International Journal of Molecular Biology & Biochemistry.
ISSN 2349-2341 Volume 4, Number 1 (2016), pp. 27-42

http://www.irphouse.com

# Plants as Latent Sources of New Antimicrobials and Resistance Modifying Agents Against Multi Drug Resistant (MDR) Strains

**Vaidehi Patel[1] *, Dr. Rajesh Patel[2]**
[1] *Department of Microbiology, Shree K. K. Patel girls science college.*
[2] *Department of life science, H.N.G.U, PATAN*

## Abstract

Antibiotic resistance has limited the use of cheap and old antibiotics, has necessitated to used higher class antibiotics. The need of higher class antibiotics need a continued search for new antimicrobial compounds. Understanding the mechanisms of resistance is important in the development of strategies to solving the problem. Active efflux of drugs, alteration of target sites and enzymatic degradations are the strategies by which pathogenic bacteria acquire or develop intrinsic resistance to antibiotics. Multi-drug resistance (MDR) pumps, capable of recognizing and expelling a variety of structurally unrelated compounds from the bacterial cell and conferring resistance to a wide range of antibiotics have since been characterized in many Gram positive and Gram negative pathogens like *Staphylococcus au reus*, *Pseudomonas ae ruginosa*, *Escherichia coli* and, more recently, in mycobacteria. The ability of some chemical compounds (called MDR inhibitors or resistance modifying agents) to modify the resistance phenotype in bacteria by working synergistically with antibiotics in vitro has since been observed. The search for such compounds which can be combined with antibiotics in the treatment of drug resistant infections may be an alternative to overcoming the problem of resistance in bacteria. Crude extracts of medicinal plants stand out as veritable sources of potential resistance modifying agents.

**Keywords:** MDR, plant extract, resistant modifying agent

## 1. 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. [1]

The increasing phenomenon of acquisition of resistance among microorganisms to antimicrobial drugs is attributed to the indiscriminate and improper use of current antimicrobial drugs. Today, clinically important bacteria are characterized not only by single drug resistance, but also by multiple antibiotic resistance - the legacy of past decades of antimicrobial use and misuse. Drug resistance presents an ever increasing global health threat that involves all major microbial pathogens and antimicrobial drugs. 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. [1]

Antibiotics that work today may not work tomorrow. It is essential to investigate newer drugs to which there is lesser resistance. 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. [2]

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. 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 .[2]

The global emergence of multi-drug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections examples include methicillin-resistant *Staphylococci*, *Pneumococci* resistant to penicillin and macrolides, vancomycin- resistant *Enterococci* as well as multi drug resistant Gram –negative organisms.[2]

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 and emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy.[2]

According to the World Health Organization, infectious diseases are the third most significant cause of mortality around the world. The burden of infectious diseases is

high in developing countries, as is the emergence of MDR pathogens due to poor health-care facilities, and over-the-counter availability and misuse of antimicrobial agents. The frequency of resistance is observed equally among Gram-negative and Gram-positive organisms, although Gram-negative bacteria are prone to develop a MDR phenotype. The high incidence rate of MDR *Pseudomonas* and *Acinetobacter* infections in critically ill patients as well as the presence of MDR *Salmonella* and *Staphylococcus aureus* in normal communities are classic examples of microbiological challenges posed in these geographic locations. [3]

Plants are historically used to treat infectious diseases. In earlier days people used to discover remedies from the local herbs. People first used plants as food and if results of ingestion were favorable, the plants were linked with some sedative and curative properties. For example, remains of the hollyhock plant, which is still an important herb in phytomedicine, are found in the ancient civilization of the Neanderthals. Scientific evidence supports the hypothesis that several plants are composed of biologically active chemical entities and several drugs in modern day medicine are actually analogues of plant origin substances. [3]

## 2. Antibiotic History, Developments and Mechanism of Actions:

Before the 20$^{th}$ centaury, medicines consisted mainly of herbs and potions. It was not until the mid nineteenth century that the first serious efforts were made to isolate and purify the active principles of these remedies. Since than many naturally occurring drugs have been obtained and their structure determined. [4]

There is evidence of antibacterial herbs or potions being used for many centuries. For example, the Chinese used mouldy soybean curd to treat carbuncles, boils, and other infections. Greek physicians used wine, myrrh and inorganic salts. In the middle ages, certain types of honey were used to prevent infections following arrow wounds.[4]

Bacteria are single cell microorganisms which were first identified in the 1670s by Van Leeuwenhoek. French scientist Paster, who demonstrated that specific bacterial strains were crucial to fermentation and that these and other microorganisms were more widespread than was previously thought. The possibility that these microorganisms might be responsible for disease began to take hold.[4]

Lister introduce ‘germ theory of disease’ and also introduced **Carbolic acid** as an antiseptic and sterilizing agent for operating theatres and wards.[4]

During the latter half of the nineteenth century, scientists such as Koch were able to identify the microorganisms responsible for the disease such as tuberculosis, cholera and typhoid. Methods of vaccination were studied and research was carried. [4]

