Fractionation, phytochemical, anti-oxidant and antimicrobial screening of *Curcuma Longa*

By

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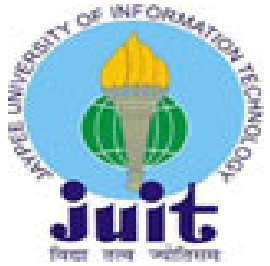
Supervisor: Dr. G. L. Gupta



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CERTIFICATE

This is to certify that the work entitled, **"Fractionation, phytochemical, anti-oxidant and antimicrobial screening of *Curcuma Longa"*** submitted by **Krittika Sharma** in partial fulfillment for the award of degree of Bachelor of Pharmacy in Department of Pharmacy, Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of Supervisor ……

Supervisor: Dr. G. L Gupta

Designation: Assistant Professor

ACKNOWLEDGMENT

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At last, I would also like to thank PhD scholar Lalit Sharma for his kind help and support.

Krittika Sharma

SUMMARY

Fractionation, phytochemical, anti-oxidant and antimicrobial screening of *Curcuma Longa*

Curcuma longa, commonly known as 'turmeric', is widely used as a spice and colouring agent, and is known for its medicinal properties. Various sesquiterpenes and curcuminoids have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant, anti-inflammatory, wound healing, anticancer and antiproliferative, antifungal and antibacterial activity. The Gram-positive and Gram-negative bacteria can be inhibited by antibiotics, either by blocking the protein synthesis or peptidoglycan synthesis in bacterial cell wall. Various studies have shown the antimicrobial effects of extracts of roots of *Curcuma longa* on various microorganisms like *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia*, *Candida albicans*, etc. Considering the above mentioned factors, the present study was conducted to evaluate the antimicrobial activity of *Curcuma longa* rhizome extracts on gram positive and gram negative bacteria. In this study, the zone of inhibition of various fractions of the extract was determined and results were reported.

Experimental work done:

- Collection and drying of plant material (Rhizomes).
- Extract preparation using soxhlet apparatus.
- Phytochemical screening of plant extract.
- Physicochemical tests.
- Fractionation of the extract using separating funnel.
- Anti- oxidant activity of *Curcuma Longa.*
- Anti- microbial assay of fractions of the extract on gram positive and gram negative bacteria.

Chapter-1

INTRODUCTION

What are microbes?

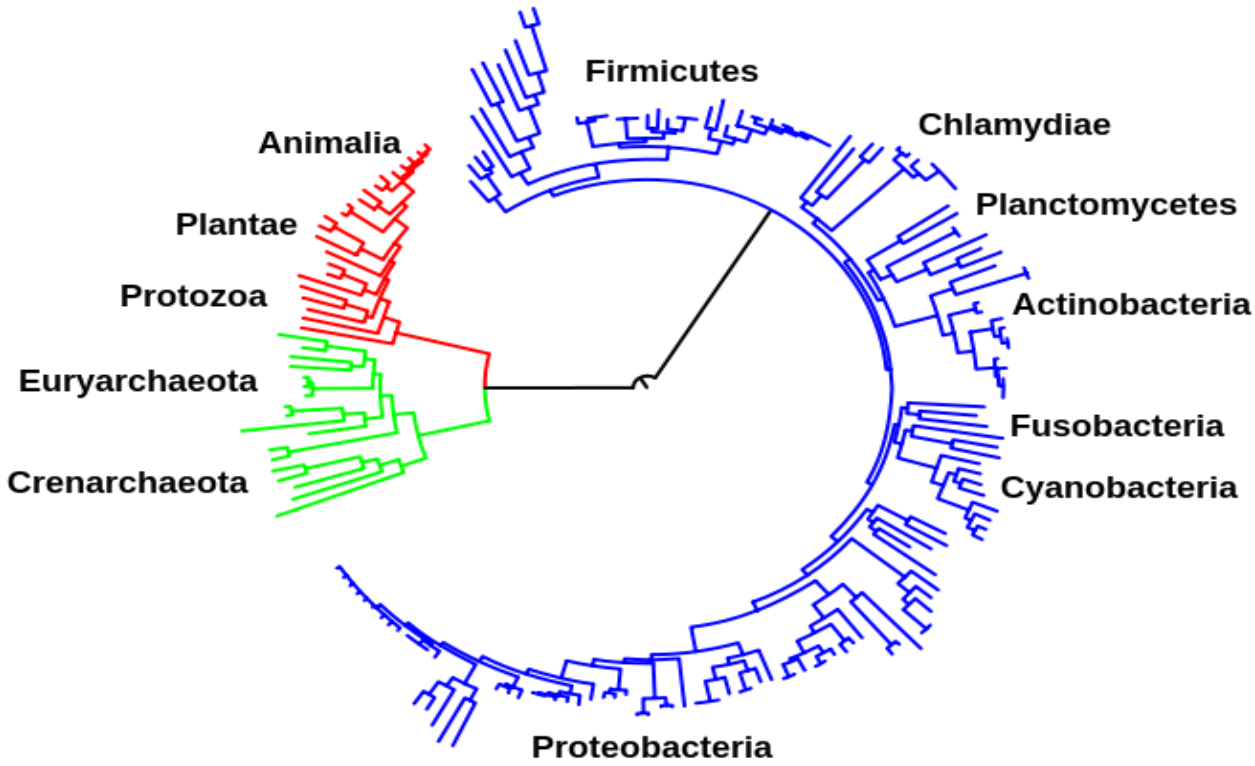
- An estimated 10 million species of bacteria live on the Earth. These, and other microbes, make up a large part of the planet's living material. Microbes thrive on land, in the oceans and on human skin. They even survive in extreme places like near deep-sea vents. People often think of microbes as just causing diseases such as fungal infections in plants or pneumonia in humans. In spite of their bad reputation, microbes also play important roles in Earth's ecosystems. For example, bacteria living in the ocean produce half of the oxygen in the atmosphere. Without them, we couldn't breathe.[1]
- The study of microorganisms is called microbiology, a subject that began with Antonie van Leeuwenhoek's discovery of microorganisms in 1675, using a microscope of his own design.
- The many species of microbes can be organized in several ways. Familiar categories include the bacteria, fungi, and viruses. There are even microscopic animals — such as dust mites — which resemble tiny insects. Microbes can also be split into two groups based upon whether they have a cell nucleus. This membrane-bound part of the cell encloses the genetic material. In addition, microbes can be classified by how they obtain and process their food. Or how they react to oxygen. Some microbes need oxygen to survive. Others — like the ones living inside our digestive system — are killed by oxygen.
- Microorganisms are very diverse and include all the bacteria and archaea and almost all the protozoa. They also include some members of the fungi, algae, and animals such as rotifers. Many macro animals and plants have juvenile stages which are also microorganisms. Some microbiologists also classify viruses as

microorganisms, but others consider these as nonliving. Most microorganisms are microscopic, but there are some bacteria such as *Thiomargarita namibiensis* and some protozoa such as *Stentor*, which are macroscopic and visible to the naked eye.[2]

- Micro-organisms are also exploited in biotechnology, both in traditional food and beverage preparation, and in modern technologies based on genetic engineering. A small proportion of micro-organisms are pathogenic and cause disease and even death in plants and animals.[2]

Classification of microbes?

Microorganisms can be found almost anywhere in the taxonomic organization of life on the planet. Bacteria and archaea are almost always microscopic, while a number of eukaryotes are also microscopic, including most protists, some fungi, as well as some animals and plants. Viruses are generally regarded as not living and therefore not considered as micro-organisms, although the field of microbiology also encompasses the study of viruses.[3]



Bacteria are colored blue, eukaryotes red, and archaea green.

Prokaryotes:-

Prokaryotes are organisms that lack a cell nucleus and the other membrane bound organelles. They are almost always unicellular, although some species such as myxobacteria can aggregate into complex structures as part of their life cycle.

Consisting of two domains, bacteria and archaea, the prokaryotes are the most diverse and abundant group of organisms on Earth and inhabit practically all environments where the temperature is below +140 °C. They are found in water, soil, air, animals' gastrointestinal tracts, hot springs and even deep beneath the Earth's crust in rocks

Bacteria:-

Almost all bacteria are invisible to the naked eye, with a few extremely rare exceptions, such as *Thiomargarita namibiensis*. They lack a nucleus and other membrane-bound organelles, and can function and reproduce as individual cells, but often aggregate in multicellular colonies. Their genome is usually a single loop of DNA, although they can

also harbor small pieces of DNA called plasmids. These plasmids can be transferred between cells through bacterial conjugation. Bacteria are surrounded by a cell wall, which provides strength and rigidity to their cells. They reproduce by binary fission or sometimes by budding, but do not undergo meiotic sexual reproduction. However, many bacterial species can transfer DNA between individual cells by a process referred to as natural transformation[3]

Archaea:-

Archaea are also single-celled organisms that lack nuclei. In the past, the differences between bacteria and archaea were not recognised and archaea were classified with bacteria as part of the kingdom Monera. However, in 1990 the microbiologist Carl Woese proposed the three-domain system that divided living things into bacteria, archaea and eukaryotes. Archaea differ from bacteria in both their genetics and biochemistry. For example, while bacterial cell membranes are made from phosphoglycerides with ester bonds, archaean membranes are made of ether lipids.

