



SYNERGISTIC ANTIBACTERIAL ACTIVITY OF FOUR MEDICINAL PLANTS COLLECTED FROM BIRBHUM, WEST BENGAL

ARNAB ROY 1 and ARABINDA NAYAK 1*

1 Gupta College of Technological Sciences, Asansol, West Bengal, India.

ABSTRACT-

Aqueous and ethanol extracts of leaves (1000 ppm) of four important plant species, *Cuminum cyminum*, *Foeniculum vulgare*, *Hemidesmes indicus* and *Catharanthus roseus* has been tested individually and in combination for their antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* through disc diffusion method. From the results we can say that *E.coli* was the most vulnerable bacteria among these bacteria was tested. After *E.coli*, *Salmonella typhi* was the most vulnerable; almost all plant extracts had activity against these bacteria. The most repellent bacteria was *Staphylococcus aureus*, plant extracts mostly ethanolic were effective. However synergistic activity was much more than the individual plant extracts. Individually, ethanolic extract of *Catharanthus roseus* had the highest zone of inhibition against *E.coli* about 1.7 cm. When two plants were mixed the highest activity was ethanolic extract of *Foeniculum vulgare* + *Hemidesmes indicus* against four bacteria like *Staphylococcus aureus* for zone of inhibition was 1.4cm, for *Bacillus subtilis*, zone of inhibition was 2.4cm. The highest activity was shown synergistically against *Escherichia coli* by the mixture of ethanolic extracts, *Cuminum cyminum* + *Hemidesmes indicus* + *Catharanthus roseus*, zone of inhibition was 2.7 cm amongst all of them.

Key words-Antimicrobial, medicinal plants, plant extracts, synergistic activity.

INTRODUCTION-

A magnificent discovery in the past century was antibiotics which pushed the medical world to a bright future. The invention of the first antibiotic accidentally found in the *Penicillium notatum* by Sir Alexander Fleming in 1928 revolutionized the medical world and changed the untreatable era of bacterial infection to treatable conditions [1]. Antibiotic word is a combination of two words "anti" and the Greek word bios (life) which means against life since they are produced by the microorganisms to kill or inhibit the growth of other microorganisms in low concentrations, however, this definition is changed over time. Antibiotics are mainly two types one that suppresses the growth (bacteriostatic) and another that kills the bacteria (bacteriocidal) [2].

Antibiotics were magical drugs until scientists found the penicillinase enzyme that drew the concept of resistance. Antibiotics are inevitable for chemotherapy however the efficacy is threatened since 1940 when the first evidence of a resistant enzyme was reported with this observation there was an urge to find new antimicrobial agents for containing the problem, over the years tetracyclines, chloramphenicol, aminoglycosides, macrolides, and other antibiotics are introduced [3].

Classification-There are many ways to classify antibiotics but the most common way to differentiate them is according to their molecular structure, mode of action, and spectrum of activity. Effectiveness, toxicity, and allergy potential side effects are similar concerning their classes. The most commonly used antibiotics are beta-lactams, cephalosporins and aminoglycosides [4].

Beta-Lactam-As we know that penicillin was the first discovered antibiotic in medical world so played a crucial beginning.

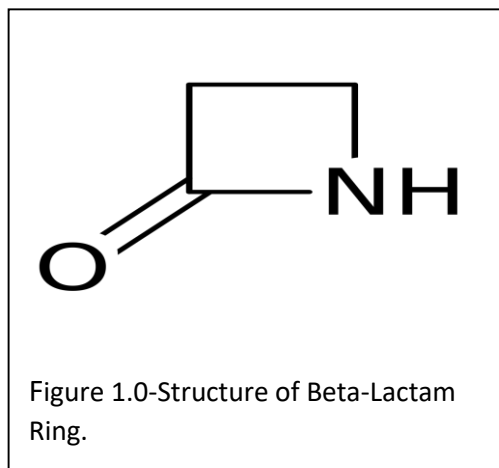


Table 1.0-different synthetic and plant based antibiotics are discovered during 1940-1960

Product class/ compounds	Discovery year
Para-aminosalicylic acid (PAS)	1943
Aminoglycosides (streptomycin)	1943
Cyclopeptides (bacitracin)	1943
Thiosemicarbazone (thiacetazone)	1944
Nitrofurans (nitrofurantoin)	1944
Cephalosporins	1945
Chloramphenicols (chloramphenicol)	1945
Tetracyclines (chlortetracycline)	1945
Diaminopyrimidines (trimethoprim)	1947/1956
Macrolides (erythromycin)	1942/1950b
Isoniazid	Around 1950
Pleurimutilin	1951

These antibiotics are having beta-lactam ring. Penicillins and cephalosporins are the major group of this class and monobactam and carbapenemes are relatively added lately. Members of this class of antibiotics contain a 3-carbon and 1-nitrogen ring that is highly reactive (FIGURE 1.0) [5]. All beta-lactam medications have a four-member ring, which is a stretched, cyclic amide that is extremely vulnerable to chemical or enzymatic hydrolysis. When the ring is broken, the hydrolyzed b-lactam medicines produce an inactive substance. Penicillin and other beta-lactam antibiotics degrade in acidic and alkaline environments or through interactions with weak nucleophiles like water or metal ions [6]. As an alternative, the beta-lactamase enzyme can hydrolyze penicillin through the same process as acid hydrolysis.

Penicillin-

The Penicillin nucleus consists of fused thiazolidine and beta-lactam rings to which side chains are attached through amide linkage penicillin G is the original penicillin clinically, having a benzyl side chain [7].

Mechanism of Action-

These bactericidal drugs act by binding to certain receptors (Penicillin Binding Proteins , PBPs), hence they are cell wall synthesis inhibitors therefore they inhibit the transpeptidase enzyme responsible for the cross linking of peptidoglycan chains. Peptidoglycans is a polymer that almost act cell wall outside the plasma membrane made up of sugars and amino acids essential for cell wall.

In the presence of these drugs bacteria cannot make the cell wall and die due to water imbibitions. Bacterias like mycoplasma not having the cell wall are very easy to produce resistance towards these drugs [8].

Present source of penicillin is *P.chrysogenam* a since naturally.

Occurring penicillin has many limitations.

Limitations-

1. Narrow spectrum of activity
2. Not orally active
3. Short duration of action
4. Have hypersensitivity activity
5. Vulnerable to penicillinase (Beta-lactamase)

For preventing tubular secretion now a days probenecid is added and particularly, some penicillin, like augmentin, are created in conjunction with non-antibiotic substances that can impede the function of the bacterial penicillinase enzyme. Actually, augmentin is a medication that contains amoxicillin (antibiotic) and clavulanic acid that has the ability to prevent the beta-lactamase enzyme from extending the duration of amoxicillin's antibacterial action [9].

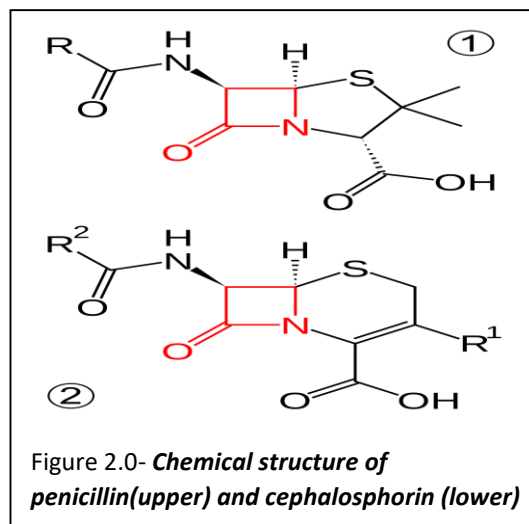


Figure 2.0- **Chemical structure of penicillin(upper) and cephalosporin (lower)**

Cephalosporins-

These are a group of semi synthetic antibiotics derived from cephalosporin-c obtained from a fungus *cephalosporium*. A structural similarity is found between penicillin and cephalosporins are that they have the same beta-lactam ring but in cephalosporins the ring is fused with dihydrothiazine.