Paul Ehrlich- father of chemotherapy, introduced the ‘principle of chemotherapy’ was that a chemical could directly interfere with the proliferation of microorganisms, at concentrations tolerated by the host. This concept was popularly known as the ‘magic bullet’. The process is one of the selective toxicity, where the chemical shows greater toxicity to the target microorganism than to the host cells. Such selectivity can be represented by a ‘chemotherapeutic index’. He had successfully developed the first example of purely synthetic antimicrobial drug, **Salvarsan** in 1910. [4]

Over the next 20 years, progress was made against a variety of protozoal diseases, but little progress was made in finding antibiotics until introduction of **proflavine** in 1934. Unfortunately, it was to toxic to be used against systemic infection. In 1935, **Prontosil** was effective against Streptococcal infections *in vivo*. The discovery of **sulfa drugs** or **sulfonamides** was a real breakthrough, as they represented the first drugs to be effective against systemic bacterial infections. [4]

Although **Penicillin** was discovered in 1928 but effectively isolated in 1940 by Florey and Chain. Penicillin was more effective than sulfonamides but not effective against all types of infections and the need for new antibacterial agents still remains. Penicillin is an example of a toxic fungal metabolite that kills bacteria and allows the fungus to compete for nutrients. The realization that fungi might be the source for novel antibiotics. [4]

In 1944, the antibiotics **Streptomycin** was discovered from a systemic search of soil organisms. This compound was the first example of a series of antibiotics known as **aminoglycoside** which are active against wide range of Gram –ve bacterial infections. The search continued leading to the discovery of **chloramphenicol** (1947), the peptide antibiotics (e.g. **bacitracin;** 1945), the tetracycline antibiotics (e.g. **chlortetracycline;** 1948), the macrolide antibiotics (e.g. **erythromycin**; 1952), the cyclic peptide antibiotics (e.g. **valinomycin**) and in 1955, the first example of a second major group of β- lactam antibiotics, **cephalosporin C.** [4]

**Isoniazid** was found to be effective against human tuberculosis in 1952. And in 1962, **nalidixic acid** (the first of quinolone antibiotic agent) was discovered. The second generation of this class of drugs was introduced in 1987 with **ciprofloxacin**. [4]

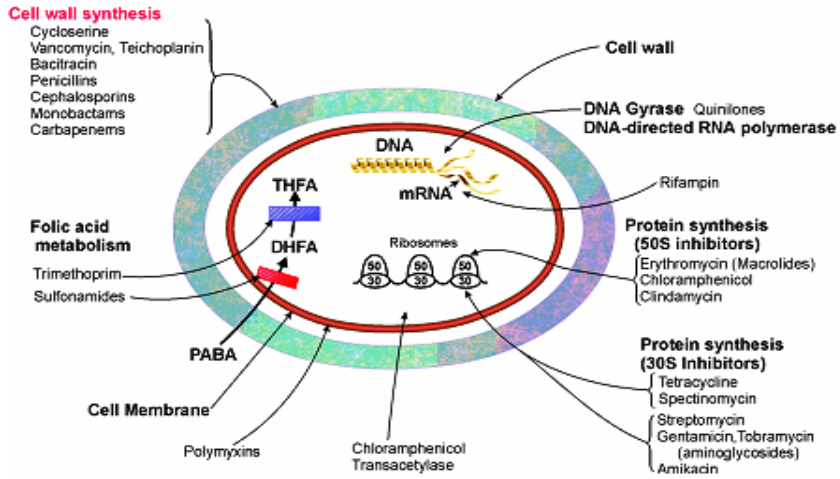


**Figure 1:** Bacterial targets of current antibiotics used in the clinic [5]

***Mechanism of action**:*

There are five main mechanisms by which antibiotics agents act.

- Inhibition of cell metabolism: Antibacterial agents which inhibit cell metabolism are called antimetabolites. These compounds inhibits the metabolism of a microorganisms but not the metabolism of the host. They can

do this by inhibiting an enzyme-catalyzed reaction which is present in the bacterial cell but not in animal cells. Example; Sulfonamides. [4]

- Inhibiting of bacterial cell wall synthesis: inhibition of cell wall synthesis leads to bacterial cell lysis and death. Agents operating in this way include penicillins, cephalosporins and vancomycin. As animal cells do not have a cell wall, they are unaffected by such agents. [4]
- Interactions with the plasma membrane: some antibacterial agents interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fatal results for the cell.example; polymyxins and tyrothricin. [4]
- Disruption of protein synthesis: disruption of protein systhesis means that essential proteins and enzymes required for the cell's survival can no longer be made. Agents which disrupt protein synthesis include the rifamycins, aminoglycosides, tetracyclines and chloramphenicol. [4]
- Inhibition of nucleic acid transcription and replication: Inhibition of nucleic acid function prevents cell division and/or the synthesis of essential proteins. Agents acting in this way include nalidixic acid and proflavin. [4]

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. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms. The worldwide emergence of *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus* and many other ß-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of *E. coli* and *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections. *Candida albicans*, also a nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis. Alarmingly, the incidence of nosocomial candidemia has risen sharply in the last decade. All this has resulted in severe consequences including increased cost of medicines and mortality of patients. [6]