Eukaryotes:-

Most living things that are visible to the naked eye in their adult form are eukaryotes, including humans. However, a large number of eukaryotes are also microorganisms. Unlike bacteria and archaea, eukaryotes contain organelles such as the cell nucleus, the Golgi apparatus and mitochondria in their cells. The nucleus is an organelle that houses the DNA that makes up a cell's genome. DNA itself is arranged in complex chromosomes. Mitochondria are organelles vital in metabolism as they are the site of the citric acid cycle and oxidative phosphorylation. They evolved from symbiotic bacteria and retain a remnant genome. Like bacteria, plant cells have cell walls, and contain organelles such as chloroplasts in addition to the organelles in other eukaryotes. Chloroplasts produce energy from light by photosynthesis, and were also originally symbiotic bacteria.

Unicellular eukaryotes consist of a single cell throughout their life cycle. This qualification is significant since most multicellular eukaryotes consist of a single cell

called a zygote only at the beginning of their life cycles. Microbial eukaryotes can be either haploid or diploid, and some organisms have multiple cell nuclei (see coenocyte).

Unicellular eukaryotes usually reproduce asexually by mitosis under favorable conditions. However, under stressful conditions such as nutrient limitations and other conditions associated with DNA damage, they tend to reproduce sexually by meiosis and syngamy.

Protists:-

Of eukaryotic groups, the protists are most commonly unicellular and microscopic. This is a highly diverse group of organisms that are not easy to classify. Several algae species are multicellular protists, and slime molds have unique life cycles that involve switching between unicellular, colonial, and multicellular forms.The number of species of protists is unknown since we may have identified only a small proportion. Studies from 2001-2004 have shown that a large number of protist diversity exists in oceans, deep sea-vents, river sediment and an acidic river which suggests that a large number of eukaryotic microbial communities have yet to be discovered.

Importance of Microbes:-

Microbes are essential components of every ecosystem. They produce the oxygen that we need to live. They break down garbage and dead organisms. They produce nutrients that plants need to grow. They even help us digest our food. In addition to these natural activities, microbes are needed for making foods like bread, wine and beer. Scientists also use microbes for practical applications. Microbes at a sewer treatment plant help breakdown the waste. Microbes can also be used to change the genetic composition of plants and animals. This gives them new traits, such as resistance to pesticides.[4]

Microbes are probably better known for the diseases that they cause. AIDS, tuberculosis, bacterial meningitis and the common cold are all caused by microbes. Greater understanding of how microbes live and function, though, has enabled scientists to

prevent and treat diseases. Vaccines, antibiotics and other drugs are powerful tools for reducing illnesses caused by microbes. Not all discoveries, however, were planned. Penicillin, an antibiotic produced by a fungus, was discovered largely by accident. In spite of the many scientific discoveries, washing and proper sanitation are still important tools in preventing diseases caused by microbes.

Antimicrobial Drugs:-

The scientific development of synthetic antimicrobial drugs began with Erlich in the 1890's, with the use of methylene blue for managing malaria, the organic arsenicals for trypanos omiasis (1904), and salvarsan 606 for syphilis (1909). 'Atebrin' was made in 1932 and used for prophylaxis of malaria; the first useful sulphonamide drug came about in 1935. Antibiotic drug use began in the 1920's when Fleming observed the anti-staphylococcal activity of Penicilliun notatum , and from this penicillin was developed for clinical use by Florey & Chain. Another important group of antibiotics was the aminoglycosides, when streptomycin was isolated from
Streptomyces griseus , in 1944. The cephalosporins were developed from Cephalosporium acremonium in 1945.

Classification:-

Antimicrobials are classified by the pathogens targeted, e.g. as antibacterials or antifungals. This
grouping may be subdivided as antibacterials also include urinary antiseptics and anti-mycobacterial drugs.
Antimicrobials, especially antibacterials, are strictly classified into chemotherapeutic agents (synthetic chemicals), and antibiotics, produced from living organisms, usually fungi.
However, 'antibiotic' is often used loosely to mean all antibacterials. Antibacterials can be further described by their:

•chemical structure (penicillins, cephalosporins)

•effect on bacterial growth (bacteriostatic or bactericidal)

•target site.

Target site classes:-

Cell wall synthesis inhibitors -

The cell wall synthesis inhibitors are bactericidal because they block synthesis of different peptidoglycan components of the wall, so growing cells lyse and die.

They do not affect eucaryotic cells, nor microbes that lack peptidoglycan.

These antibiotics are:

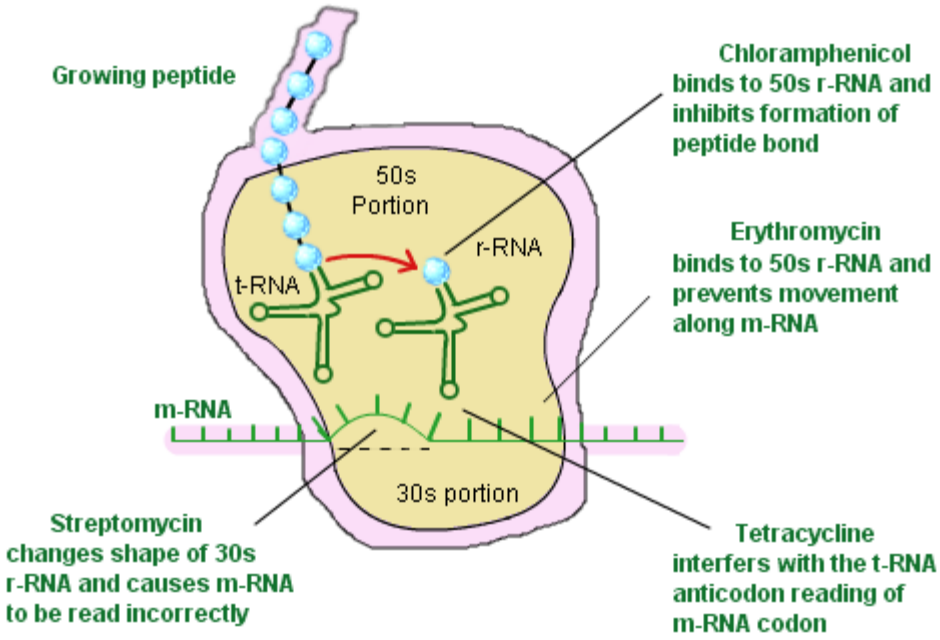
•Beta-lactams: penicillins, cephalosporins, carbapenems, monobactams

•Glycopeptides: vancomycin, teicoplanin

•Polypeptide: bacitracin, cycloserine

Protein synthesis inhibitors: -

Protein synthesis inhibitors act on varying stages of protein synthesis.



Inhibition of Protein Synthesis by Antibiotics

If these are unique to bacteria (e.g. affect the bacterial 70S ribosome rather than the eucaryotic 80S ribosome) they are selectively toxic. However, eucaryotic mitochondrial protein synthesis occurs on 70S ribosomes and can be affected. This group includes:

- Aminoglycosides: streptomycin, gentamicin, tobramycin, netilmicin, amikacin, spectinomycin, neomycin.

- Tetracyclines: tetracycline, doxycycline, minocycline , linezolid, chloramphenicol
- Lincosamides: clindamycin, lincomycin .
- Macrolides: erythromycin, roxithromycin, azithromycin, clarithromycin, spiramycin , streptogramins ,fusidic acid.

Aminoglycosides cause ineffective proteins to form and so are bactericidal. All the others in this group have a reversible (bacteriostatic) action and so protein synthesis begins again when antibiotic levels decrease.

Nucleic acid synthesis inhibitors -

Nucleic acids are made by all cells so the possibility of selective drugs toxic only for microbes is limited. Some pathways have distinct features that can be targeted, or some enzymes are sufficiently different for a selective effect to occur:

•Folic acid synthesis: a precursor of purines and pyrimidines is folic acid which microbes can only synthesise; humans obtain folic acid in food. Sulphonamides and trimethoprim interfere with folic acid synthesis.