History-

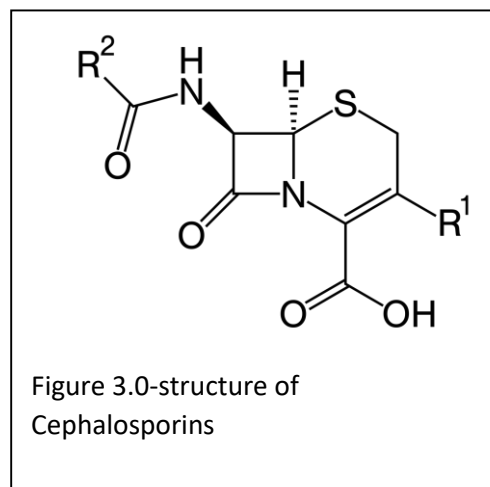
Guisepe Brotzu initially identified the earliest recognized member of this class of antibiotics from the fungus *cephalosporium acremonium* in 1945. Although Edward Abraham was the one who was able to extract the component, Guisepe Brotzu was the one who initially isolated the medicine. According to the target organism, they are separated into generations (1st–5th), however later versions become increasingly more effective against Gram-negative infections.

Mechanism of Action-

Cephalosporins feature a variety of side chains that allow bind to various penicillin-binding proteins (PBPs), bypass brain barrier, withstand degradation by bacterial strains produce penicillinase, and ionise to make it easier to enter negative bacterial cells [10,11].

Indications-

The majority of Gram-positive cocci are covered by first-generation cephalosporin but some of the exceptional Gram-negative bacteria are *E. coli*, *P. mirabilis*, and *K. pneumonia*. Second-generation cephalosporins are much more towards Gram-negative bacteria like *M. catarrhalis* and *H. influenza*. Third-generation cephalosporins are those which have increased coverage against Gram-negative bacteria but decreased coverage against the majority of Gram-positive bacteria, they are mainly effective towards *Enterobacteriaceae*, *Neisseria spp.*, and *H. influenza*. Fourth-generation cephalosporins are like third generation but providing better protection against Gram-negative bacteria with antibiotic resistance, such as those bacteria that produce the enzyme beta-lactamase. Methicillin-resistant Staphylococci and penicillin-resistant Pneumococci are both covered by fifth-generation cephalosporins [12].



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Lower respiratory tract infections can be treated first with second generation cephalosporins. cephalosporins should be taken with an aminoglycoside, ureidopenicillin, or quinolone in the event of a serious infection. In lower respiratory tract infections, cefuroxime has demonstrated good clinical effectiveness and tolerance [13].

Adverse Effects-

1. Hypersensitivity Reaction
2. Drug-induce Immune Hemolytic Anemia (DIIHA)
3. Vitamin K Deficiency
4. Increase Nephrotoxicity of Aminoglycosides

5. Pseudomembranous Colitis [14].

Carbapenems-

After realization the potential of beta-lactamase enzyme which can easily produce resistance against penicillin hence the efficacy was greatly threatened creating an emergency situation. This ominous scenario prompted scientists to start search for beta-lactamase inhibitors on a large scale [15].

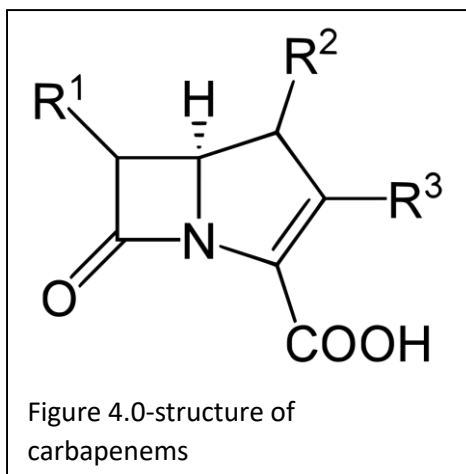
History-

Scientists in 1976, efforts paid off when olivanic acids, produced by the Gram-positive bacteria *Streptomyces clavuligerus* inhibits beta-lactamase. Unfortunately, since these acids were unstable chemically, they could not effortlessly enter the bacterial cell. These obstacles caused a slowdown.

More research on the olivanic acids is done after important to note that two Better beta-lactamase been found. These clavulanic acids came from *S* well as thienamycin from *Streptomyces cattleya* isolated reports, thienamycin was the first "carbapenem"

Chemical Structure-

The 4:5 fused ring lactam of penicillins with a double C-2 and C-3 but with carbon replacing sulphur at C-1 is "carbapenem." Thienamycin's hydroxyethyl side chain departure from the traditional penicillin and cephalosporin structures, which all have an acylamino substituent on the beta-lactam ring; the stereochemistry of this hydroxyethyl side chain is a key characteristic of carbapenems and is crucial for activity



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Indications-

These classes have wide spectrum of activity including Gram-positive Cocci, Gram-negative rods well as anaerobes. These drugs should be used with Aminoglycosides to treat Pseudomonas infections. Advantage of using Carbapenems is that they are resistant to beta – lactamase enzyme and also reliably efficacious against ESBL (extended spectrum Beta-lactamase) producing organisms hence they are drug of choice for ESBL-producing bacteria [19].

Adverse Effects-

1. Cholestatic liver injury
2. Skin Rashes
3. Hypersensitivity Reaction
4. Vomiting [20].

Monobactams-

25 years ago, the first N-thiolated beta-lactams were obtained from natural sources by two separate groups. Because they lack a carboxylic acid component and contain a flexible monocyclic ring attached directly to the beta-lactam ring, they were given the name "Monobactams".

History-

The first monobactam was launched under the name 1984. Carumonam, pirazmonam, and tigemonam substances. Monobactams are almost solely against Gram-negative bacteria, and their antipseudomonal activity is what makes them clinical settings [21].

It was first announced that this class of antibiotics discovered by Skyes and her colleagues. The was acquired from the *Chromobacterium violaceum*

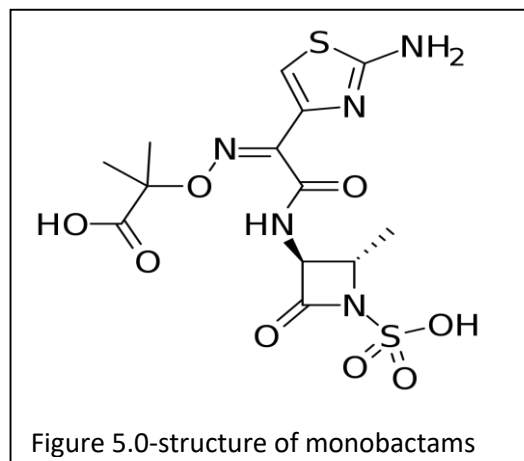


Figure 5.0-structure of monobactams

aztreonam in are other effective valuable in had been medication bacteria [22].

Indications -

Most commercially successful drug in this class is **aztreonam**. Aztreonam has been proven to be beneficial in clinical studies for the treatment of Gram-negative infections, such as uncomplicated Gonorrhoea, Septicemia, intra-abdominal, joint and bone, skin, and soft tissue infections, urinary tract infections, and lower respiratory tract infections [23].

Macrolides-

History-

In 1952 scientists saw the discovery and isolation of J. an M. McGuire metabolic byproduct of a fungus that lives in soil *Erythraean saccharopolyspora*, this fungus used to be referred to as *Streptomyces erythraeus*, a member of the actinomycete bacteria belong to the genus Saccharopolyspora.

Chemical Structure-

Characteristics of macrolides include 14-, 15-, or 16-lactose rings with a peculiar deoxy linked L-cladinose desosamine sugars (figure 5.0). They have broader antibacterial activity than penicillin and very useful to patients who have the allergy to the penicillin. Several agents, such as antibiotics, antifungal medications, and immunosuppressants, are included in this family substances [24].