## 3. Antibiotic Resistance and Its History of Development:

**Antibiotic resistance** is a form of drug resistance whereby some (or, less commonly, all) sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics; pathogens resistant to multiple antibiotics are considered multidrug resistant (MDR) or, more colloquially, **superbugs.**

Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century, in particular as it pertains to pathogenic organisms (the term is especially relevant to organisms that cause disease in humans). A World Health Organization report released April 30, 2014 states, "this serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country". Antibiotic resistance–when bacteria

change so antibiotics no longer work in people who need them to treat infections–is now a major threat to public health. [8]

In the last fifty years, only two novel classes of antimicrobial compounds such as oxazolidinone and cyclic lipo-peptide have passed clinical trials and are available for clinical use. These agents have undergone analogue development and six drugs were introduced in market in the last decade; however, they are not useful for MDR pathogens due to the rapidly evolving mechanisms of resistance among bacteria. Most other compounds do not proceed to clinical trials due to lack of sustained activity and higher toxicity rates. It is clearly understood that progress in the field of drug discovery is far behind what is required to keep up with present day needs. The situation necessitates the development of new antimicrobial agents in rapid fashion. [3]

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 [10]. 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 and emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy. [2]

Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. 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. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains.(6)

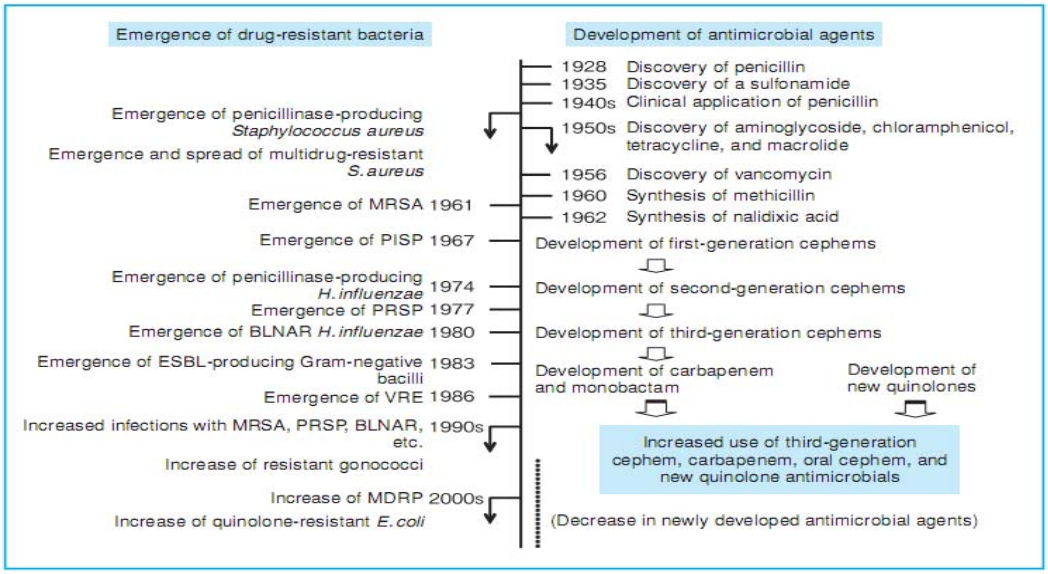


**Figure 2 :** Trend of development of antimicrobial agents and emergence of drug-resistant bacteria [9]

In the simplest cases, drug-resistant organisms may have acquired resistance to first-line antibiotics, thereby necessitating the use of second-line agents. Typically, a first-line agent is selected on the basis of several factors including safety, availability, and cost; a second-line agent is usually broader in spectrum, has a less favorable risk-benefit profile, and is more expensive or, in dire circumstances, may be locally unavailable. In the case of some MDR pathogens, resistance to second- and even third-line antibiotics is, thus, sequentially acquired, a case quintessentially illustrated by *Staphylococcus au reus* in some nosocomial settings. Some pathogens, such as *Pseudomonas aeruginosa*, also possess a high level of intrinsic resistance.

Genes for resistance to antibiotics, like the antibiotics themselves, are ancient. However, the increasing prevalence of antibiotic-resistant bacterial infections seen in clinical practice stems from antibiotic use both within human medicine and veterinary medicine. Any use of antibiotics can increase selective pressure in a population of bacteria to allow the resistant bacteria to thrive and the susceptible bacteria to die off. As resistance towards antibiotics becomes more common, a greater need for alternative treatments arises. However, despite a push for new antibiotic therapies, there has been a continued decline in the number of newly approved drugs. Antibiotic resistance therefore poses a significant problem. [4,7]

Certain antibiotic classes are more highly associated with colonisation with "superbugs" compared to other antibiotic classes. A superbug, also called multiresistant, is a bacterium that carries several resistance genes. The risk for colonisation increases if there is a lack of susceptibility (resistance) of the superbugs to the antibiotic used and high tissue penetration, as well as broad-spectrum activity against "good bacteria". In the case of MRSA, increased rates of MRSA infections are seen with glycopeptides, cephalosporins, and especially quinolones. In the case of colonisation with *Clostridium diff icile*, the high-risk antibiotics include cephalosporins and in particular quinolones and clindamycin. [4,7]