•RNA polymerase: inhibited by rifamycins (rifampicin, rifabutin).

•DNA structure: disrupted by nitroimidazoles (e.g. metronidazole).

•topo-isomerase: blocked by quinolones (norfloxacin, ciprofloxacin and others). [4]

Cell membrane function inhibitors-

Drugs that destroy the selective permeability of membranes will kill both microbial and human cells. As a result, they will be relatively toxic if given systemically. Colistin acts like a detergent, disrupting the cell membrane phospholipid. The polyene antifungal drugs (e.g. amphotericin B and nystatin) act by damaging sterols in eucaryotic membranes; they are particularly toxic to fungi through their action on ergosterol but also affect human cells.

Uncertain targets -

The target of some anti-mycobacterial drugs is uncertain: isoniazid may act on mycolic acid synthesis, which would explain its specific activity, while ethambutol may inhibit RNA synthesis.

Characteristics:-

Physicochemical properties

These are important in relation to the effectiveness and mode of administration of a drug, particularly whether they are stable to gastric acid and are absorbed from the gut and so can be given orally; if unstable or not absorbed, they need injection. Other important factors are whether the drug will cross barriers within the body - into cells, into the brain across the blood-brain barrier, into other protected tissues like prostate, or into cysts.

Spectrum and type of activity

The spectrum of activity is the range of organisms against which an antimicrobial is usually active. The minimal inhibitory concentration (MIC) is the smallest concentration of antimicrobial which is bacteriostatic, reversibly inhibiting bacterial growth, so re-growth occurs if the antimicrobial is removed by excretion or inactivation. By contrast, the minimal bactericidal (or fungicidal) concentration (MBe, MFC) is the smallest concentration irreversibly killing the microbes, so they do not re-grow if the antimicrobial is removed.

Mechanisms of resistance

Some bacteria are innately resistant to certain antibiotics because they lack a target site or are impermeable to the antibiotic; other bacteria acquire resistance, by one of three mechanisms:

1. Altered target site - These may result in lower affinity for the antibiotic, or additional target enzymes may emerge unaffected by the drug.

2. Altered uptake - Effective drug concentration in the bacterial cell can be decreased either by decreasing permeability or by actively pumping the drug out of the bacterial cell.

3. Antibiotic-inactivating enzymes - These occur particularly against penicillins, cephalosporins and aminoglycosides.

Resistance spreads between bacteria in three genetic ways:

1. Chromosomal mutation, usually random, causes an altered protein, e.g. a ribosomal protein (streptomycin resistance) or altered enzyme (sulphonamides). Selection by the antibiotic after each cell division will result in a resistant population.

2. Transmissible plasmids are small circular DNA units replicating independently of the chromosome, and transmissible between cells. They have four advantages over chromosomal mutation: transfer between bacteria is more rapid than cell division; resistance to numerous individual antibiotics can be carried at once; resistance to several classes of antibiotic can be carried at once; and one class of plasmid can enter numerous genera, e.g. TEM-l beta-lactamase in enteric Gram-negative rods, in N. sonorrhoeae and in H. influenzae.

3. Transposons, called 'jumping genes', move from the security of the chromosome to the mobility of a plasmid, and from one plasmid to another

Resistance also spreads between bacteria in three physical ways:

1.Conjugation (by direct contact)

2.Transduction (by phages)

3.Transformation (uptake of free DNA).

Pharmacokinetics

The pharmacokinetics of a drug describes its behaviour in the body: absorption, distribution, protein binding, serum and tissue concentrations, serum half-life,

metabolism and excretion. Important factors that will alter the effective half-life of the drug (and its toxicity) include the age of the patient, concurrent diseases particularly of organs which metabolise or excrete the drug (usually liver and kidney), genetic factors (slow and fast drug metabolism) and interaction with other drugs.

Side effects & toxicity

Even safe effective antibiotics like penicillins fall short of the ideal of a 'magic bullet' which would not affect humans yet would eradicate germs by a single 'dosa sterilisa magna' (great sterilising dose). Side effects and toxicity are often similar within a group of antibiotics, e.g. all
aminoglycosides are ototoxic (ear) and nephrotoxic (kidney), but in varying degrees.

Specific anti-microbials

Cell Wall Synthesis Inhibitors

Penicillins

The penicillins all have a similar structure, with different drugs being created using different side chains. The side chains of the natural product can be modified chemically to give a wider spectrum of activity. A common bacterial drug resistance is via betalactamases.

Pharmacokinetics -

Some penicillins are stable to gastric acid and are absorbable (penicillin V, ampi/amoxicillin, flucloxacillin) and so can be given orally. Others must be given by injection: penicillin G, ticarcillin and piperacillin. All penetrate widely, except to CSF and brain tissue, and all
have relatively short half-lives, are little metabolised and are excreted renally.

Toxicity - usually minimal, almost limited to hypersensitivity, chiefly rash or fever, and very rarely, anaphylaxis.

Cephalosporins

Structure of cephalosporins differs from the penicillins in having a six-member ring attached to the beta-Iactam ring. The different side chains give the different cephalosporins, which are often classified into first, second, third and fourth 'generations' by their date of introduction; more logical classifications exist but are less used.

Pharmacokinetics -

Cephalosporins behave like penicillins, although the later drugs have longer half-lives, especially ceftriaxone, and penetrate well to CSF and brain.

Spectrum-

All are broad-spectrum, active against many Gram-positive, Gram-negative and anaerobic bacteria. Enterococci are resistant to all. In general first generation are best against Gram-positive bacteria, second generation best against anaerobes, and third generation best against Gram-negative rods. [4]

Toxicity -

Low toxicity, similar to the penicillins.

Other β-Iactams

Aztreonam is a mono-bactam, i.e. a single β-lactam ring. Its action and pharmacokinetics are like an injectable cephalosporin, but its spectrum is like the aminoglycoside gentamicin, i.e., solely Gram negative, including many pseudomonads. Its use is restricted by its high cost.

Imipenem, meropenem and ertapenem are carbapenems are structurally similar to penicillin.

Their actions and pharmacokinetics are like an injectable cephalosporin, but their spectrum is very wide, although they are inactive against MRSA, E.faecium, and some Gram-negative rods. Their use is restricted by policy and cost to serious systemic infections before precise microbial diagnosis.

Vancomycin and Teicoplanin

Vancomycin and teicoplanin are glycopeptides and are bactericidal to Gram-positive bacteria.

Pharmacokinetics - Neither is absorbed from the gut, so vancomycin is given intravenously, by slow infusion to decrease the side effects of headache and flushing (the 'red man' syndrome).

Teicoplanin is also given intramuscularly. Distribution of both is wide into most fluids and tissues, but suboptimal into CSF and brain. Their half-lives are long, hence 12or 24-hourly dosing. Excretion is renal and, because of toxicity, serum levels are usually monitored, though safe levels have not yet been established.

Spectrum – their use is limited to severe Gram-positive (including MRSA) infections, and oral treatment of unresponsive C. difficile associated colitis.

Linezolid and streptogramins are active against most glycopeptide-resistant Gram-positive bacteria.

Toxicity -

The main toxic effect is hearing loss, but phlebitis or neutropenia can also occur.

Nephrotoxicity is uncommon with current preparations.

Protein synthesis inhibitors

All act on the 30S or 50S bacterial ribosome. Only aminoglycosides are bactericidal.

Aminoglycosides

The aminoglycosides contain streptamine or a streptidine-containing amino cyclitol, with side chains that are modified to produce the individual drugs. Gentamicin is actually a mixture of three related molecules. [4]

Pharmacokinetics -

Aminoglycosides are not absorbed from the gut, so must be injected for systemic use. They are not metabolised significantly and have a relatively long half-life, about 3 hours, so usually are given once- or twice-daily. Excretion is renal. Penetration is relatively poor into bone, lung and sputum, and non-existent into CSF and brain.

Spectrum -

Bactericidal with a broad Gram-negative spectrum, however their uptake into cells is prevented by anaerobiosis so they are ineffective against anaerobes. They are mainly used against enteric Gram-negative rods; gentamicin, tobramycin and amikacin are also active against pseudomonads. Streptomycin was used for tuberculosis but is now used mainly in Gram-negative zoonoses including brucellosis, plague and tularaemia.

Toxicity

Aminoglycosides have both renal and ototoxicity, related mainly to total dose. This is reflected later in peak than in trough levels, which should be monitored carefully, especially in the old, the underweight and those with renal or auditory impairment.

Chloramphenicol

Chloramphenicol is a natural product that is now chemically synthesised. Action is on bacterial protein synthesis at the 50S ribosome, and is usually bacteriostatic only.

