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SYNERGISTIC ACTIVITY OF ANTIBIOTICS AND BIOACTIVE PLANT EXTRACTS AGAINST *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens.

KEYWORDS: Synergy, Antibiotics, Drug resistance, medicinal herbs.

INTRODUCTION

Infectious diseases caused by bacteria and fungi affect millions of people worldwide. Throughout the history of mankind, infectious diseases have remained a major cause of death and disability. Today, infectious diseases account for one-third of all deaths in the world; the World Health Organization estimates that nearly 50,000 people die each day throughout the world from infectious diseases. The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind. Different antibiotics exercise their inhibitory activity on different pathogenic organisms. The development and spread of resistance to currently available antibiotics is a worldwide concern.

The increasing phenomenon of acquisition of resistance among microorganisms to antimicrobial drugs is attributed to the indiscriminate and improper use of current antimicrobial drugs.[1] Today, clinically important bacteria are characterised not only by single drug resistance, but also by multiple antibiotic resistance - the legacy of past decades of antimicrobial use and misuse.[2] Drug resistance presents an ever increasing global health threat that involves all major microbial pathogens and antimicrobial drugs.[3,4] These are difficult to treat and are responsible for a variety of infectious diseases. For over a decade, the pace of development of new antimicrobial agents has slowed down while the prevalence of resistance has grown at an astronomical rate. The rate of emergence of antibiotic resistant bacteria is not matched by the rate of development of new antibiotics to combat them.[5]

Antibiotics that work today may not work tomorrow. It is essential to investigate newer drugs to which there is lesser resistance.[6] As resistance to old antibiotics spreads, the development of new antimicrobial agents has to be expedited if the problem is to be contained. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy.[7] The steadily increasing bacterial resistance to existing drugs is a serious problem, and therefore there is a dire need to search for new classes of antibacterial substances, especially from natural sources. Unlike synthetic drugs, antimicrobials of plant origin are not

associated with side effects and have a great therapeutic potential to heal many infectious diseases.[8,9] Sometimes the use of single antibiotic does not produce the desired effective inhibitory effects and to overcome this, a combination of drugs often exercises their synergistic effect which surpasses their individual performance. The synergistic effect may be due to certain complex formation which becomes more effective in the inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or by causing its lyses or death.

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents.Gram negative bacteria Pseudomonas which can cause infections in the blood, lungs (pneumonia) or other parts of the body after surgery. Multi-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 multi factorial, 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.[10]

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'. 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 utilised as therapeutic agents.[11] from these microbes resistant to antibiotics, *Pseudomonas aeruginosa* 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 multi-drug-resistant *P. aeruginosa* strains resistant to different antimicrobial agent classes. Perhaps, this high degree of multi-drug resistance related to the presence of antibiotic efflux systems which provide resistance to multiple antimicrobial agents.[12]

P. aeruginosa is the most important toxigenic pathogen within the genus *Pseudomonas* because of the quantity and types of invasive infections it produces, as well as the noteworthy morbidity and mortality associated.[13] This Gram-negative bacterium has the ability to survive in adverse environments and develop multiple antibiotic resistance mechanisms. Among them, the most representative are the expression of chromosomal-encoded AmpC β-lactamase, the reduction of porin channels, the production of extended-spectrum β-lactamase and the mutation of topoisomerase II and IV.[14] It must be considered that several resistant mechanisms can coexist in one strain and just one of them can be effective against numerous antimicrobials.[15] The resistant mechanisms in *P. aeruginosa* are related to enhancement of the mortality rate of patients infected with this pathogen.[16] Furthermore, this rate is higher among patients infected with resistant strains and received inappropriate initial empirical treatment.[14] Additionally, the rising indiscriminate use of antimicrobials in health centers or by people who practice self-medication could lead susceptible patients to get infected by multi-drug-resistant microorganisms[17,18] The emergence of antibiotic resistance and related toxicity issues limit the use of these drugs, and generate a renaissance in phytotherapy research.[19] To address this challenge, there is growing interest in identifying and evaluating antimicrobial compounds in extracts of medicinal plants as a new source of drugs and alternative treatment approach.[20]

REVIEW OF LITERATURE

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 drugs are of natural product origin.[21] 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 phyto- chemicals, then, represents a starting point for antimicrobial drug discovery. Phyto- chemical 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.[22]

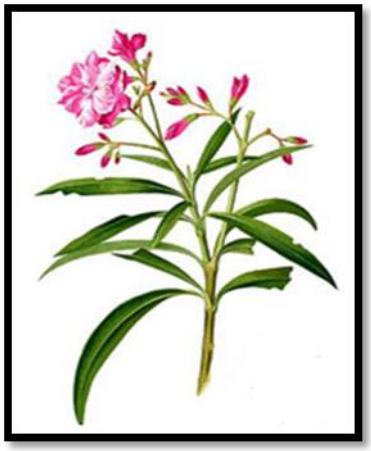
Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity.[23] The use of plant extracts and phytochemicals, both with known antimicrobial 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.[24]