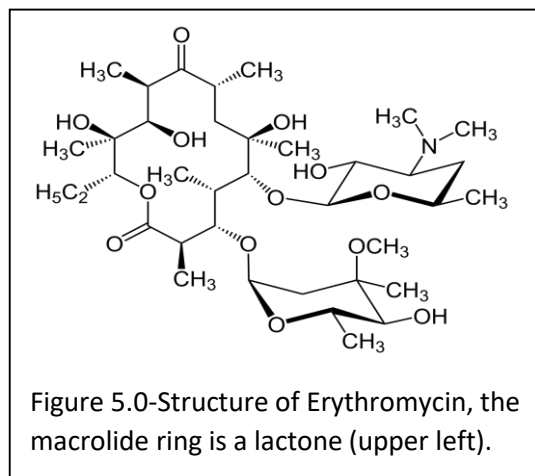


Figure 5.0-Structure of Erythromycin, the macrolide ring is a lactone (upper left).

macrocyclic and D-spectrum of those bioactive prokinetics, of

Mechanism of Action-

Macrolides either eliminate or prevent microbial growth by efficiently preventing the creation of bacterial proteins, by attaching to the bacterial ribosome, preventing polypeptide chains from gaining more amino acids when protein is being produced. More succinctly, they bind 50s ribosome and block the translocation of peptide chain from A (acceptor) to peptidyl (p) site [25].

Indications-

The majority of their antibacterial activity is directed towards Gram-positive cocci and unusual infections, and they have great tissue penetration. The concentrations of macrolides in epithelial lung fluid are at least ten times greater than those in serum.

Even so, macrolides are typically wide-ranging spectrum, some strains of bacteria like Streptococcus but the bacteria Pneumoniae are resistant to antibiotics, Erythromycin, as an example [26]. Macrolides are frequently employed in the treatment of Chlamydial and Gonococcal infections, two sexually transmitted diseases. Like other antibiotics, the majority of macrolide use is determined by the target organism's susceptibility and resistance status.

Macrolides have also been a mainstay in the treatment of atypical pneumonia, which is typically brought on by pathogens like *Mycoplasma pneumoniae*, Legionella, and *Chlamydia pneumoniae*.

Adverse Effects-

Macrolides frequently accumulate in the liver which can recycle it back into the bile. Additionally, they have the ability to trigger inflammation. Clinical professionals typically advise dispensing small doses [27].

Aminoglycosides-

Aminoglycosides are strong, all-purpose antibiotics that work by preventing the creation of new proteins.

History-

Since streptomycin was initially identified from *Streptomyces griseus* and put into clinical use in 1944, the class has been a cornerstone of antibacterial chemotherapy. Over the years many drugs in this class were introduced like neomycin (identified from *S. fradiae* at 1949), kanamycin (identified from *S. kanamyceticus* at 1957), gentamicin (identified from *Micromonospora purpurea* at 1967), netilmicin (derived from sisomicin at 1967), tobramycin (derived from *S. tenebrarius* at 1967), and amikacin were among the additional members that were introduced over the years (derived from resistance of the class after so many efforts arbekacin and plazomicin were discovered [28]).

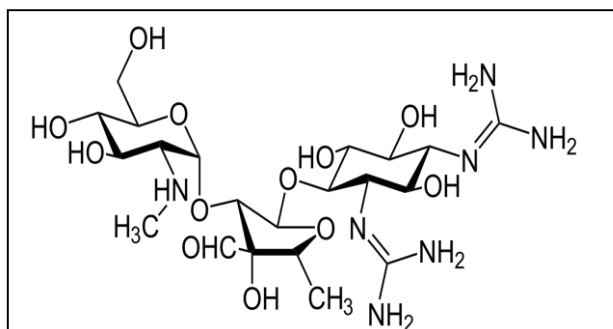


Figure 6.0- structure of Aminoglycosides

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Mechanism of Action-

The antibacterial spectrum of aminoglycosides is very extensive. By attaching to a ribosomal subunit, they can prevent bacteria from synthesising proteins. More specifically they bind to 30S and 50S ribosomes and freeze initiation, interfere with polysome formation and cause misreading of mRNA code [29].

Indications-

Various Gram-positive and Gram-negative microorganisms can be abolished by aminoglycosides. The Enterobacteriaceae family, which includes *Serratia spp.*, *Proteus spp.*, *Klebsiella pneumonia* even also *Morganella spp.*, *Providencia spp.*, and *Escherichia coli* are particularly sensitive to aminoglycosides. Additionally, aminoglycosides are effective against the pathogens that cause the plague (*Yersinia pestis*) and tularemia (*Francisella tularensis*)

The class also effectively combats isolates of *Staphylococcus aureus* that are methicillin- and vancomycin-resistant, as well as *P. aeruginosa* and, to a lesser extent, *Acinetobacter baumannii*. Numerous species of Mycobacterium, such as *Mycobacterium tuberculosis*, *M. fortuitum*, *M. chelonae*, and *M. avium*, are also vulnerable to aminoglycosides [30].

Adverse Effects-

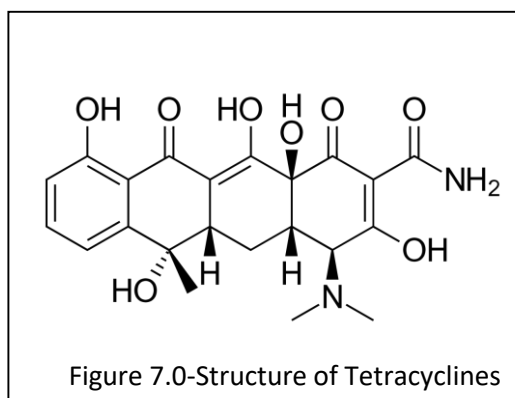
1. **Ototoxicity-** Ototoxicity is the pharmacological adverse reaction affecting the inner ear or auditory nerve, characterized by cochlear or vestibular dysfunction.
2. **Nephrotoxicity-** Nephrotoxicity is defining as rapid deterioration in the kidney function due to toxic effect of medications and chemicals.
3. **Neuromuscular Blockade-** Neuromuscular-blocking drugs block neuromuscular transmission at the neuromuscular junction, causing paralysis of the affected skeletal muscles. This is accomplished via their action on the post-synaptic acetylcholine receptors [31].

Tetracyclines-

A group of broad-spectrum antibiotics known as tetracyclines are used to manage and treat a number of infectious illnesses. Groups are divided according to their origin.

History-

Benjamin Duggar first found tetracycline in a soil genus *Streptomyces* in 1945[32]. Chlorotetracycline was this class's original member. Members of this class are identified by the suffix "-tetracycline" and feature four (4) hydrocarbon rings (figure 7.0).



bacterium of the genus *Streptomyces* (Aureomycin). Chlorotetracycline and

Classification-

Tetracycline, chlortetracycline, oxytetracycline, and demeclocycline are medications in this class that are naturally occurring. Lymecycline, methacycline, minocycline, rolitetracycline, and doxycycline are semi-synthetic tetracyclines. There is a substance from the glycylicycline subclass called tigecycline. Ervacycline, sarecycline, and omadacycline are among a group of more recent tetracyclines in this group [33].

Mechanism of Action-

The ribosome is their target for antibacterial action against bacteria. They prevent this bacterial organelle from synthesizing proteins by preventing the addition of amino acids to polypeptide chains. They bind to 30S ribosomal subunit and inhibit the binding of aminoacyl-tRNA to the A (acceptor) site [34].

Indications-

These medications can be used to treat a variety of illnesses, including rickettsial infections, ehrlichiosis, anaplasmosis, leptospirosis, amebiasis, actinomycosis, nocardiosis, brucellosis, melioidosis, tularemia, chlamydial infections, pelvic inflammatory disease, syphilis, traveler's diarrhoea, early Lyme disease, acne. They cover *Vibrio vulnificus*, *Borrelia recurrentis*, *Mycobacterium marinum*, *Mycoplasma pneumoniae*, and *Staphylococcus aureus* (including Methicillin-resistant *S. aureus*)[MRSA] (susceptible strains), Prophylaxis against Meningococci is also possible [35].

Adverse Effects-

Tetracyclines should be taken by patients before or after meals for improved absorption. Tetracyclines have been demonstrated to induce tooth discolouration in patients younger than eight (8) years of age, hence all of them are only advised for those over this age [36].

Quinolones-

Members of this family of antibiotics have a complicated nomenclature. Members of this class of antibiotics have a complicated name, although they are frequently referred to by the suffix-oxacin, such as floxacin, ciprofloxacin, and levofloxacin [37].