Pharmacokinetics -

Chloramphenicol is lipophilic; it is orally absorbed and has wide penetration including into the interior of the eye, the CSF and brain. It is metabolised by the liver and excreted renally. It can also be given by injection.

Spectrum

Like its pharmacokinetics, the spectrum is extremely broad and includes most bacteria, chlamydiae, rickettsiae and mycoplasmata. It is also cheap.

Toxicity -

The use of this antibiotic is limited by two toxicities. An unpredictable irreversible marrow aplasia causing aplastic anaemia occurs rarely (1 in 30000) and is fatal without marrow transplantation. If liver function is impaired, chloramphenicol levels rise above normal, and dose-related reversible marrow hypoplasia can occur; newborns who fail to metabolise chloramphenicol adequately die from toxic complications ('grey baby syndrome').

Lincosamides

Clindamycin and lincomycin are bacteriostatic. They are orally absorbed, but are also injectable, and widely distributed apart from CSF and brain. They are metabolised by the liver and excreted renally.

Spectrum

They are mainly used against anaerobes or staphylococci. Clindamycin is a reserve drug in toxoplasmosis.

Toxicity

Toxic effects include allergy, a metallic taste, and initiation of pseudomembranous enterocolitis associated with C. difficile overgrowth.

Macrolides

The macrolides have an unusual macrocyclic lactone ring with sugars attached. There are four macrolides in clinical use: erythromycin, roxi-thromycin, azithromycin and clarithromycin. Their action is bacteriostatic.

Pharmacokinetics -

Erythromycin is inactivated by gastric acid so is protected by enteric-coating or given as a salt or ester. Intravenous use often causes thrombophlebitis. The half-life is about 2 hours, and excretion is hepatic; as a result, care is needed in liver failure but dosage is unaltered in renal impairment.

Spectrum

Macrolides are mainly used against Gram-positive and unusual bacteria: chlamydiae, legionellae, mycoplasmata and non-tuberculous mycobacteria.

Toxicity

- These are very safe antibiotics with low toxicity.

Tetracyclines

The tetracyclines have a basic structure of four fused rings with side-chain changes to produce different drugs. They are bacteriostatic. [4]

Pharmacokinetics -

Tetracyclines are orally absorbed yet are also injectable; they are widely distributed including CSF and brain. Metabolism occurs in the liver, and excretion is renal.

Spectrum-

The spectrum of tetracyclines is very broad, including most pathogenic bacterial genera (except Pseudomonas), plus chlamydiae, mycoplasmata and rickettsiae, but is not deep, with many resistant bacterial strains. The use of tetracyclines as growth promoters added to livestock feed increased the numbers of resistant strains. Use is, therefore, chiefly for unusual bacteria.

Toxicity-

This is low, apart from deposition in immature bone and teeth (causing discoloration), and the usual allergy or gut intolerance.

Nucleic acid synthesis inhibitors

Nitroimidazoles

Metronidazole and tinidazole are nitroimidazoles, with a unique mode of bactericidal action, acting as electron acceptors and producing intermediate compounds toxic to bacterial DNA.

Pharmacokinetics-

They are well absorbed, penetrate widely, are metabolised by the liver, and excreted by the kidney.

Spectrum-

Their spectrum includes almost all pathogenic anaerobes (except some cocci), microaerophilic bacteria and some parasites. Their principal use is in prophylaxis and treatment of anaerobic bacterial infections, and in amoebiasis, giardiasis and trichomoniasis.

Quinolones

Structurally, the quinolones (e.g. norfloxacin and ciprofloxacin) are fluoro-quinolone carboxylic acid derivatives with two six-member rings, and distinctive side chains. Action is bactericidal by inhibiting bacterial DNA gyrase, hence preventing supercoiling of DNA. [5]

Pharmacokinetics-

Quinolones are well absorbed and distributed, but serum concentrations are low. Their half-lives are relatively long, about 4 hours, and excretion is renal.

Spectrum-

Older drugs mainly kill Gram-negative bacteria including pseudomonads, with poor activity against Gram-positive and anaerobic bacteria, now improved with broad-spectrum quinolones. Norfloxacin is used mainly in urinary and gut infections, while ciprofloxacin is used chiefly in serious systemic Gram-negative infections. Numerous other quinolones are available in different countries.

Toxicity can affect the CNS with headache, mood changes and fits.

Trimethoprim and sulphonamides

Sulphonamides are derived from sulphanilamide, a single ring compound structurally similar to, and competing with, an intermediate in folic acid synthesis, para-aminobenzoic acid. They are commonly used in combination with trimethoprim, which inhibits the next step to tetrahydrofolic acid in folic acid synthesis. Co-trimoxazole is sulphamethoxazole plus trimethoprim.

Pharmacokinetics-

All have good absorption, wide distribution, long half- lives (to 10 hours) and renal excretion.

Spectrum-

Chiefly Gram-negative rods were sensitive, but resistance is now common. Uses are mild urinary or respiratory infections, and unusual infections including P. jirovecii pneumonia, nocardiosis, chancroid and typhoid fever.

Toxicity -

This is mainly allergy with rash and fever. Megaloblastic anaemia is uncommon and reversed by folinic acid.

Rifamycins

These are red-coloured derivatives of a natural product, rifamycin B.

Pharmacokinetics-

All are well absorbed orally, penetrate widely including the CNS, and are cleared by liver metabolism and excretion mainly in bile.

Spectrum-

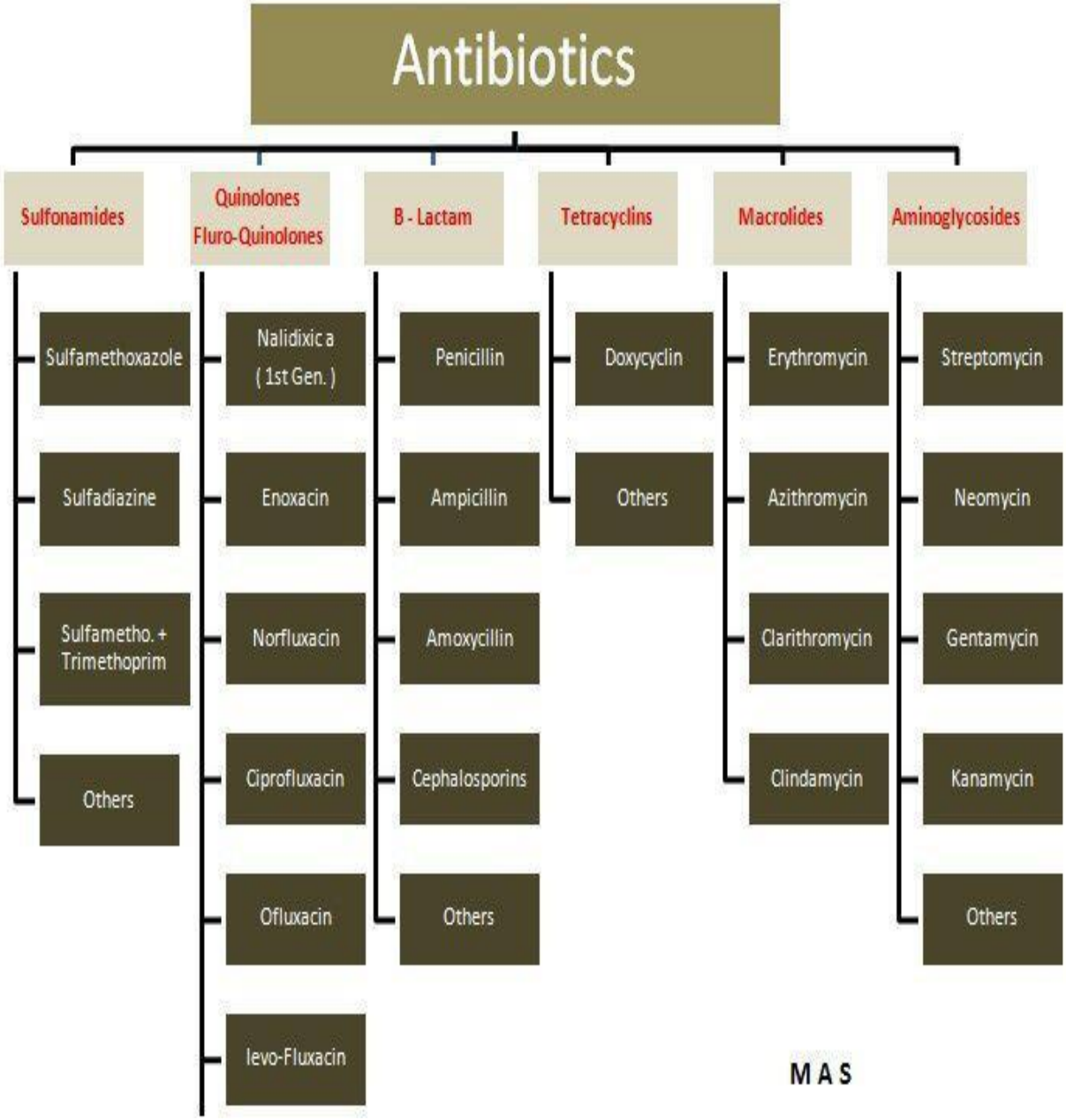
Because rifamycins enter cells they are used against intracellular organisms such as mycobacteria. Rifampicin is a first line drug for TB and leprosy, and also used for

resistant (especially MRSA) staphylococcal infections (with fusidic acid) and as prophylaxis in contacts
of meningococcal and Haemophilus meningitis. Rifabutin is used in combination with other drugs (e.g. ethambutol, clarithromycin) in the treatment and prophylaxis of atypical my cobacterial infections in AIDS.