## 4. Cause of MDR:

- Although there were low levels of preexisting antibiotic-resistant bacteria before the widespread use of antibiotics, evolutionary pressure from their use has played a role in the development of multidrug-resistant varieties and the spread of resistance between bacterial species. The widespread use of antibiotics both inside and outside medicine is playing a significant role in the emergence of resistant bacteria.
- In some countries, antibiotics are sold over the counter without a prescription, which also leads to the creation of resistant strains.
- Household use of antibacterials in soaps and other products, although not clearly contributing to resistance, is also discouraged (as not being effective at infection control).
- Unsound practices in the pharmaceutical manufacturing industry can also contribute towards the likelihood of creating antibiotic-resistant strains.
- The procedures and clinical practice during the period of drug treatment are frequently flawed — usually no steps are taken to isolate the patient to prevent

re-infection or infection by a new pathogen, negating the goal of complete destruction by the end of the course.

## 5. Mechanism of Antibiotics Resistance in Pathogenic Bacteria :

Resistance to antimicrobials is as a result of three main strategies namely enzymatic inactivation, modification of target sites and extrusion by efflux. While chemical modifications could be significant in antibiotic resistance, exclusion from the cell of unaltered antibiotic represents the primary strategy in denying the antibiotic, access to its targets and this is believed to enhance resistance even in cases where modification is the main mechanism. [2]

***Alteration of drug targets:***

Chemical modifications in the antibiotic target may result in reduced affinity of the antibiotic to its binding site. This is a mechanism employed by a number of pathogenic bacteria in evading the effect of antibiotics. Modifications are usually mediated by constitutive and inducible enzymes. Resistance to macrolides, lincosamide and streptogramin B antibiotics ($MLS_B$ resistance) in pathogenic *Streptococcus* species is a result of methylation of the $N^6$ amino group of an adenine residue in 23S rRNA. This is presumed to cause conformational changes in the ribosome leading to reduced binding affinity of these antibiotics to their binding sites in the 50S ribosomal subunit. Beta-lactams antibiotics function by binding to and inhibiting the biosynthetic activity of Penicillin Binding Proteins (PBPs), thereby blocking cellwall synthesis. In *S. aureus* and *S. pneumoniae*, resistance to □β-lactams can be a result of mutations leading to the production of PBP2a and PBP2b respectively. The two proteins have a reduced affinity for □β-lactams and yet they take over the functions of normal PBPs in the presence of inhibitory levels of □β-lactams. This mechanism of resistance is also responsible for □β-lactam resistance in non- β- lactamase producing *Haemophillus influenza*. [2]

***Enzyme inactivation:***

The production of hydrolytic enzymes and group transferases is a strategy employed by a number of pathogens in evading the effect of antibiotics. Genes that code for antibiotic degrading enzymes are often carried on plasmids and other mobile genetic elements. The resistance to β-lactam antibiotics by both gram negative and gram positive bacteria has long been attributed to β-lactamases. These enzymes confer significant antibiotic resistance to their bacterial hosts by hydrolysis of the amide bond of the four membered β-lactam ring. Resistance to aminoglycosides in Gram-negative bacteria is most often mediated by a variety of enzymes that modify the antibiotic molecule by acetylation, adenylation or phosphorylation. [2]

***Antibiotic efflux:***

It is now widely recognized that constitutive expression of efflux pump proteins encoded by house-keeping genes that are widespread in bacterial genomes are largely responsible for the phenomenon of intrinsic antibiotic resistance. Several studies have shown that active efflux can be a mechanism of resistance for almost all antibiotics.

The majority of the efflux systems in bacteria are non-drug-specific proteins that can recognize and pump out a broad range of chemically and structurally unrelated compounds from bacteria in an energy-dependent manner, without drug alteration or degradation. The consequence of this drug extrusion is that, it leads to a reduced intracellular concentration of the antimicrobial such that the bacterium can survive under conditions of elevated antimicrobial concentration. The MIC of the drug against such organisms will be higher than predicted.[2]

Multi-drug resistance efflux pumps are ubiquitous proteins present in both Gram-positive and Gram-negative bacteria as either chromosomally encoded or plasmid encoded. Although, such proteins are present constitutively in bacteria, the continued presence of the substrate induces over-expression. This increased transcription is responsible for the acquired resistance. In Gram-negatives bacteria, the effect of the efflux pumps in combination with the reduced drug uptake due to the double membrane barrier is responsible for the high inherent and acquired antibiotic resistance often associated with this group of organisms.[1,2,4]

The MDR pumps of pathogenic bacteria known so far, belong to five families of transporters namely; the major facilitator super-family (MFS), the adenosine triphosphate (ATP)-binding cassette (ABC) super-family, the small multi-drug resistance (SMR) family and the resistance-nodulation-cell division (RND) super-family and the multi drug and toxic compound extrusion (MATE) family. [1,2,4]