History-

Scientists looking for antimalarial medications originally found this class of antibiotics called nalidixic acid. In the early 1960s, nalidixic acid was found to be a quinine development process contaminant. Since its discovery in the early 1960s, its basic structure has undergone a number of alterations, which have facilitated the invention and synthesis of numerous derivatives with proven antibiotic efficacy.

Classification-

The basic molecule has given rise to two main classes of compounds: quinolones and naphthyridones, which include the antibiotics cinoxacin, norfloxacin, ofloxacin, ciproxacin, and temafloxacin, sparfloxacin, nalidixic acid, enoxacin etc. Recent generations of quinolones have an additional ring structure that allows them to extend their antibacterial spectrum of activity to some particularly anaerobic bacteria that were previously to quinolones[38].

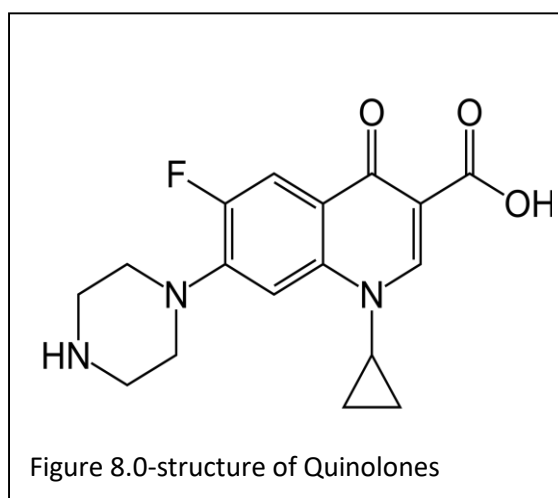
Mechanism of Action-

Quinolones are bactericidal antibiotics that kill bacteria destroying them directly. They work by blocking the of bacterial type II topoisomerases, DNA gyrase, and topoisomerase IV, turning them into hazardous that result in long-lasting double-strand breaks in the chromosome of the bacteria. For the bacteria to normally, DNA topoisomerases are necessary for processes like DNA replication, transcription, recombination, and condensed DNA remodeling [39].

Adverse Effects-

The most frequently reported quinolone side effects are gastrointestinal ones, like nausea, vomiting, and diarrhea. Due to the quinolones's impact on the gut flora, antibiotic-associated colitis is a frequent side effect of quinolone treatment. Compared to other antibiotics, the use of quinolones may lead to greater rates of *Clostridium difficile* infection.

Arthralgias are another frequent side effect that goes away on its own when medication is stopped, especially in the paediatric population. Additionally, some patients could have severe anaphylaxis and cutaneous reactions, ranging from a mild rash and/or photosensitivity to Stevens-Johnson syndrome or toxic epidermal necrolysis [40].



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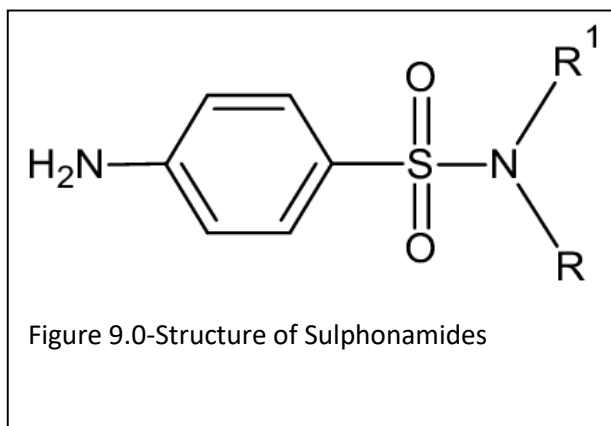
function

Sulphonamides-

According to reports, sulphonamides were the first class of antibiotics employed in therapeutic treatment, and they continue to play a crucial role in both human and veterinary care. Sulphonamides are widely used to treat a variety of infections, including tonsillitis, septicemia, meningococcal meningitis, bacillary dysentery, and some urinary tract infections [41].

Chemical Structure-

The conventional structure of a tertiary SN aniline group with R¹/R that can also be alkyl, an aryl, or a heteroaryl group, as well sulphur atom with two doubly bound additionally bonded to a nitrogen atom substituted amine).



consists of an hydrogen, an as a central oxygens that is (existing as a

Mechanism of Action-

Sulphonamides are structural counterparts competitive antagonists in the synthesis of of PABA and folic acid, which is necessary for the continued creation of DNA in bacteria. Given that SN and PABA have similar chemical structures, SN can inhibit and take the place of PABA in the enzyme dihydropteroate synthetase, whose activity is crucial for the production of folate. This prevents the formation of dihydrofolate and tetrahydrofolate, which in turn prevents bacterial DNA growth and cell division or replication [42].

Indications-

Since the drug's side effects prevent cell division, SN medicines are bacteriostatic rather than bactericidal. The sulphonamide medication sulfamethazine (SMZ), which serves as an antibacterial agent to treat livestock illnesses such gastrointestinal and respiratory tract infections, is frequently used in veterinary medicine.

Another often used sulphonamide medicine is sulfadiazine (SDZ), which is used with the anti-malarial medication pyrimethamine to treat toxoplasmosis in warm-blooded animals. They inhibit both Gram-positive and Gram-negative bacteria, including *Nocardia*, *E. coli*, *Klebsiella*, *Salmonella*, *Shigella*, and *Enterobacter*, as well as *Chlamydia trachomatis* and some Protozoa. Sulphonamides can obstruct malignant cell agents, according to studies.

The original antibacterial sulphonamide is a class of synthetic antimicrobial drugs that include the sulphonamide group (also spelled sulfonamide by some Workers).

Adverse Effects-

1. Hemolysis
2. Aplastic anemia
3. Crystalluria

4. Hematuria [43].

Oxazolidinones-

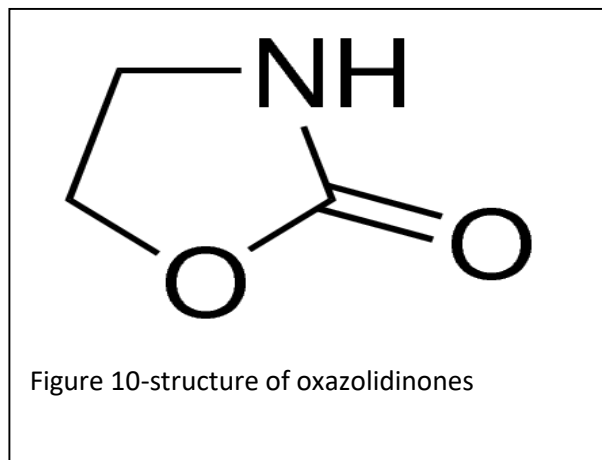
A class of synthetic antibiotics known as oxazolidinones has just given usage approval. Only in 2000 was linezolid, the first member to be synthesized, given approval for use in clinical trials .

History-

The first oxazolidinones were synthesized at de Nemours & Co. in the middle of the 1980s, were subsequently used by Pharmacia (now New York, NY, USA). They are a totally class of bacteriostatic drugs that have no natural product compounds.

Chemical Structure-

The oxazolidone ring with the S configuration substituent at C5, the acylaminomethyl group C5, and the N-aryl substituent are specifically included in the pharmacophoric core (ring B). The para-substitution broadens the antibacterial range, whereas the meta-fluoro substitution on the B ring improves biological activity [44].



E.I. du Pont and they Pfizer Inc., synthetic congeners in

Indications-

In-vitro activity (generally bacteriostatic) against numerous significant resistant pathogens, such as meticillin-resistant *Staph aureus*, vancomycin-resistant Enterococci, and penicillin-resistant *Strep. Pneumoniae*, is demonstrated by linezolid. In addition to having a nearly perfect oral absorption and favourable pharmacokinetic and toxic effect profiles, linezolid is a parenteral medication. Clinical studies support the efficacy of linezolid in the treatment of vancomycin-resistant Enterococci infections, skin and soft tissue infections, and pneumonia. For the treatment of severe infections brought on by Gram-positive bacteria that are resistant to glycopeptides and streptogramin, linezolid holds promise as an alternative [45].