Toxicity-

Rifampicin has few toxic side effects but numerous drug interactions. It colours body fluids blood-red. [5]

Classification of antibiotics:-


Antibiotics
Sulfonamides
Quinolones
Fluro-Quinolones
B - Lactam
Tetracyclins
Macrolides
Aminoglycosides
Sulfamethoxazole
Sulfadiazine
Sulfametho. + Trimethoprim
Others
Nalidixic a (1st Gen.)
Enoxacin
Norfluxacin
Ciprofluxacin
Ofluxacin
levo-Fluxacin
Penicillin
Ampicillin
Amoxycillin
Cephalosporins
Others
Doxycyclin
Others
Erythromycin
Azithromycin
Clarithromycin
Clindamycin
Streptomycin
Neomycin
Gentamycin
Kanamycin
Others
MAS

Different strains of bacteria:-

E. coli

There are many types of *E. coli*, and most of them are harmless. But some can cause bloody diarrhea. Some strains of *E. coli* bacteria (such as a strain called O157:H7) may also cause severe anemia or kidney failure, which can lead to death.

Other strains of *E. coli* can cause urinary tract infections or other infections.

You get an *E. coli* infection by coming into contact with the feces, or stool, of humans or animals. This can happen when you drink water or eat food that has been contaminated by feces.

E. coli can get into meat during processing. If the infected meat is not cooked to 160°F (71°C), the bacteria can survive and infect you when you eat the meat. This is the most common way people in the United States become infected with *E. coli*. Any food that has been in contact with raw meat can also become infected.

Other foods that can be infected with *E. coli* include:

- Raw milk or dairy products. Bacteria can spread from a cow's udders to its milk. Check the labels on dairy products to make sure they contain the word "pasteurized." This means the food has been heated to destroy bacteria.
- Raw fruits and vegetables, such as lettuce, alfalfa sprouts, or unpasteurized apple cider or other unpasteurized juices that have come in contact with infected animal feces.

Human or animal feces infected with *E. coli* sometimes get into lakes, pools, and water supplies. People can become infected when a contaminated city or town water supply has not been properly treated with chlorine or when people accidentally swallow contaminated water while swimming in a lake, pool, or irrigation canal.

The bacteria can also spread from one person to another, usually when an infected person does not wash his or her hands well after a bowel movement. *E. coli* can spread from an infected person's hands to other people or to objects.[6]

The main symptoms of an *E. coli* infection are:

- Bloody diarrhea.
- Stomach cramps.
- Nausea and vomiting.

Some people do not notice any symptoms. Children are more likely than adults to have symptoms. Symptoms usually start 3 or 4 days after you come in contact with the *E. coli*.

Most people get better in about a week. They often don't see a doctor and don't know that *E. coli* caused their problems.

When *E. coli* causes serious problems with the blood or kidneys, symptoms include:

- Pale skin.
- A fever.
- Weakness.
- Bruising.
- Passing only small amounts of urine[6]

Staphylococcus aureus

Staph is short for *Staphylococcus*, a type of bacteria. There are over 30 types, but *Staphylococcus aureus* causes most staph infections (pronounced "staff infections"), including

- Skin infections
- Pneumonia
- Food poisoning

- Toxic shock syndrome
- Blood poisoning (bacteremia)

Skin infections are the most common. They can look like pimples or boils. They may be red, swollen and painful, and sometimes have pus or other drainage. They can turn into impetigo, which turns into a crust on the skin, or cellulitis, a swollen, red area of skin that feels hot.

Anyone can get a staph skin infection. You are more likely to get one if you have a cut or scratch, or have contact with a person or surface that has staph bacteria. The best way to prevent staph is to keep hands and wounds clean. Most staph skin infections are easily treated with antibiotics or by draining the infection. Some staph bacteria such as MRSA (methicillin-resistant Staphylococcus aureus) are resistant to certain antibiotics, making infections harder to treat.[6]

Chapter-2

Curcuma Longa

Common name- Turmeric, Haldi

Family- Zingiberaceae

Synonyms- *Curcuma domestica*, Turmeric.

Description

Curcuma longa is a perennial plant with roots or tubers oblong-palmate, and deep orange inside. Leaves about 2 feet long, lanceolate, long, petioled, tapering at each end, smooth, of a uniform green. Flowers are dull yellow, three or five together surrounded by bracteolae. It is propagated by cuttings from the root. In fresh state, the roots have an aromatic and spicy fragrance, which by drying gives way to a more medicinal aroma.[7]

Origin and distribution

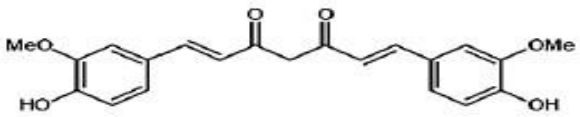
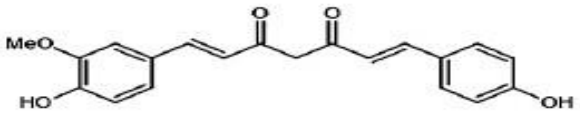
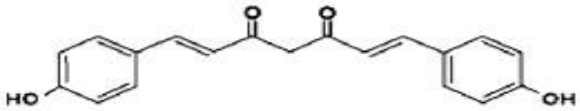
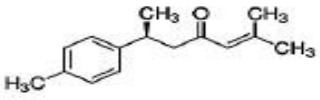
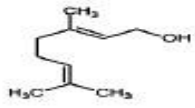
South America (specially Peru), Sothern asia, Southern China, India (especially Bengal, Madras), Indonesia, other countries with a tropical climate.

Used parts: Root, rhizome

Phytochemicals: [8]

1,8-cineole, 2-bornanol, 2-hydroxy-methyl-anthraquinone, 4-hydroxy-cinnamoyl-(Feruloyl)-methane, Alpha-atlantone, Alpha-pinene, Alpha-terpineol, Ar-turmerone, Arabinose, Ascorbic-acid, Ash, Azulene, Beta-carotene, Beta-pinene, Beta-sesquiphellandrene, Bis-(Para-hydroxy-cinnamoyl)-methane, Bis-desmethoxycurcumin, Bisabolene, Borneol, Boron, Caffeic-acid, Calcium, Caprylic-acid, Caryophyllene, Chromium, Cineole, Cinnamic-acid, Cobalt, Copper, Cuminyl-alcohol, Curcumene, Curcumenol, Curcumin, Curdione, Curlone, Curzerenone, Curzerenone-c, Cyclo-isoprenemyrcene, D-alpha-phellandrene, D-camphene, D-camphor, D-sabinene, Dehydroturmerone, Desmethoxycurcumin, Di-p-coumaroyl-methane, Dicinnamoylmethane, Didesmethoxycurcumin, Diferuloyl-methane, Dihydrocurcumin, EO, Eugenol, Feruloyl-p-coumaroyl-methane, Gamma-atlantone, Guaiacol, Isoborneol, L-alpha-curcumene, L-beta-curcumene, Limonene, Manganese, Monodesmethoxycurcumin, Niacin, Nickel, O-coumaric-acid, P-coumaric-acid, P-cymene, P-methoxycinnamic-acid, P-tolymethylcarbinol, Phosphorus, Protocatechuic-acid, Resin, Riboflavin, Syringic-acid, Terpinene, Terpineol, Thiamin, Turmerone, Ukonan-a, Ukonan-b, Ukonan-c, Ukonan-d, Vanillic-acid, Zingiberene.