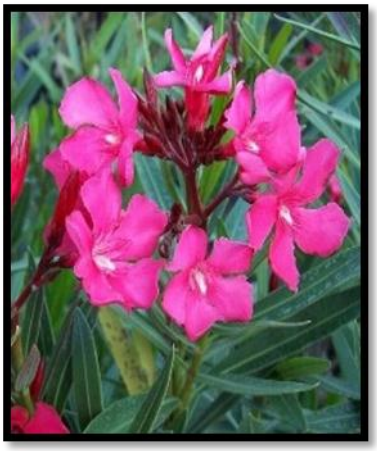
In Palestine, there are numerous medicinal plants described for treatment of many diseases. Herbal medicine is considered an integral part of the Palestinian

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.[25]

The following are some of the medicinal plants that have been studying its effect against some clinically isolated bacteria.

***Nerium oleander*:** *Nerium oleander* most commonly known as nerium, is a shrub or small tree in the dogbane family Apocynaceae, cultivated worldwide in temperate and subtropical areas as an ornamental and landscaping plant. It is the only species currently classified in the genus Nerium. 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.[21] 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.[26]





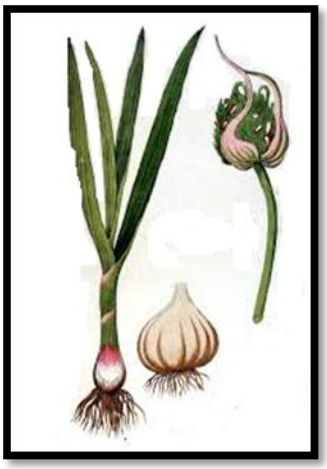
The leaves and the flowers are cardiotonic, diaphoretic (is excessive sweating commonly associated with shock and other medical emergency conditions), diuretic, anticancer, antibacterial, antifungal and expectorant. And also the flowers, leaves, leaf juice, bark and roots have been used against corns, warts, cancerous ulcers, carcinoma, ulcerating or hard tumors.[27] 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.[28] 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, anti hyperglycemic, antifungal, cytotoxial and insecticidal activity.[29]

1. ***Withania somnifera****: Withania somnifera* belongs to Solanaceae family commonly known as Ashwagandha/Indian ginseng/winter cherry.[30] The species name somnifera means "sleep-inducing" in Latin.[31] The name, ashwagandha, is a combination of the Sanskrit words ashva, meaning horse, and gandha, meaning smell, reflecting that the root has a strong horse-like odor.[32] The main active constituents of Withania somnifera are steroidal lactones, alkaloids, flavonoids, tannin etc. The major chemical constituents of these plants, with anolides, are mainly localized in leaves. [33] 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.[34] Its roots, leaves and seeds are used in Ayurvedic and Unani medicines, to combat diseases ranging from tuberculosis to arthritis. The pharma- cological 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 Lactare' which of is the galactagogue.[35] Also have several medicinal properties such as sedative, hypotensive, aphrodisiac, bradycardiac, respiration stimulatory, antiperoxidative, cardiotonic, radiosensitizing and thyro-regulatory effects.[36]

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.[37] The roots of *Withanian somnifera* are highly valued, and are used either alone or in combination with other medicinal plants to treat a variety of ailments. It is also used as a general tonic to increase and to improve overall health and longevity. Regular consumption of ashwagandha is believed to prevent diseases in individuals of different ages and with various health conditions.Preclinical studies showed that ashwagandha was an effective immunomodulatory agent and inhibited the myelosuppression induced by diverse immunotoxins (namely, cyclophosphamide, azathioprin, and prednisolone) in mice. Ashwagandha was effective in preventing myelosuppression with all three immunotoxins. The body weight, along with the levels of hemoglobin, red blood cells, white blood cells, and platelets, was restored.

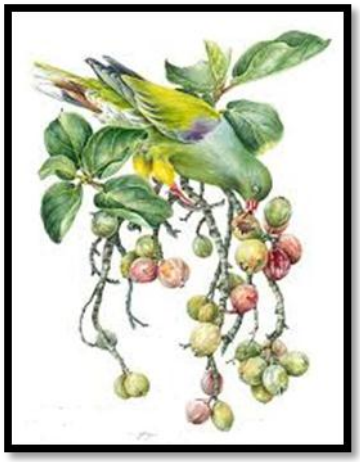
2. ***Allium sativum****: Allium sativum*; commonly known as garlic, is a species of the onion family Alliaceae. Allium sativum is a natural plant being used as a food as well as folk medicine for centuries in all over the world, in 1996, Reuter et al. described garlic a plant with various biological properties like antimicrobial, anti-cancer, antioxidant. As well as different properties such as antiviral, antifungal, expectorant, anti-septic, anti- histamine.[28] It 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.[38]



