinations of Vit. C and Ceftazidime were showed the highest synergistic activity against *S. aureus*.

While, a combinations of Paracitamol and loperamid Hcl with Nalidixic acid and Co-trimoxazole was showed the highest synergism against *E. coli*. However, acombination of Vit. C with antibiotics did not show synergistic activity against *E. coli*.

A combination of paracitamol and loperamid Hcl with Amikacin showed the highest synergistic activity against *P. aeruginosa*. While the combination of Vit. C with each of Ceftriaxone, Amikacin and Neomycin were showed the highest synergistic activity against *P. aeruginosa*.

Conclusion

On the basis of the antibacterial assay of this study *S. aureus* was found the more (susceptible to the employed plant extracts) than *E. coli* and *P. aeruginosa*.

All plant extracts were evaluted for their MIC against *E. coli, S.aureus* and *P. areuginosa*, The MIC value for each of methanolic extract of *E. camaldulensis* against *E. coli* was 3.125 mg/ml. And the methanol and aquatic extract of *F. sycomorus* (leaves) against *S.aureus* was from 6.25-3.125 mg/ml. And the ethanol extract of *E. camaldulensis* against *P. areuginosa* was 6.25 mg/ml . Suggesting that very small amount of the extracts are required to inhibit the growth of the bacteria thus *E. camaldulensis* (methanol extract), leaf extract of *F. sycomorus* (methanol and aquatic extract) and *E. camaldulensis* (ethanol extract) had very potent activity against *E. coli, S.aureus* and *P. areuginosa*, Respectively.

Ethanolic plant extracts were showed antimicrobial and synergistic activity with antibiotics better than methanolic and aquatic extracts.

The strongest effect agaist *E. coli* was recorded when *F. sycomorus* (leaves and bark) were mixed with Ofloxacin. And the strongest effect on *S. aureus* was observed when *A. sativum* was combined with Ofloxacin and Tetracyclin. The strongest effect againest *P. areuginosa* was observed when Ceftazidime was combined with most plant extracts, especially with *F. sycomorus* (leaves and bark); when the extracts of *N. oleander*, *A. herba-alba* and *W. somnifera* were combined with Amikacin and also when the extract of *W. somnifera* and *L. camara* were mixed with Neomycin.

Vitamin C alone did not show any antibacterial activity against all tested bacteria. It is likely that used distilled water as solvent has reduced the effectiveness it.
Paracetamol showed antibacterial activity against *S. aureus* and *P. aeruginosa*, especially at a concentration of 10 µM (inhibited zone=11mm). Loperamide Hcl was showed antibacterial activity against *S. aureus*, *P. aeruginosa* and *E.coli*, at a concentration of 100 µM, 10 µM and 10 µM, respectively (inhibited zone= 12, 13 and 12, respectively).

The synergistic activity of plant extracts and Non-antibiotic drugs was the best among the aqueous extracts of *L. camara* and each of Paracetamol, loperamid Hcl and vitamin C against *E.coli*. Aswell, the best synergistic activity among the aqueous extracts of *A. herba-alba* and each of Paracetamol and loperamid Hcl was against *S. aureus*. And the best synergistic activity was observed between *N. oleander* and Paracetamol (at a concentration of 50 and 10µM) against *P. aeruginosa*.

Regards the synergistic activity between the antibiotics and non-antibiotic drugs, the best synergistic activity was recorded between Ampicillin and each of paracetamol and loperamide Hcl against *S. aureus*, and among Nalidixic acid and each of paracetamol and loperamide Hcl. In addition synergistic activity was observed withCo-trimoxazole and each of paracetamol and loperamide Hcl against *E. coli*; Amikacin and paracetamol and loperamide Hcl against *P. aeruginosa*.

Recommendation

Based on the findings of this study the following recommendations are suggested:

1- The extracts of these plants should be further analyzed to isolate the specific antibacterial principles in them.

2- Research on the effectiveness of other parts of the plant as the roots or flowers, etc..

3- Toxicity studies of the effective plants should also be done to determine the safety indices of the extracts. Clinical trials should be carried out to explore the potential of these plant extracts in the treatment of these infectious diseases.

4- Determine the activity of these plant extracts on the types of fungi as *Candida albicans*, in addition to the synergistic activity of these medicinal plants with antibiotics and Non-antibiotics.

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