Active constituents present in *Curcuma Longa [9]*

Phytoconstituents	Structure
Curcumin I	
Curcumin II (demethoxycurcumin)	
Curcumin III (bis-demthoxycurcumin)	
Ar-tumerone	
α-Phellandrene	
Sabinene	
Geraniol	

Side Effects and Toxicity

No significant toxicity has been reported following either acute or chronic administration of turmeric extracts at standard doses. At very high doses (100 mg/kg body weight), curcumin may be ulcerogenic in animals, as evidenced by one rat study.

Traditional view [10]

Curcuma Longa vibrant yellow spice, derived from the rhizome of the plant, has a long history of use in traditional medicines of China and India . Use of curcumin as a folk remedy continues today. As part of the ancient Indian medical system, ayurveda, a poultice of turmeric paste is used to treat common eye infections, and to dress wounds, treat bites, burns, acne and various skin diseases . In Northern India, women are given a tonic of fresh turmeric paste with powder of dried ginger roots and honey in a glass of hot milk to drink twice daily after childbirth. A poultice of turmeric is also applied to the perineum to aid in the healing of any lacerations in the birth canal . Powdered turmeric is taken with boiled milk to cure cough and related respiratory ailments and roasted turmeric is an ingredient used as an antidysenteric for children . This ancient remedy is also used to treat dental diseases, digestive disorders such as dyspepsia and acidity, indigestion, flatulence, ulcers, as well to alleviate the hallucinatory effects of hashish and other psychotropic drugs . In food and manufacturing, curcumin is currently used in perfumes and as a natural yellow coloring agent, as well as an approved food additive to flavor various types of curries and mustards . Recent emphasis on the use of natural and complementary medicines in Western medicine has drawn the attention of the scientific community to this ancient remedy.

Dosage

Doses of 500-8,000 mg of powdered turmeric per day have been used in human studies. Standardized extracts are typically used in lower amounts, in the 250-2,000 mg range[10]

Pharmacokinetics

Little is known about the pharmacokinetic pathway of curcumin . Doses up to 5μg/ml of curcumin added to microsome- and hepatocyte suspensions disappeared within 30 minutes.
In rats, 40-75% of orally administered curcumin is excreted in the feces. Blood levels of less than 5 μg/ml indicate poor absorption from the gut.

Available data suggests that curcumin is poorly absorbed from the gut, rapidly metabolized and excreted in feces. [11]

In humans the estimated bioavailability of curcumin after oral administration is 65%. Cytochrome P 450 isoenzyme 1A1 is inhibited by curcumin and is metabolized by glucuronidation .
In a phase I clinical study subjects had average peak serum concentrations of 0.5, 0.6 and 2 μM after taking 4-, 6- or 8-g doses of curcumin. Urinary excretion of curcumin was not detectable

Pharmacological activity of *Curcuma Longa*

Anti-inflammation

Curcuminoids inhibit LOX, COX, phospholipases, leukotrienes, prostaglandins, thromboxane, nitric oxide [49, 50], elastase, hyaluronidase, collagenase, monocyte chemoattractant protein-1, interferon inducible protein, TNF and interleukin-12 .

Curcuminoids decrease prostaglandin formation and inhibit leukotriene biosynthesis via the lipoxygenase pathway.

Anti-depression

The effect of curcumin was investigated in a rat chronic mild stress (CMS) model. In comparison with normal rats, rats suffering the CMS procedure have a significant lower intake of sucrose, increased IL-6, TNF-α levels, CRF- and cortisol levels. Treatment with ethanolic extract increased the sucrose intake to normal control levels, reduced the CMS-induced increase in serum IL-6 and TNF-α levels and reduced the CRF levels in serum and medulla oblongata to lower than normal. It also lowered the cortisol levels in serum to normal levels . [12]

Side effects

The Food and Drug Administration classifies turmeric as a substance Generally Recognized as Safe . No major side effects have been reported in the clinical studies

No side-effects were reported in patients with rheumatoid arthritis treated with 1200 mg/day of

curcumin for two weeks .

In a phase I trial with 25 subjects, who had various high-risk cancerous conditions, no toxic reactions were observed. The subjects received up to 8 g of curcumin a day for

3 months .

In a clinical study in patients with irritable bowel syndrome dry mouth and flatulence was reported by approximately 25% of the patients. In another study two of 19 patients treated with 2500 mg of curcumin per day, complained of gastric irritation. No other adverse effects were reported.

Mild side-effects as nausea, diarrhoea, headache, tiredness and sleepiness have been reported in the turmeric group (2 g/day) as well as in the other groups (placebo and comparitive herbal combination). [13]

Rare cases of allergic contact dermatitis have been reported. In an 18-month study on the topical use of curcumin to treat skin and mucous membrane cancers, scalp itching was observed in 1 patient of 62 patients. Patch testing led to allergic reactions (not further classified) in persons who were regularly exposed to the substance or who already had

dermatitis of the finger tips. Few allergic reactions (skin rash) occurred to people not previously exposed to curcumin

Drug interactions

Because curcumin was found to stimulate the gall bladder, the use of curcumin or turmeric is contraindicated in patients with obstruction of
the biliary tract .

Cautions

The use of Curcuma longa in children under the age of 18 years cannot be recommended. As relevant data on the use during pregnancy and lactation is lacking, Curcuma longa can not be recommended in these cases. [14]

Advantages

- Mild side effects
- Reversible side effects
- Do not need a prescription

Chapter-3

Objectives

- Literature review
- Collection and drying of plant material (leaves).
- Extract preparation using soxhlet apparatus.
- Phytochemical screening of plant extract.
- Physicochemical tests.
- Anti oxidant activity *of Curcuma Longa*.
- Anti- microbial activity of *Curcuma Longa*.

Work done

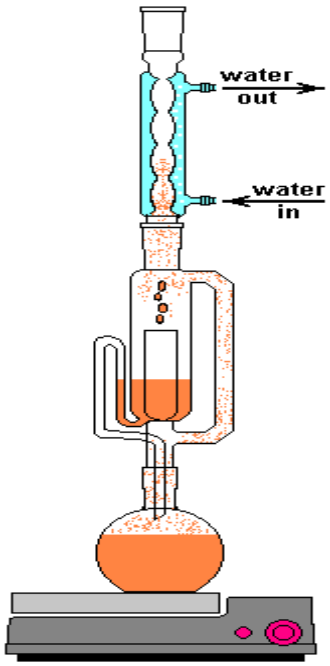
- Plant collection – whole plant of *Curcuma Longa* was collected from Dumehar region.
- Drying of plant – air drying.
- Then the rhizomes of the dried plant were collected.
- Extraction using soxhlet
- Phytochemical screening of the plant: It was done to test the extract for its chemical constituents. The plant extract was tested using various reagents for different tests.
- Physicochemical tests were performed to check the extract for impurities.
- Anti oxidant activity of curcuma longa was checked using DPPH assay .
- Anti microbial activity against gram positive and gram negative bacteria using agar medium.

Materials

The plant *curcuma longa* was collected from Dumehar region. Extraction was performed using soxhlet assembly. Rotatory evaporator was used to remove the solvent and then lypholysation was done using a lypholyser. Defatting of the extract was done with Hexane. Then ethanol and water in the ratio of 1:1 were used for the hydroalcoholic extraction.[16]

Methods

Soxhlet extraction



- The 22g of dried powdered rhizomes of *curcuma longa* were packed in the thimble using some cotton. Organic solvent (hexane) was placed in the flask which was placed on heating mantle(temperature maintained at 20 to 30 degree celsius), continuous water supply was provided to the condenser to keep the system cool and extraction was done as follows:

- Defatting of plant – it was done with hexane for 48 hours ; the leaves were placed in the assembly of the soxhlet along with the solvent (hexane).Temperature maintained was 20 degree Celsius.
- Methanolic extraction of plant – it was done for 10 days. Solvent used was methanol temperature maintained was 30 degree celsius .
- The solvent was removed from the plant extract using rotator evaporator at 50 degree Celsius.
- Lypholysation of the plant extract was done and stored at 4 degree celsius.

Phytochemical Tests:

The test for Carbohydrates (Molisch Test): Few drops of α-napthol was added to each portion dissolved in distilled water, this was then followed by the addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for 2 min. Formation of a red or dull color at the interphase of the two layers was a positive test.

The test for Tannins: About 0.01g of each extract was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2ml of the filtrate occurrence of blue-black, green or blue –green precipitate indicates the presence of tannins.

The test for phenols: Few drops of extract was mixed with 3-4 drops of ferric chloride solution. Presence of bluish black color precipitate indicates presence of phenols.[17]

The test for Saponins*:* Some portion of each extract was boiled with 5ml of distilled water, filtered. To the filtrate, about 3ml of distilled water was further added and shaken vigorously for 5 min. Frothing which persisted on warming was taken as an may be the presence of saponins.