Adverse Effects-

Although following the regular recommended procedures for taking linezolid is typically safe, cases when therapy is prolonged are more likely to result in adverse effects such as myelosuppression, which can cause anaemia and thrombocytopenia [46].

Glycopeptides-

Originally derived as natural compounds, glycopeptide antibiotics—often abbreviated as GPAs—have evolved over the past 20 years into semi-synthetic derivatives with better action and pharmacokinetic characteristics. Glycopeptides are naturally created from a cyclic peptide of 7 amino acids to which 2 sugars are attached, hence the term glycopeptides. Semi-synthetic derivatives of natural products make up second generation GPAs [47].

History-

Yim and Associates do a good job of presenting of different types of glycopeptides. The Food Administration (FDA) first gave telavancin approval for clinical use in 2009. This derivative differs from the parent substance by hydrophobic and a hydrophilic group to the structure.

Indications-

Life-threatening infections brought on by resistant Gram-positive organisms like *Staphylococcus aureus*, Enterococcus species, *Clostridium difficile* are routinely treated using antibiotics (GPAs). They are last-resort for treating Methicillin-resistant *Staphylococcus aureus* (MRSA), which is now a major contributor to both hospital-acquired infections and community-acquired infections and is associated with high rates of morbidity and mortality. First-generation GPAs are organic compounds made from a variety of actinomycetes that have had their non-ribosomal heptapeptides glycosylated.

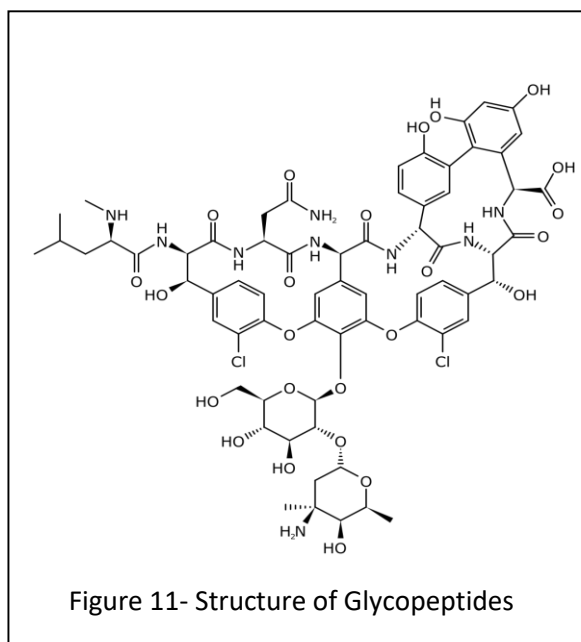


Figure 11- Structure of Glycopeptides

the structures and Drug (Vibativ) adding a vancomycin multidrug- and glycopeptide medications

Adverse Effects-

1. Nephrotoxicity
2. Ototoxicity
3. Red Man Syndrome [48]

Resistance to Antibiotics-

The medical community believed that once antibiotics were developed, the war against infectious diseases was over. The battle has, however, appeared to turn in favour of the bacteria as a result of the widespread development of antimicrobial agent resistance among bacteria. In the world, infectious diseases are today a major cause of morbidity and mortality. In the top 10 causes of morbidity and mortality, according to a World Health Organization (WHO) assessment of these illnesses, are lower respiratory infections, diarrheal illnesses, HIV/AIDS, and malaria [49].

Antimicrobial resistance has greatly exacerbated the effects of infectious diseases, increasing the number of infections as well as the expense of healthcare. There is established antimicrobial resistance to every one of the many antimicrobial medicines we have at our disposal for possible infection therapy, and this resistance develops

quickly when a new drug is given the go-ahead. The WHO's Global Action Plan on Antimicrobial Resistance was introduced in 2015 as a result of these worries [50].

It would be beneficial to distinguish between resistance and persistence before going over the various components of antibiotic resistance. Any progeny cells of a bacteria that is resistant to one antimicrobial agent would likewise be resistant (unless additional mutations occurred in the meantime). Nevertheless, persistence refers to bacterial cells that lack resistance genes yet are not drug-susceptible. The persistence is certainly caused by the possibility of latent (stationary growth phase) bacterial cells in bacterial populations, which are generally unaffected by most antimicrobial drugs [51, 52].

Origin of Resistance-

Bacteria as a class or species are not always equally vulnerable to or resistant to a given antibiotic. Within bacterial groups that are closely related, resistance levels might vary substantially. The least drug concentration necessary to stop bacterial growth is known as the minimum inhibitory concentration, or MIC, and it is used to determine both susceptibility and resistance. The range of average MICs for a specific antibiotic across the same bacterial species makes up the susceptibility in actuality. A species is deemed to have intrinsic resistance to a medicine if its average MIC falls within the range of resistance. The degree of resistance will vary based on the species and the genes gained, as bacteria may also acquire resistance genes from other related organisms [53, 54].

Natural Resistance-

Natural resistance may be intrinsic (present in all members of the species) or inherent (the genes are naturally occurring in the bacteria, but are only expressed to resistance levels after exposure to an antibiotic). A feature known as intrinsic resistance can be described as being universally present within a bacterial species, unaffected by prior antibiotic exposure, and unconnected to horizontal gene transfer. Reduced outer membrane permeability (most notably caused by lipopolysaccharide, or LPS, in Gram-negative bacteria) and naturally occurring efflux pumps are two of the most frequent bacterial processes implicated in intrinsic resistance. Induced resistance is frequently caused by multidrug-efflux pumps as well [55, 56].

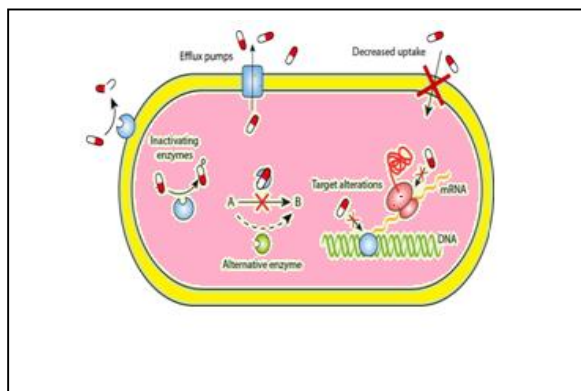
Acquired resistance- It is possible for bacteria to acquire genetic material that imparts resistance by transformation, transposition, and conjugation (all referred to as horizontal gene transfer, or HGT), as well as through alterations to their own chromosomal DNA. The purchase could be either short or long-term. The most frequent way to acquire foreign genetic material is by plasmid-mediated transmission of resistance genes; bacteriophage-borne transmission is rather uncommon. Certain bacteria, including *Acinetobacter* species, are naturally competent and can thus take genetic material straight from their environment. Genetic material may be moved internally by integrins and insertion sequences, and stressors (such as hunger, UV radiation, chemicals, etc.) on the bacteria are frequently responsible for genetic changes (substitutions, deletions etc.). Bacteria typically experience one mutation per 10^6 to 10^9 cell divisions, and the majority of these mutations are detrimental to the cell [57].

Mechanism of Resistance-

Bacteria have developed a number of ways that make them resistant to antibiotics. These processes can alter the antibiotic chemically, make it inactive by physically removing it from the cell, or alter the target site so that the antibiotic does not recognize it.

❖ Methods –

- A. Limiting uptake of a Drug.
- B. Modifying Drug target.
- C. Inactivating the Drug.
- D. Active Drug efflux [58].



➤ Limiting uptake of a Drug.

The capacity of bacteria to restrict the uptake of antimicrobial drugs varies naturally. Some kinds of chemicals are blocked by the Gram-negative bacteria's LPS layer's structure and activities. Because of this, certain bacteria have inbuilt resistance to specific classes of powerful antimicrobial medicines [59].

Due to the high lipid content of the outer membrane of mycobacteria, hydrophobic drugs—such as rifampicin and fluoroquinolones—have easier access to the cell than hydrophilic ones [60].

Hence, all medications that target the cell wall, such as beta-lactams and glycopeptides, are fundamentally resistant to bacteria that lack a cell wall, such as mycoplasma and related species. Drug access restrictions are less common for Gram-positive bacteria because they lack an outer membrane.