***Some characterized efflux proteins of pathogenic bacteria:***

The NorA protein of *S. aureus* is the best studied chromosomally encoded pump in pathogenic gram-positive bacteria. It is present in S. epidermidis but appears to be absent in *Enterococcus faecalis* or in gram-negative organisms, such as *E. coli* and *K. pneumoniae*. Over expression of the N or A gene in *S. aureus* confers resistance to chloramphenicol and hydrophilic fluoroquinolone antimicrobials. [2]. QacA is a member of the major facilitator super-family of transport proteins, which are involved in the uniport, symport, and antiport of a wide range of substances across the cell membrane. The QacA multidrug exporter from *S. aureus* mediates resistance to a wide array of monovalent or divalent cationic, lipophilic, antimicrobial compounds. QacA provides resistance to these various compounds via a proton motive force-dependent antiport mechanism. [2]. The mefA efflux protein of *S. pyogenes* is a hydrophobic 44.2 kDa transposon encoded protein, of the Major Facilitator super family that mediates efflux of macrolides resulting in the M phenotype in *S. pyogenes*. It shares a 90% amino acid homology with MefE of S. pneumoniae that also mediates the efflux of macrolides. [2]. PmrA (pneumococcal multidrug resistance protein) efflux of *S. pneumonia* is a chromosomally encoded protein of the Major facilitator family that confers a resistance profile in *S. pneumonia* similar to that of N or A in *S. aureus*. The efflux protein which is not expressed constitutively in pneumococcal strains is responsible for low-level fluoroquinolone resistance in pneumococci. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties,

safety and efficiency. [9]. But plant extracts as antimicrobials are rarely used as systemic antibiotics at present, this may be due to their low level of activity, especially against Gram-negative bacteria. Here we are trying to investigate an alternative approach to the treatment of bacterial infections by combining antimicrobial agents with crude plant extracts against different pathogens. [10]

**Synergism** is a positive interaction created when two agents combined and exert an inhibitory effect that is greater than the sum of their individual effects. Combination therapy can be used to expand the antimicrobial spectrum, to prevent the emergence of resistant mutants, to minimize toxicity and to obtain synergistic antimicrobial activity, it could be an alternative to monotherapy for patients with invasive infections that are difficult to treat, such as those due to multi-resistant species and for those who fail to respond to standard treatment. [12]

***Plants as source of new antimicrobials and resistance modifying agents:***

Natural products are rich source of biologically active compounds. Many of today's medicines are either obtained directly from a natural source or were developed from a lead compound originally obtained from a natural source. Usually, the natural source has some form of biological activity and the compound responsible for that activity is known as the active principle. Such a structure can act as a lead compound. Most biological active natural products are secondary metabolites with quite complex structures. This has an advantage in that they are extremely novel compounds [4].

The alkaloid berberine is a common component of a variety of plant species, particularly in the family of *Berberidaceae*. Berberine alkaloids, which are cationic antimicrobials produced by a variety of plants are readily extruded by MDRs. Berberine found to synthesize an inhibitor of N or A MDR pump of a human pathogen *Staphylococcus A ureus*. The inhibitor was identified as 5′ - methoxyhydnocarpin , previously reported as a minor component of chaulmoogra oil, a traditional therapy for leprosy. [13]

The interaction between water extracts of *Psidium guajava, Rosmarinus officinalis, Salvia f ruticosa, M ajorana s yriaca, Oc imum basilucum, Syz ygium a romaticum, Laurus nobilis* and *Rosa damascena* alone and then synergy testing of these extracts with known antimicrobial agents of different mechanisms (protein synthesis inhibition: oxytetracycline HCl and gentamicin sulfate; cell wall synthesis inhibition: penicillin G and cephalexin; folic acid synthesis inhibition: Sulfadimethoxine as sodium; and nucleic acid synthesis inhibition: enrofloxacin) using both well-diffusion and microdilution method. This study was conducted against five *S. aureus* isolates; one is Methicillin-resistant *Staphylococcus aureus* (MRSA) and 4 Methicillin-sensitive Staphylococcus aureus (MSSA). The results of the conducted experiments using well-diffusion method demonstrate that these plants showed in vitro interactions between antimicrobial agents and plant extracts were additive against the five strains of *S. aureus,* while using microdilution method showed synergistic effects between combination of antibiotics and plant extracts with significant reduction in the MICs of the test antibiotics against these strains of S. aureus. This change in MIC was noticed in all plant extracts against test antibiotics including these plants showed weak antibacterial activity by well diffusion method. Also our results showed that

synergism effect between antimicrobial agent and plant extract was occurred in both sensitive and resistant strains but the magnitude of minimum fold inhibition in resistant strains especially MRSA strain was higher than the sensitive strains. [10]

Multidrug resistant (MDR) strains of *Escherichia coli, K lebsiella pneumoniae* and *Candida albicans*. ATCC strains of *Streptococcus mutans, Staphylococcus aur eus, Enterococcus f aecalis, Str eptococcus bovi s, P seudimonas aer uginosa, Salmonella typhimurium, E scherichia coli, K lebsiella pneu moniae* and *Candida albicans* that showed resistance against *Acacia nilot ica, Syz ygium ar omaticum* and *Cinnamum zeylanicum,* whereas they exhibited strong resistance to the extracts of *Terminalia arjuna* and *Eucalyptus globulus*. Community acquired infections showed higher sensitivity than the nosocomial infections against these extracts. The most potent antimicrobial plant was *A. nilotica.*[6]