The test for Flavonoids: Few quantity of the each portion was dissolved in water and filtered. To 5ml of each of the filtrate, 3ml of Lead ethanoate solution was then added. Appearance of buff-colored ppt indicates the presence of flavonoids.

The test for Alkaloids: Few quantity of the each portion was stirred with 5ml of 1% aq. HCl on water bath and then filtered. Of the filtrate, 1ml was taken individually into 2 test tubes. To 1ml, Mayer's Reagent was added and appearance of buff-colored ppt will be an indication for the presence of alkaloids.

The test for cardiac glycosides (legals test) - the test extract was treated with pyridine followed by the addition of sodium nitropuside solution, red colour indicate the presence of cardiac glycosides.[17]

Physicochemical tests

Ash test-

Ignite a crucible of platinum, quartz or porcelain at 500-550˚C for 1 hour,

↓

Allow it cool in a desicator and weigh it accurately

↓

Place few gms of sample in the crucible and weigh it accurately

↓

Take off or slide the lid of crucible if necessary, heat the crucible gently first then raise the temperature gradually.

↓

Ignite at 500-550˚C for not less than 4 hours to incinerate until it is free from charred material, cool in a desiccator and weigh accurately. incinerate the residual to constant weigh, cool in a desicator and weigh accurately.

Acid insoluble ash-

Add carefully 25 ml of dil. Hcl to the ash (obtained as directed under the ash limit test), boil gently for 5 min, collect the insoluble matter on the filter paper for quantitative analysis, wash thoroughly with hot water and dry the residue together with the filter paper.

Ignite it for 3 hours in a crucible of quartz which has been prepared as directed in the ash limit test and whose weight is already known.cool it in a desiccators and weigh accurately.

If the measured amount is larger than the specified value ignite until a constant weight is obtained.[18]

Water soluble ash

To crucible with total ash add 25ml of water and boil for 5 min followed by collection of insoluble matter in sintered glass crucible/ filter paper .wash with hot water and ignite in crucible for 15 minutes at a temperature not exceeding 450˚C. Subtract weight of the residue in mg from weight of total ash.

Moisture content

The most convenient procedure for determining the mass of the sample before and after drying is to place it in a tared container where it will remain throughout the test. The mass of the container and sample are determined and the mass of the container subtracted. If the mass of the test sample is not determined immediately after preparation, place the moisture-tight cover on the container to prevent evaporation. Dry to constant mass at 110 ± 5°C.

Fractionation using separating funnel:-

1) 5g of extract was loaded in separating funnel along with water.

2) Hexane was mixed in the separating funnel and shaked properly. The mixture was allowed to stand for 3-4 hours. Hexane layer was collected from the bottom of the separating funnel and named as fraction 1.

3) Same procedure was followed for other solvents like chloroform , ethyl acetate , methanol and the fractions were named as 2, 3, 4 respectively.

4) At last water layer was collected and named as fraction 5.

Anti – oxidant assay:-

1) DPPH assay:-

Preparation of DPPH solution: - 1.5*10^-4 M concentration of DPPH solution was prepared in methanol.

The free radical scavenging capacity of various fractions of *Curcuma longa* L. was evaluated

First, 4 mL of test sample in MeOH (final concentrations were 12.5, 25, 50, and 100 μg/mL, respectively) were mixed with 1 mL of 1.5×10-4 M DPPH solution. After 30 min of incubation at room temperature, the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation:

Scavenging rate= [1-(A1-A2)/A0] ×100%

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract, A2 was the absorbance without DPPH.[19] [20]

Anti microbial assay:-

Cultures of bacteria were grown on nutrient broth at 37°C for 12–14 h and were maintained on nutrient agar slants at 4°C. The extracts were dissolved in ethylene glycol, membrane-filter (0.47 mm) sterilized and tested for antibacterial activity using disc diffusion method. A concentration of 2000 mg/disc was chosen based on available literature. Sterile 6-mm diameter filter paper discs were impregnated with 2000 mg of the sterile test material, and placed onto nutrient agar surface spread with 0.1 ml of bacterial culture. The plates were incubated at 37°C for 12–14 h. The experiments were carried out in triplicate. The results (mean value $n = 3$) were recorded by measuring the zone of growth inhibition around the discs. For comparison, standard antibiotics gentamycin and ampicillin inhibiting bacterial cell wall biosynthesis were included in the assay.[21]

Chapter- 4

Results:

After extraction 8 g of extract was obtained.

% yield obtained :- (wt. of extract obtained / total sample loaded) * 100.

= (8/ 22) * 100

= 36.36%

Phytochemical tests

Sr. No.	COMPOUND	TESTS	RESULT
1)	ALKALOIDS	HAGER'S + MAYER'S +WAGNER'S+DRAGENDOFF'S	NEGATIVE
2)	FLAVONOIDS	ALKALINE REAGENT	POSITIVE
3)	VOLATILE OILS	SUDAN 3	POSITIVE
4)	CARBOHYDATE	MOLISCH'S + BENEDICT'S	POSITIVE
5)	SAPONIN	FROTH FORMATION	POSITIVE

6)	**TANINS**	**GELATIN TEST**	**POSITIVE**
7)	**PHENOLS**	**FERRIC CHLORIDE**	**POSITIVE**

Physicochemical tests

Test	Experimental value	Standard value(as per WHO)
Moisture content	6.4%	Not more than 10%
Total ash content	4.9%	Not more than 7%
Acid insoluble ash	2.1%	Not more than 2.5%
Water soluble ash	10.8%	Not more than 12

Fractionation of extract:

5g of extract was loaded and amount of fractions obtained is:

Hexane: 52 mg

Chloroform: 72.2 mg

Ethyl acetate: 122 mg

Methanol: 1.2 g

Water: 2.2 g

Anti oxidant assay

No. of samples	Concentration of samples(mg/50ml)	Absorbance	abs	abs	Mean absorbance	% inhibition
Blank	0	0	0	0	0	0
1	2.9850	0.073	0.082	0.074	0.076	92.00
2	1.9900	0.122	0.137	0.142	0.133	86.75
3	0.9950	0.367	0.333	0.370	0.356	64.54
4	0.4975	0.641	0.614	0.638	0.631	37.15
5	0.2487	0.777	0.784	0.776	0.779	22.41