Example-

Substances frequently enter cells in big outer membrane bacteria through porin channels. Hydrophilic compounds can typically access Gram-negative bacteria through their porin channels. Porin mutations that alter the selectivity of the porin channel and decreased porin abundance are the two main ways that porin alterations can restrict medication absorption. It is known that members of the Enterobacteraceae family can develop resistance by having fewer porins (and sometime stopping production entirely of certain porins). These bacteria all work together to decrease the quantity of porins as a defence against carbapenems [61].

➤ Modifying Drug target-

There are several parts of the bacterial cell that may be targeted by antimicrobial substances, and there are just as many targets that the bacteria could modify to confer resistance to those medications. Changes in the structure and/or number of PBPs are one method of resistance to the beta-lactam medicines utilised almost exclusively by Gram-positive bacteria (Penicillin-Binding Proteins). PBPs are transpeptidases that help build the peptidoglycan that makes up the cell wall [62].

The amount of drug that can bind to that target is affected by changes in the number of PBPs (increase in PBPs with decreased drug binding ability or decrease in PBPs with normal drug binding). The capacity of the medicine to bind may be reduced or completely inhibited by a structural alteration (such as PBP2a in *S. aureus* after the *mecA* gene was acquired) [63].

➤ Drug inactivation-

Bacteria can inactivate medications in one of two ways: by actually degrading the drug, or by adding a chemical group to the drug. The broad category of drug-hydrolyzing enzymes known as beta-lactamases. Tetracycline can also be rendered inactive by hydrolyzation through the tetX gene [64].

The most often used chemical groups for drug inactivation through transfer to the drug are acetyl, phosphoryl, and adenylyl. Transferases have been found to exist in huge numbers. The most widely utilised method is acetylation, which has been shown to be effective against aminoglycosides, chloramphenicol, streptogramins, and fluoroquinolones. It is well known that the aminoglycosides are the main targets of phosphorylation and adenylation [65].

▪ Beta-lactamase-

The beta-lactam class of antibacterial agents is the most popular. This drug class's members all have a core structure in common that is made up of a four-sided beta-lactam ring. Three common pathways account for resistance to beta-lactam medications: (1) blocking the interaction between the drug and the target PBP, typically by altering the drug's affinity for the PBP (this is accomplished by adding new PBPs to the system or changing existing PBPs); (2) the presence of efflux pumps that can expel beta-lactam drugs; and (3) hydrolysis of the drug by beta-lactamase enzymes [66, 67].

➤ Drug Efflux-

The genes for efflux pumps are found on the chromosomes of bacteria. Some are constitutively produced, while others are triggered or over expressed in response to specific environmental cues or when an appropriate substrate is present (high-level resistance is typically caused by a mutation that alters the transport channel). The main purpose of the efflux pumps is to remove hazardous molecules from the bacterial cell, and many of these pumps will move a wide range of compounds (multi-drug [MDR] efflux pumps). What carbon source is available affects the resistance capability of many of these pumps.

Most bacteria have a wide variety of efflux pumps. The ATP-binding cassette family, the multidrug and toxic compound extrusion (C) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family are the five main families of efflux pumps in bacteria, which are categorised based on structure and energy source [68,69].

In every region of the world, antibiotic resistance is increasing to dangerously high levels. Our ability to cure widespread infectious diseases is being threatened by the emergence and global dissemination of new resistance

mechanisms. As antibiotics lose their effectiveness, a rising number of infections, including gonorrhoea, blood poisoning, pneumonia, and tuberculosis, are become difficult to cure and occasionally becoming incurable [70].

Scientists are looking for an alternative option to tackle this emergence situation, When natural products are utilised correctly, they can be a powerful alternative to the synthetic medicine.

Globally, the use of herbal medicine has been increasingly popular in recent years. These products are not covered by the Food and Drug Administration's regulations and are not examined to the same extent as conventional medications. Patients frequently combine well-known medicines with herbal supplements.

Being a natural medicine , there is a advantage that they cannot produce any side effects in any form of administration effects unlike other synthetic medicines also they have naturally acquired antioxidants that will make it effective against most of the bacteria.

The majority of the research found synergy both within plants and between plants and antibiotics, according to the in vitro data. It has been demonstrated that combined chemicals and whole plant extracts are more potent antimicrobials than isolated components.

Common name of *Cuminum cyminum* is Jira(Cummin), belonging to the family Umbelliferae. Cummin fruits contain 2.5-4% volatile oil(falvonoids), 10% fixed oil, and proteins. Volatile oil contains trace amounts of cuminic alcohol, hydrated cuminaldehyde, and hydro-cuminine, but mostly 30-50% cuminaldehyde (chief constituent), the activities are – Stimulant, Carminative and Anti-diarrheal .

Common name of *Foeniculum vulgare* is Fennel, belonging to the Umbelliferae. Fruits of Fennel consists of 3-7 per cent volatile oil(flavonoids) is a ketone, fenchone(about 20%) and a phenolic ether anethole (about 50%) have most of pharmacological activity due to Fenchone (Chief constituent),the activities are Carminative, Aromatic, Stimulant, expectorant and Flavoring agent.

Common name *Hemidesmes indicus* is Anantmool, belonging to family Asclepladaceae. Roots are the most prominent source of the glycosides, tannins and saponins and the chief constituent vanillin about 0.2% vol. The pharmacological activities are -Flavouring agent, Blood purifier, Anti-inflammatory tonic and Increase lactation in cows.

Common name of *Catharanthus roseus* is Nayantara(Madagascar periwinkle), belonging to the family of Apocynaceae . Roots of this plant is the source of two major alkaloids Vincristine and vinblastine use to treat cancer, the pharmacological activities Anti-cancer, Diabetes, Sore throat and Eye irritation.



MATERIALS -

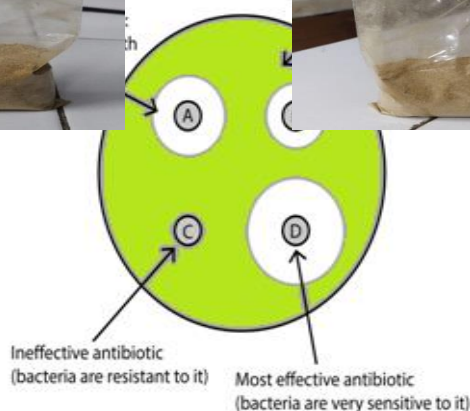
- ❑ *Cuminum cyminum*
 - Seeds
- ❑ *Foeniculum vulgare*
 - Seeds
- ❑ *Hemidesmes indicus*
 - Roots
- ❑ *Catharanthus roseus*
 - Roots

Methodology –

- ✓ Disc method (zone of Inhibition)

Plant material-

- ✓ In the present study, different parts of medicinal plants namely, the seeds of *Cuminum cyminum*, the seeds of *Foeniculum vulgare*, the roots of *Hemidesmes indicus* and the roots of *Catharanthus roseus* were collected in Nov 2022 from Birbhum district West Bengal the plant was authenticated from Gupta College of Technological Sciences Asansol (Voucher specimen no.YMA 3) by Dr. Manik Baral.
- ✓ The collected plant parts were dried for one week.
- ✓ Ground into a coarse powder with the help of a suitable grinder.
- ✓ The powder was stored in an airtight container and kept in a cool, dark and dry place.



Preparation of different part extracts- The crude extracts of the several plant species were produced separately using ethanol (95% ethanol) and distilled water, as detailed below.

➤ Solvent extraction :

1. 50 gm of different parts of each plants was taken in 250 ml beaker
2. 100 ml of ethanol (95%) was added

3. Mixture of ethanol-plant parts were kept at room temperature (48 h)
4. Rapidly stirred using glass rod (every 8 h)
5. Filtered through Whatman No. 1 filter paper (after 48 h)
6. Each filtrate was concentrated by using vacuum evaporator.



*Mixing
Filtering*

Aqueous extraction:-

1. 50 g of air-dried powder
100ml distilled water
2. Boiled for 6 hours
3. Filtered through eight layers of muslin cloth (2 h.)
4. Centrifuged at 5000 rpm for 15 min.
5. Supernatant was collected and concentrated.
6. A greasy final material obtained for each plant.