Pseudomonasis wide spread in nature, inhabiting soil, water, plants, and animals (including humans). *Pseudomonas aer uginosa* has become an important cause of infection, especially in patients with compromised host defense mechanisms. Total 5 MDR isolates of *Pseudomonas* sp. were selected for further studies on the basis of their resistance to more than 60% antibiotics. Hot and cold extracts of solvents with increasing order of polarity from petroleum ether, chloroform, acetone, methanol and water were used for study. Combined effect of herbal extracts of *Foeniculum vulgare Miller* and antibiotics on susceptibility of Multidrug resistant (MDR) isolates showed promising effect although individual extracts were not effective against any isolates. Concept of this synergism provides a new thought of antibiotics and bioactive plant extracts in development combined antimicrobial therapy for effective management of MDR Pseudomonas isolates. [14]

The studies were carried out to evaluate antibacterial activity of 35 aqueous herbal extracts against a total of 20 clinical *Klebshiella s p.* isolates. The maximum antibacterial activity was found as 90% in crude extracts of *Syzygium a romaticum* (leaf) and *Citrus limon L.* (fruit) followed by 85% in *Spondias pinnata* (leaf). Sensitivity of these isolates was also evaluated for eight commercial antibiotic discs following disc diffusion assay where most of the isolates found to develop resistance against multiple commercial antibiotics. 85% of isolates exhibited resistant to chloramphenicol and erythromycin and 80% were resiatant to sulfamethoxazole and cephradine. The isolates showed their resistance between 55-60 % to the other four antibiotic discs, viz; gentamycin, streptomycin, ciprofloxacin and azithromycin. Among 35 herbal extracts tested, 19 herbal extrats were found to possess antimicrobial activity in all multi-drug resistant isolates. Therefore these herbal extracts could be used in future direction as alternative therapeutic agents for the treatment of human diseases caused by *Klebsiella sp*. [15]

The emergence of antibiotic-resistant bacteria such as *Staphylococcus aureus* calls for inventive research and development strategies. Inhibition of this bacterial pathogenesis may be a promising therapeutic approach. The screening of antimicrobial compounds from endophytes is a promising way to meet the increasing threat of drug-resistant strains of human and plant pathogens. In the study, a novel endophytic fungus, *Colletotrichum gloeosporioides*, was isolated from the medicinal plant *Vitex n egundo L.* Extracts of *C. gloeo sporioides* were obtained using hexane,

ethyl acetate and methanol solvents. The fungal extracts exhibited an effective antimicrobial activity against bacterial and fungal strains. The extracts were also analysed for antibacterial activity against methicillin-, penicillin- and vancomycin-resistant *S. aur eus* strains (1–10). The methanol extract showed an effective antibacterial activity against S. aureus strain 9, with a minimal inhibitory concentration of 31.25 $mgmL^{-1}$. The synergistic action of endophytic fungal extract with antibiotics such as methicillin, penicillin and vancomycin was observed against S. aureus strain 6. The fractional inhibitory concentration index of methanol extract with methicillin, penicillin and vancomycin was 1.0, 0.5 and 0.375, respectively. These results clearly indicate that themetabolite of endophytic fungus *C. gloeosporioides* is a potential source of new antibiotics. [17]
Some isolated pure compounds of plant origin have been reported to have resistance modifying activities in vitro. Examples of some of the compounds are given in below Table. This has prompted the search for such compounds from a variety of medicinal plants. Some of the compounds which have been observed to have direct antimicrobial activity have also been shown to be potentiate against the activity of antibiotics when used at low MIC levels.

**Table 1:** some antibiotic resistance modifying compounds from plants [18,19,20]

| **Compound** | **Plant source** | **Antibiotics potentiated** |
|---|---|---|
| Ferruginol<br>5-Epipisiferol | *Chamaecyparis lawsoniana* | Oxacillin, Tetracycline, Norfloxacin<br>Tetracycline |
| Carnosic acid carnosol | *Rosmarinus officinalis* | Erythromycin |
| Ethyl gallate  | *Caesalpinia spinosa* | β-lactams |
| Epicatechin gallate<br>Epigallocatechin gallate | *Camellia sinensis* | Norfloxacin, Imipenem, Panipenem<br>  β –Lactams |

Plants used for medicinal purpose for thousands of years and most of the world still depends on them. This study describes the chemical and biological studies of *Terminalia arjuna*, a plant belonging to the family *Combretaceae*. Here the ethanolic leaf-fruit extract of *Terminalia a rjuna* used to observe the cytotoxicity and antibacterial activity. To ascertain the bioactivity of the extract Brine shrimp lethality test was done and it was observed that $LC_{50}$ obtained for *T. arjuna* extract was 44.157 µg/ml, which was found to be quite lower than the previous studies, indicating that the prepared extract was rich in bioactive compounds. To observe antibacterial activity four Gram-negative and two Gram-positive bacteria were tested using agar well diffusion method. The results indicate that antibacterial activity of the extract were concentration dependent ranging from 0.5-10mg/ml. The striking and distinctive feature of observed antibacterial activity of *T. arjuna* extract is that it exhibited decent activity against the multi-drug resistant Gram-negative bacteria *Coliform s pp, Klebsiella pneumoniae, Pseudomonas aer uginosa* even at low concentrations( 3mg/ml). Minimum Inhibitory Concentration(MIC) was predicted for the extract and it was varied from 3-20mg/ml. [21]