Anti microbial assay

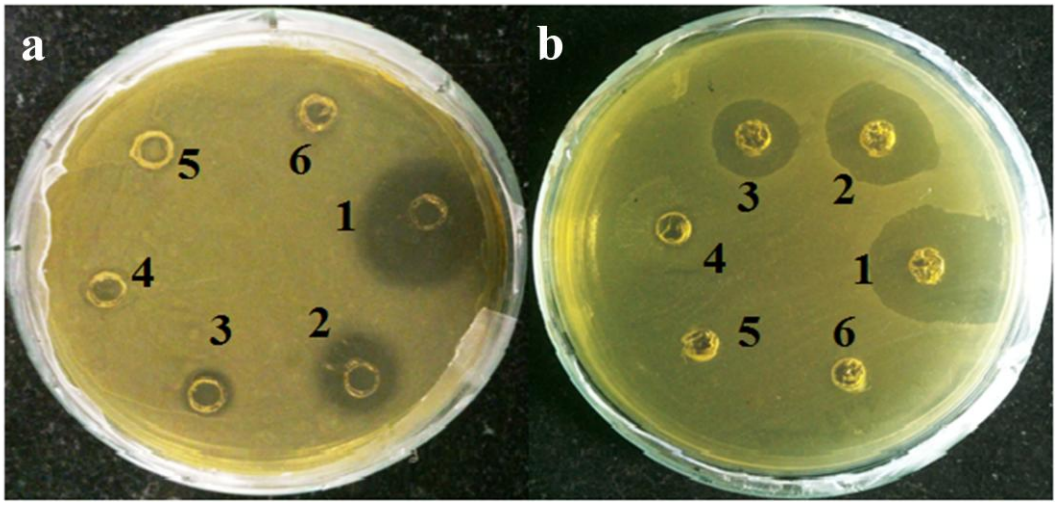
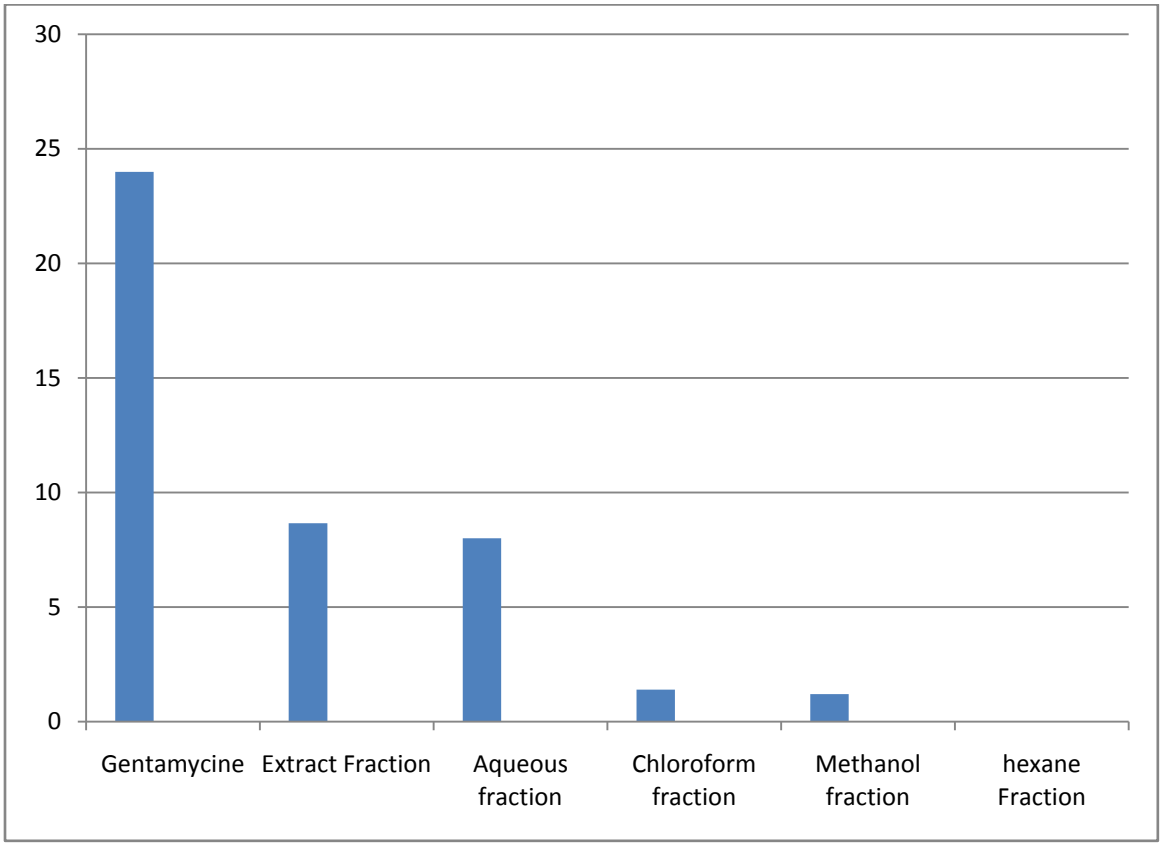


Figure 1.Qualitative growth inhibition assay of bacteria on agar plates with effect on (a) *E.coli* and (b) *S.aureus*after 24 h incubation with various extract of curcuma longa.

1. Standard
2. Curcuma Longa rhizome extract
3. Aqueous fraction of C. Longa
4. Chloroform fraction of C. Longa
5. Methanol fraction of C. Longa
6. Hexane fraction of C. Longa

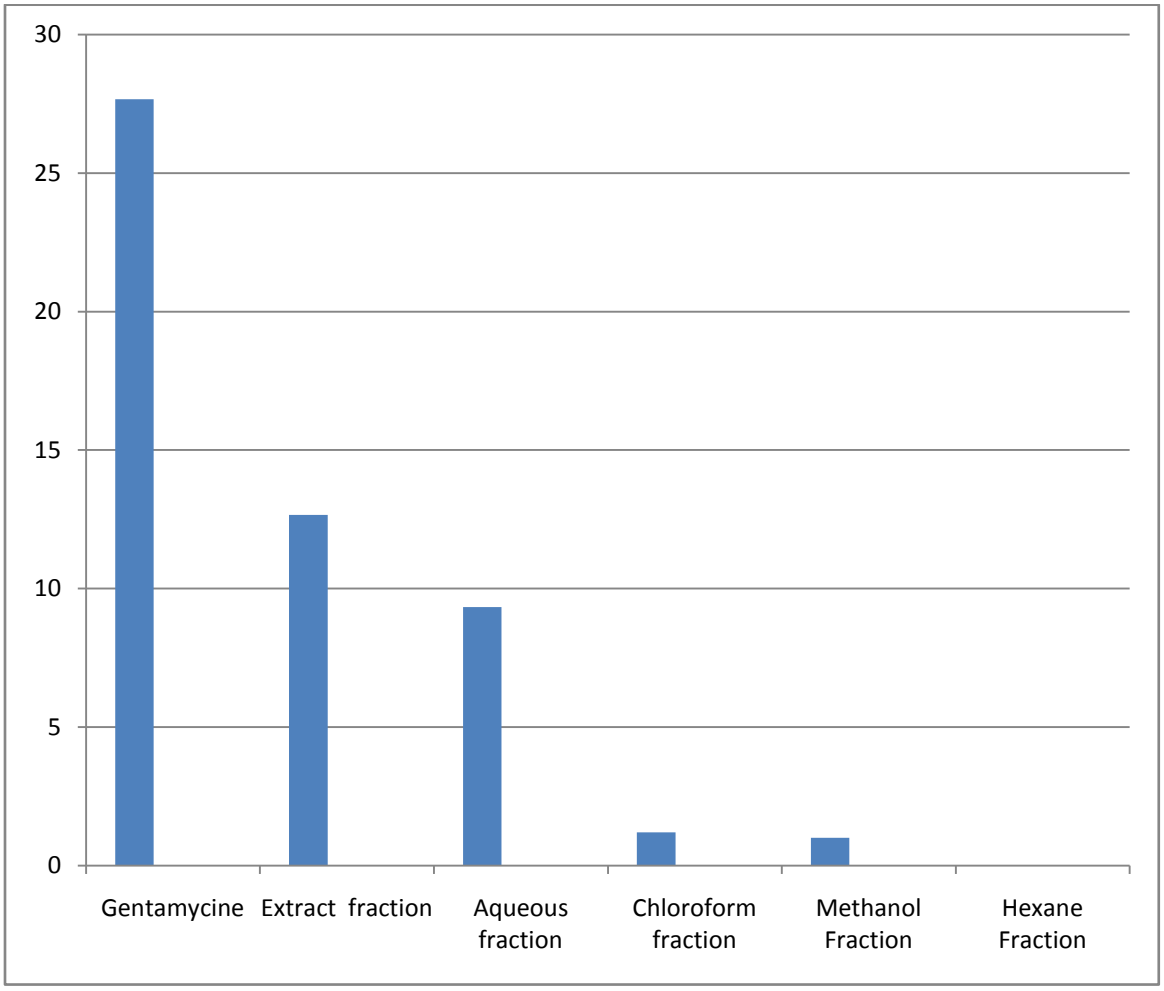
Table: Zones of inhibition of gram positive S. Aureus and gram negative E. coli:

Sr no.	Name of drug	Zone of inhibition In (mm) mean
1	Gentamycine (30 mcg)	24.00
2	Extract fraction	8.66
3	Aqueous fraction	8.00
4	Chloroform fraction	1.4
5	Methanol fraction	1.2
6	Hexane fraction	0.00



S. Aureus

S.N..	Name of drug	Zone of inhibition in (mm) mean
1	Gentamycine (30 mcg)	27.67
2	Extract fraction	12.66
3	Aqueous fraction	9.33
4	Chloroform fraction	1.2
5	Methanol fraction	1.0
6	Hexane fraction	0.0



Discussion and Conclusion:

Anti microbial infections are the most common type of infections prevalent in the world in recent times. Viral, fungal, bacterial infections are the most common one. There are certain treatment for these infections like the antibiotics etc but the most commonly used drugs has problem as the microorganisms has acquired resistance against them and they are no longer affective.

Curcuma Longa is well known herb and traditionally used for its anti inflammatory property and also possess certain other properties like anti cancer, anti bacterial, anti oxidative etc. In this study we focused on phytochemical study , anti oxidant property and anti microbial activity of fractions of extract of Curcuma Longa. The phytochemical study proved the presence of flavonoids, carbohydrates etc. Anti oxidant assay was done using DPPH method and % inhibition was found to be 18.4 mcg/ml. Anti microbial assay was done by cup plate method on gram positive and negative bacteria and zone of inhibition of fractions in gram positive S. Aureus was found to be maximum in case of extract and aqueous fractions 12.66mm and 9.33mm respectively and same was found in gram negative bacteria. These fractions of extract can be used to develop efficient therapeutic anti microbial therapy.

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