Concentrated Greasy Material

of each plant parts was placed In

Boiling

Centrifuging

Preparation of stock

1. 200 each mg standard electronic
2. 5 ml of both extracts
3. One or two
4. Each extract was made up to 200ml adding by distilled water
5. Stock solution is formed of 1000 ppm and stored.



Concentrated Greasy Material

and test solutions -

of both extracts was taken in a measuring flask by using balance ethanol and water is added to the respectively drops of emulsifier were added



5 ml of ethanol is added



stock solution(1000 ppm)

Bacterial susceptibility testing

❖ Bacterial culture-The antibacterial activity test involved four bacterial species: *Staphylococcus aureus*, *Bacillus subtilis* [Gram-positive], *Escherichia coli*, *Salmonella typhi* [Gram-negative]. Before usage, the organisms were sub-cultured for 24 hours while being kept on

an agar slope at 4°C. These microorganisms were first collected from the collection of microbial type cultures (MTTC) from Gupta College of technological sciences.

Antibacterial assay (Zone of Inhibition) -

A sterile glass spreader was used to evenly distribute the standard inoculums of each bacterium, which ranged from 1 to 2×10^7 CFU (Colony Forming Units)/ml with 0.5 McFarland standards, onto the surface of sterile Muller-Hinton (MH) agar plates. A sterile paper disc that had been previously soaked with extracts at a predetermined concentration (1mg/ml/disc) was delicately placed in the centre of the seeded and tagged MH agar. As a control, sterile paper discs were used that contained only physiological saline. Three replicates were kept for each test solution. A reference standard for antibiotics was employed, which was 10 ug/disc of amoxicillin. Individual tests were conducted using 1000 ppm concentrations of the ethanolic and aqueous test extracts against test organisms. The unprocessed aqueous leaf extracts were combined in groups of two, three or four in equal parts. Individual plant extracts (ethanolic & aqueous) were also examined for antibacterial activity as a point of reference. The preparation of various aqueous combinations followed the same process. Five millimetre diameter Whatman No. 1 filter paper discs were dipped in each test solution, dried in a hot air oven, and then utilised for the antibacterial assay. After 24 hours of aerobic incubation at 37°C, the plates were checked for zone of inhibition. A ruler was used to measure each zone of inhibition and compare it to the control [71].

RESULTS-

Table-1:- Effect of antibacterial reference standards on selected bacteria

Standard Antibiotic	Concentration (ug/ml/disc)	Test bacteria	Zone of inhibition(cm)
Amoxicillin	10	<i>Staphylococcus aureus</i>	2.7
		<i>Bacillus subtilis</i>	2.5
		<i>Escherichia coli</i>	3.1

<i>Salmonella typhi</i>	2.9
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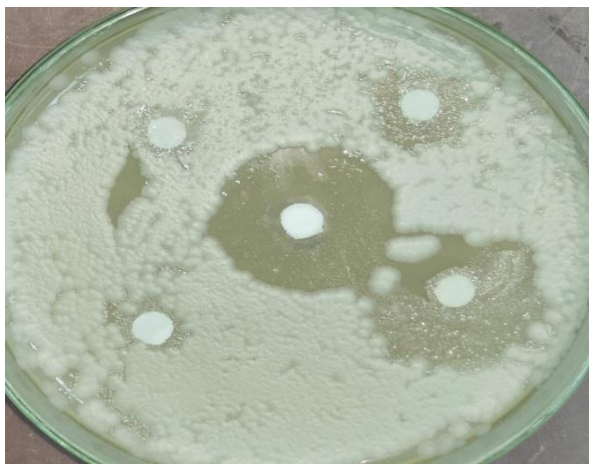
Table 2:- Effect of crude and aqueous and ethanolic extracts selected plant parts of on different bacteria (24h)

Plant species	Aqueous part extract				Ethanolic part extract			
	SA	BS	EC	ST	SA	BS	EC	ST
<i>Cuminum cyminum</i>	-	1.0	1.2	1.4	0.9	1.4	1.5	1.1
<i>Foeniculum vulgare</i>	0.7	-	1.2	0.7	1.2	-	1.4	1.2
<i>Hemidesmes indicus</i>	-	-	1.0	-	1.0	1.2	1.4	-
<i>Catharanthus roseus</i>	-	0.9	-	0.8	-	1.5	1.7	1.2

SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, EC: *Escherichia coli*, ST: *Salmonella typhi*, zone of inhibition will be measured in cm.



Pictures



demonstrating Disc diffusion method and Zone of Inhibition

Table 3: Synergistic activity of aqueous and ethanolic extracts of selected plant

SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, EC: *Escherichia coli*, ST: *Salmonella typhi*, A-*Cuminum cyminum*, B- *Foeniculum vulgare*, C- *Hemidesmes indicus*, D- *Catharanthus roseus*, zone of inhibition was in cm.



us part extract

	BS	EC	S'
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A+B	0.9	-	1.9	-	1.6	-	2.0	0.9
A+C	-	1.6	-	1.5	1.5	0.9	-	2.0
A+D	1.0	-	1.2	-	1.7	1.9	1.4	1.7
B+C						2.4	2.1	1.9
B+D						1.9	-	2.0
C+D						2.3	1.4	-



Pictures demonstrating Disc diffusion method and Zone of Inhibition

Table 4- Synergistic activity of aqueous and ethanolic extracts of selected plant parts in combination of three against bacteria

Combination of plant extracts tested	Aqueous part extract				Ethanolic part extract			
	SA	BS	EC	ST	SA	BS	EC	ST
A+B+C	0.9	2.1	1.7	-	1.2	0.8	-	1.8
A+B+D	1.0	-	1.5	1.8	1.5	2.0	1.9	2.1
B+C+D	-	1.8	-	1.5	1.7	-	2.3	2.2



A+C+D	0.7	-	1.5	1.0	-	1.7	2.7	0.8
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SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, EC: *Escherichia coli*, ST: *Salmonella typhi*,

A-*Cuminum cyminum*, B-
Hemidesmes indicus, D-
inhibition was in cm.



Foeniculum vulgare, C-
Catharanthus roseus, zone of

Pictures demonstrating Disc diffusion method and Zone of Inhibition

DISCUSSION-

In this study, the ability of four widely accessible medicinal plants to inhibit the growth of two Gram-negative and two Gram-positive bacteria was examined respectively. *S. aureus*, *B. subtilis*, *E. coli*, *S. typhi*, were all shown to be extremely susceptible to the antibiotic amoxicillin, with average diameter inhibition zones measuring 2.7, 2.5, 3.1 and 2.9 cm respectively.

Zone of inhibition of individual plant extracts was comparatively less than the synergistic ones. Leaf extracts, ethanolic and aqueous when tested individually for their antibacterial activity, showed various degrees of activity. Ethanolic plant extract had higher degree of activity than aqueous, ethanolic extract of *Catharanthus roseus* had the highest zone of inhibition against *E.coli* about 1.7 cm and also bacteria was much more vulnerable towards ethanolic extracts on the other hand aqueous extracts had also sufficient activity the highest zone of inhibition was for *Cuminum cyminum* against *salmonella typhi* about 1.4 cm. Aqueous extracts had activity against almost every bacteria expect *Staphylococcus aureus*, only *Foeniculum vulgare* had slight activity against this bacteria about only 0.7 cm. Ethanolic extracts had activity against bacteria varies from 0.9-1.7cm, for aqueous plant extracts less number was achieved about 0.7-1.4cm.

The common antibiotic amoxicillin has an appropriate standard diameter zone of inhibition for sensitive organisms of >21 mm (NCCLS 1993). The average zone of inhibitions against these food-borne diarrheagenic bacteria for plant extracts, however, ranged from 0.7 to 2.0 cm in aqueous extracts and from 0.7 to 2.3 cm in ethanolic extracts. When compared to control antibiotics, these values are resistant and/or intermediately sensitive. The fact that the plant extracts are in crude form and only contain trace levels of bioactive chemicals, however, may be the cause of the low values recoded for the extracts. The bioactivity of crude extracts of

medicinal plants within this range of the diameter zone of inhibitions has also been observed by a number of researchers at the same time [72].