Resistance modifying activities of plants crude extracts: the basis for isolation of potentially useful compounds. If the isolation of resistance modifying compounds from plants is to be realistic, screening for such activities in crude extracts is the first step in identifying leads for isolation of such compounds, and some plants have provided good indications of these potentials for use in combination with antimicrobial therapy. Aqueous extracts of tea (*Camellia sinensis*) have been shown to reverse methicillin resistance in MRSA and also, to some extent, penicillin resistance in beta-lactamase-producing *Staphylococcus aureus*. [22]

Antibiograms of 11 isolated bacteria (MDR) (*GPs, Enterococcus faecalis* and *Staphylococcus aureus*; and *GNs, Acinetobacter baumannii, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris* and *Pseudomonas aeruginosa*) were ascertained by the disc-diffusion method, and antibacterial effectivity of plant extracts (9 tropical flowering plants *Anogeissus acuminata, Azadirachta indica, Bauhinia variegata, Boerhaavia diffusa, Punica granatum, Soymida febrifuga, Terminalia chebula, Tinospora cordifolia* and *Tribulus terrestris*) was monitored by the agar-well diffusion method. This study examines effectivity of for possible use as source of antimicrobials for multidrug resistant (MDR) bacteria, along with main-stream antibiotics. Pathogenic bacteria were isolated from urine samples of patients attending and admitted in the hospital. Isolated bacteria were floridly MDR to most antibiotics of the day. Methanol extracts of 9 plants were used, and extracts of 3 plants, *A. acuminata, P. granatum* and *S. febrifuga* at least caused 25–29 mm as the maximum size of zone of inhibition on bacterial lawns. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of methanol extracts of 9 plants were recorded. The methanol extract of *A. acuminata* had 0.29 mg/ml as the lowest MIC value and 0.67 mg/ml as the lowest MBC value, against MDR *S. aureus*, signifying effectivity; but, it had the highest MIC value. [23]

The leaf extracts of *Psidium guajava, Phyllanthus niruri, Ehretia microphylla* and *Piper betle (P. betle)* showed antibacterial activity against the Gram-positive *methicillin-resistant Staphylococcus aureus* and *vancomycin-resistant Enterococcus. P. betle* showed the highest antibacterial activity for these bacteria in the disk diffusion (16–33 mm inhibition diameter), minimum inhibitory concentration (19–156 mg/mL) and minimum bactericidal concentration (312 mg/mL) assays. *P. betle* leaf extracts only showed remarkable antibacterial activity for all the Gram-negative multidrug-resistant bacteria (extended spectrum *b-lactamase-producing, carbapenem-resistant Enterobacteriaceae* and *metallo-b-lactamase-producing)* in the disk diffusion (17–21 mm inhibition diameter), minimum inhibitory concentration (312–625 mg/mL) and minimumbactericidal concentration (312–625 mg/mL) assays. [24]

## 6. Outlook of the New Study:

While there is an abundance of published data validating the antimicrobial activity of medicinal plants commonly used in folk medicine, this has not resulted in the identification of commercially exploitable plant derived antibacterial agents. The majority of plant derived antimicrobial compounds generally have higher MICs than

bacterial or fungal produced antibiotics, thus limiting their therapeutic potential. It has already been established that crude extracts of some medicinal plants and some pure compounds from such plants can potentiate the activity of antibiotics in vitro. This search for antibiotic resistance modulators in plants represents a new dimension to addressing the problem of antibiotic resistance. The chemical diversity available in plants still remains largely uninvestigated for potentials in improving the clinical efficacy of antibiotics. Most interestingly are medicinal plants and food plants which are inadvertently used with antibiotics in common community practices providing opportunities for interactions. As many medicinal plants still remain unexplored, there are enormous opportunities for the discovery of novel resistance modifying compounds of plant origins. Screening of antibiotic resistance modifying compounds from plants sources are expected to provide the basis for identifying leads for the isolation of therapeutically useful compounds. This could in future be followed by in vivo assessments to determine the clinical relevance of such compounds. This represents a potential area of future investigation.

## Reference

[1] Chanda, S., & Rakholiya, K. (2011). Combination Therapy□: Synergism Between Natural Plant Extracts And Antibiotics Against Infectious Diseases. *Science Against Microbial Pathogens: Communicating Current Research And Technological Advances*, 520–529.

[2] Sibanda, T., & Okoh, A. I. (2007). The Challenges Of Overcoming Antibiotic Resistance: Plant Extracts As Potential Sources Of Antimicrobial And Resistance Modifying Agents. African Journal Of Biotechnology, 6(25), 2886–2896.