Different garlic extracts demon- strated 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

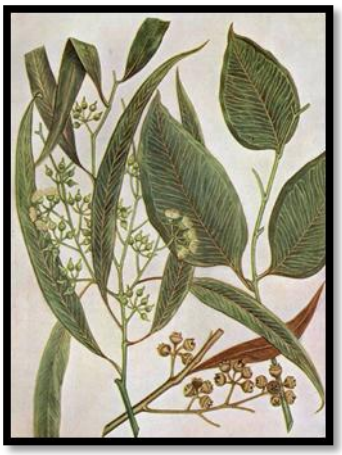
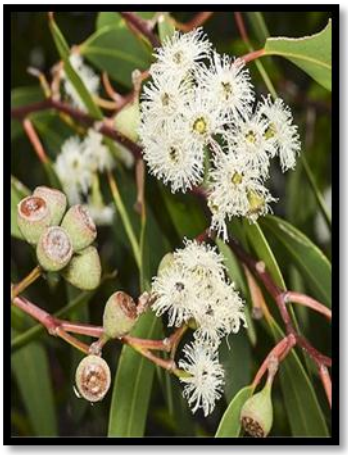
thiosulfinate compound of garlic reported for its antibacterial activity. Allicin is proved to be anti-bacterial as it inhibits RNA synthesis.[28] Allicin is formed by the action of an enzyme when garlic is chopped. Allicin is responsible for the characteristic odor of garlic and is mostly destroyed during cooking. Garlic contains about 2000 active compounds including various sulfur-containing chemicals which may release H2S. It reduces blood pressure, suppresses blood platelet aggregation, and has antiatherosclerotic properties. As of 2015, clinical research to determine the effects of consuming garlic on hypertension found that consuming garlic produces only a small reduction in blood pressure (4 mmHg),[39][40]41] and there is no clear long-term effect on cardiovascular morbidity and mortality.[41] A 2016 meta-analysis indicated there was no effect of garlic consumption on blood levels of lipoprotein(a), a biomarker of atherosclerosis.[65] Because garlic might reduce platelet aggregation, people taking anticoagulant medication are cautioned about consuming garlic.[42,43,44] A 2016 meta-analysis of case-control and cohort studies found a moderate inverse association between garlic intake and some cancers of the upper digestive tract.[45] Another meta-analysis found decreased rates of stomach cancer associated with garlic intake, but cited confounding factors as limitations for interpreting these studies.[46] Further meta-analyses found similar results on the incidence of stomach cancer by consuming allium vegetables including garlic. [47,48] A 2014 meta-analysis of observational epidemiological studies found that garlic consumption was associated with a lower risk of stomach cancer in Korean people.[49]

3. ***Ficus sycomorus*:** The common name of *Ficus sycomorus* is fig. The Sycamore Belongs to family Moraceae 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 1 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.[50] 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.[51] The fruit extracts of *Ficus sycomorus* L exhibited antitumor activity in the potato disc bioassay. it had significant antibacterial activity, but no antifungal activity.[52] Latex sap or bark extracts are drunk for chest complaints, glandular problems, pharyngitis and diarrhoea. Bark powder is sprinkled on burns. Decoctions of 2 hand-sized pieces, are drunk for diarrhoea. Fruit infusions are drunk for tuberculosis. Two handfuls of bark are boiled with 3 glasses of water and the vapour inhaled, as a galactagogue. One handful of fallen leaves is boiled with 1 cup of water, and the filtrate is drunk for dysentery.

4. ***Eucalyptus camaldulensis**: E. camaldulensis*, commonly known as the river red gum.[53] *E. camaldulensis* is an important ethnomedicinal plant belonging to the family Myrtaceae. 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.[54] 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.[55]

The essential oil obtained from various species of eucalyptus is a very powerful antiseptic, especially when it is old, because ozone is formed in it on exposure to air. It has a decided disinfectant action, destroying the lower forms of life.[56] The oil can be used externally, applied to cuts, skin infections etc; its vapours can also be inhaled for treating blocked nasal passages; it can be gargled for sore throat; and can also be taken internally for a wide range of complaints.[56] Some caution is advised, however, because like all essential oils, it can have a deleterious effect on the body in larger doses.[56] A kino- resin is exuded from the tree.[57,58] It can also be obtained from the tree by making incisions in the trunk.[56,59] This resin contains tannin and is powerfully astringent, it is used internally in the treatment of diarrhoea and bladder inflammation.[56,57,59] externally it is applied to cuts etc.

Clinical manifestation of *Pseudomonas aeruginosa.*

Pseudomonas aeruginosa is a classic opportunist pathogen belonging to the genus Pseudomonas.[60] It is an obligate aerobe, motile, rod- shaped, and measuring about 0.6 x 2 µm. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains. 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.[61]

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.[62]

CONCLUTION

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 and 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.[63] *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.[62] *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.[64]

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