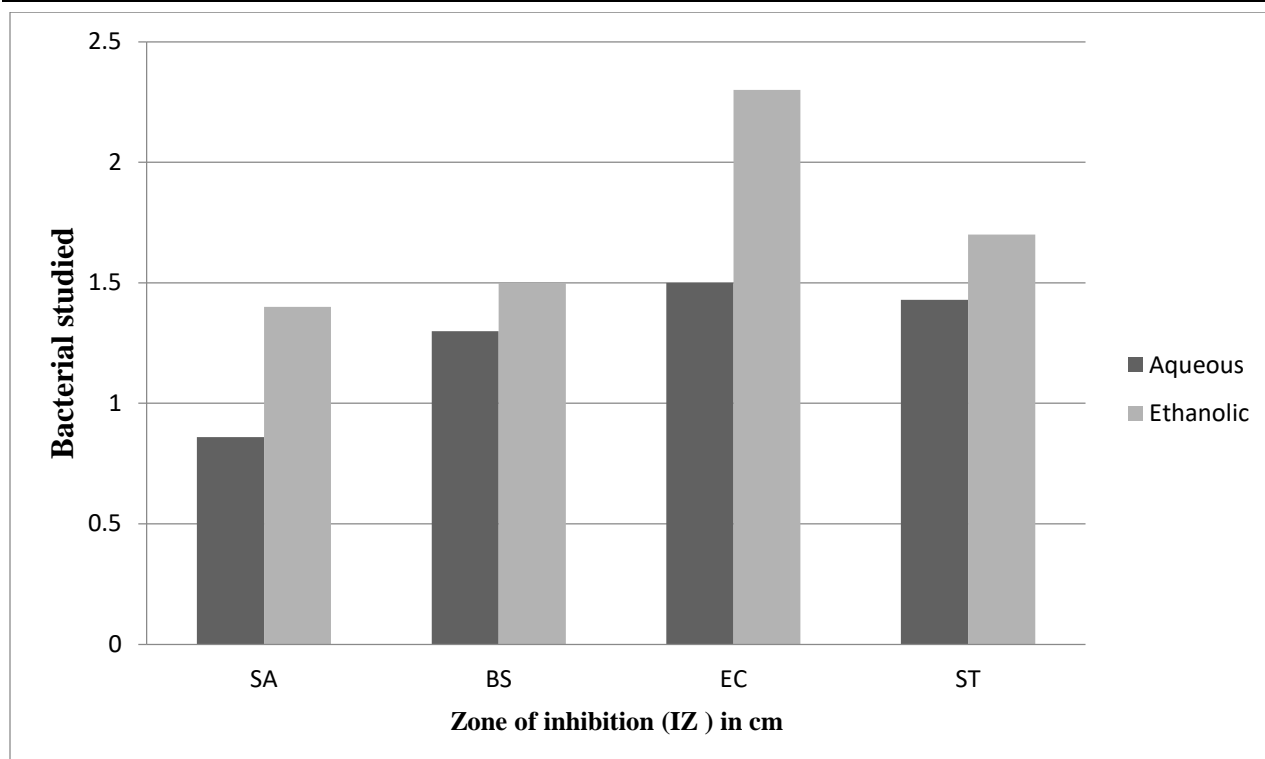
For synergistic effect two same amount of plant extract either it is ethanolic or aqueous was mixed properly and tested against four different types of bacteria and found out that highest activity was ethanolic extract of B +C against four bacteria like for SA IZ was 1.4cm, for BS IZ was 2.4cm, for EC IZ was 2.1cm and for ST IZ was 1.9cm, so these results shown that mixture of ethanolic extract of two plants have synergistic activity against all of four bacteria. Second highest was ethanolic extract A+D against four different bacteria like for SA IZ was 1.7cm, for BS IZ was 1.9cm, for EC IZ was 1.4cm and for ST IZ was 1.7cm, so these two plant mixture of ethanolic extract have activity against all four bacteria. Lowest activity was observed for ethanolic extract when these two plant was mixed like A+B this mixture was slightly effective against SA (IZ was 0.9cm), for EC IZ was 2.0cm, for ST IZ was lesser than SA only about 0.8cm and on the other hand there was no activity against BS. For Mixture of two ethanolic extract of plants IZ was varied from 0.9-2.4cm [73].

For aqueous extract, the synergistic activity of two plant mixture IZ was varied from 0.8-2.1cm. Most of the mixture was not effective against bacteria, but highest activity found out between aqueous extract of C+D against ST, the IZ was 1.7cm and second highest was A+B and C+D against common bacteria EC, IZ was 1.9 and 1.7 respectively [74].

For three mixtures of plant extracts the highest synergistic effect was shown between mixture of ethanolic extracts of A+C+D against EC IZ was about 2.7 cm, second highest was mixture of ethanolic extracts of B+C+D against EC IZ was about 2.3 cm and the mixture of ethanolic extract of A+B+D had activity against all four bacteria like for SA IZ was 1.5cm, for BS IZ was 2.0cm, for EC IZ was 1.9cm and for ST IZ was 2.1cm. IZ for ethanolic extracts was varied for 1.2-2.5cm.

IZ of Mixture of three aqueous extracts was varied from about 0.9-2.1cm. Highest activity was found out when three aqueous extracts of plants was mixed like A+C+D against EC, IZ was about 2.1cm [75].

SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, EC: *Escherichia coli*, ST: *Salmonella typhi*,
A- *Cuminum cyminum*, B- *Foeniculum vulgare*, C- *Hemidesmes indicus*, D- *Catharanthus roseus*
,IZ- Zone of Inhibition



Synergistic activity of aqueous and ethanolic extracts of selected medicinal plant leaves, in combination of four, against different bacteria (24 h). Values of mean of three replicates (Refer Tables for abbreviations)

CONCLUSION-

World scientists thought that we have enough medicines to fight harmful bacteria and can protect the society from its effects but corona opened their eyes urged them to find alternative ways to develop effective medicines. Mainly there were three sources from which they can develop the medicines like Animal, Mineral and Plants. Due to plants have natural antioxidants and phytochemicals they were the first choices, also plants can be used synergistically.

Plants are one of the most significant sources of medicines. The significance of healing plants in improving human health capacity. Unpleasant and challenging conditions have been documented regularly worldwide from ancient times. Secondary metabolites, which could serve as drug sources and are useful therapeutically, are common in medicinal plants. The use of plant extracts as therapeutic agents is growing [76].

From the tables we can say that *E.coli* was the most vulnerable bacteria among these bacteria tested. After *E.coli*, *Salmonella typhi* was the most vulnerable almost all plant extracts had activity against this bacteria. The most repellent bacteria was *Staphylococcus aureus*, plant extracts mostly ethanolic were effective. However synergistic activity was much more than the individual plant extracts. Individually, ethanolic extract of *Catharanthus roseus* had the highest zone of inhibition against *E.coli* about 1.7 cm.

When two plants were mixed the highest activity was ethanolic extract of *Foeniculum vulgare* + *Hemidesmos indicus* against four bacteria like for *Staphylococcus aureus* zone of Inhibition was 1.4cm, for *Bacillus subtilis* zone of Inhibition was 2.4cm. The highest activity was shown synergistically against *E.coli* by the mixture of

ethanolic extracts *Cuminum cyminum* + *Hemidesmes indicus* + *Catharanthus roseus*, zone of Inhibition was 2.7 cm amongst all of them.

Results of the study suggest that the tested four plants possess significant antimicrobial potential. Study justify the folklore antimicrobial use of drug, thus these four plants can be used as potent antimicrobial compound individually and also synergistically in common conditions.

Purified extracts that may be employed in next research to more fully characterizes pharmacological and toxicological characteristics. Nonetheless further investigations and isolation of compound is necessary to establish the exact constituent responsible for antimicrobial activity.

Finally, medicinal plant extracts have various properties that affect one of the most essential pharmaceutical industries, that is antibacterials (medical, residential, and commercial), with a market value of over \$50 billion. As a result, these plants and its derivatives have numerous applications that extend beyond traditional medical folklore. Because of scientific and technological breakthroughs, we may now use these extracts as modern medical adjuvants, recognising their potential. We now understand how to expand on the progression of knowledge and even provide more practical applications for it [77].

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