[3] Farooqui, A., Khan, A., Borghetto, I., Kazmi, S. U., Rubino, S., & Paglietti, B. (2015). Synergistic Antimicrobial Activity Of Camellia Sinensis And Juglans Regia Against Multidrug-Resistant Bacteria. *Plos One*, *10*, E0118431.

[4] Patrick, Graham L. (2006). *An Introduction To Medicinal Chemistry* (First Indi, P. Part B Chapter 9 And Part D Chapter 16). Oxford University Press.

[5] Sumitra chandra, Kalpana Rakholiya, (2011). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. *science against microbial pathogens: communicating current research and technological advances A.mendez-vilas (Ed.)* , 520-529.

[6] Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A., Ali, S. M., … Khan, A. U. (2009). Antimicrobial Activity Of Five Herbal Extracts Against Multi Drug Resistant (Mdr) Strains Of Bacteria And Fungus Of Clinical Origin. *Molecules*, *14*, 586–597.

[7] Antibiotic resistant (2015). retrieved from Http://wikipedia/antibiotic-resistance, retrived date september 10, 2015

[8] WHO, Fact sheet N°194, (April 2014) retrieved from http://www.who.int/drugresistance/publications/infographic-antimicrobial-resistance-2014043

[9] Tomoo Saga, Keizo Yamaguchi (2009), History of Antimicrobial Agents and Resistant Bacteria. Japan medical association journal (JMAJ ) 52(2): 103–108.

[10] Ghaleb Adwan and mohammad Mhanna (2008). Synergic effects of plant extracts and antibiotics on Staphylococcus aureus strains isolated from clinical specimens. *Middle-East Journal of scientific research 3 (3): 134-139.*

[11] Sevil Toroglu (2011). In-vitro antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. Journal of Environmental Biology, 32 (1) 23-29.

[12] Aiyegoro Olayinka, Afolayan Anthony J And Okoh Anthony (2009). Synergistic interaction of Helichrysum pedunculatum leaf extracts with antibiotics against wound infection associated bacteria. *Biol Res 42: 327-338.*

[13] Frank stermitz, Peter Lorenz et al. (2000). Synergy in a medical plant: Antimicrobial action of berberine potentiated by 5' – methoxyhydnocarpin, a multidrug pump inhibitor. *Proceedings of the N ational A cademy of Sciences,vol.97 (4), 1433-1437.*

[14] Vijay N. Charde, Pratik M. Bezalwar, Ashok V. Gomashe (2014). In Vitro Management of MDR Pseudomonas Sp. By Combined Activity of Antibiotics and Foeniculum Vulgare Miller Extract. *International Jour nal of Research Studies in Biosciences (IJRSB), 2 ( 11),62-70.*

[15] Rehnuma Sharmeen, Md. Nazmul Hossain et al (2012). In-vitro antibacterial activity of herbal aqueous extract against multi-drug resistant Klebsiella sp. isolated from human clinical samples. *International C urrent P harmaceutical Journal , 1(6): 133-137.*

[16] Bassam abu-shanab, ghaleb adwan, dahood abu-safiya (2004), antibacterial activities of some plant extracts utilized in popular medicine in palestine. *Turk j biol, 28, 99-102.*

[17] U.S. Ezhil Arivudainambi, Thangarajan Durai Anand et al. (2011). Novel bioactivemetabolites producing endophytic fungus Colletotrichumgloeosporioides againstmultidrug-resistant Staphylococcus aureus. *FEMS Immunol Med Microbiol 61 (2011) 340–345.*

[18] Smith ECJ, Williamson EM et al (2007). Antibacterial and modulators of bacterial resistance from the immature cones of Chamaecyparis lawsoniana.*Phytochem. 68(2), 210-217.*

[19] Oluwatuyi M, Kaatz GW, Gibbons S (2004). Antibacterial and resistance modifying activity of Rosmarinus officinalis. *Phytochemistry. 65 (24), 3249-3254.*

[20] Shibata H, Kondo K, et al (2005). Alkyl Gallates, Intensifiers of β- Lactam susceptibility in Methicillin-resistant Staphylococcus aureus. *Antimicrob. Agents Chemother. 49(2),549-555.*

[21] Abdullah-Al-Emran, Farzana Ahmed et al (2011), Investigation of Antimicrobial Activity of Ethanolic Leaf- Fruit Extract of Terminalia arjuna against Multi-Drug Resistance (MDR) Bacteria in Bangladesh. *Journal of Applied Environmental and Biological Sciences. 1(5)90-95.*

[22] Stapleton PD, Shah S, Anderson JC Hara Y, Hamilton-Miller JMT, Taylor PW (2004). Modulation of □β-lactam resistance in Staphylococcus aureus by

catechins and gallates. International Journal of Antimicrob. Agents. 23(5): 462-467.

[23] Monali P. Mishra, Sibanarayan Rath, Shasank S. Swain et al (2015), In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. *Journal of K ing Saud Univer sity – Sci ence.* Retrieved from http://dx.doi.org/10.1016/j.jksus.2015.05.007

[24] Demetrio L. Valle Jr., Jeannie I. Andrade et al (2015), Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. *Asian Pacif c Journal of Tropical Biomedicine. 5(7): 532–540.*