Chapter 7
MICROBIOLOGICAL ANALYSIS OF MEDICINAL PLANTS

7.1 Introduction:
The study of unicellular, Multi cellular or non-cellular microscopic organism is known as microbiology. Microbiology encompasses varied sub-disciplines together with medical specialty, mycology, parasitological, and medicine. There are 2 sorts of organisms eukaryotic and prokaryotic. The microorganisms in which cell organelles are bound by cell membrane are known as eukaryotic. E.g. fungi, protests. On the other hand side the organism which do not consist of bounded cell membrane are known as prokaryotic cell. Prokaryotic organisms embrace eubacteria and archeaebacteria.

Microbiologists historically relied on culture, staining, and research. However, but 125th of the microorganisms present in common environments are often cultured in isolation mistreatment current suggests that. Microbiologists perform analysis through extraction or detection of nucleic acid, either from deoxyribonucleic acid or ribonucleic acid sequence.

The branches of microbiology are often classified into pure and applied sciences. Supported Taxonomy, biology are often classified into

1. Bacteriology,
2. Mycology,
3. Protozoology, and
4. Phycology

A variety of biopolymers, like polysaccharides, polyesters, and polyamides, are made by microorganisms. Microorganisms are used for the biotechnological production of biopolymers with tailored characteristics appropriate for rich-value medicinal uses like tissue engineering and drug delivery. Microorganisms are used for the biogenesis of xanthan, alginate, cellulose, cyanophycin, poly (gamma-glutamic acid), levan, hyaluronic acid, organic acids, oligosaccharides and polysaccharide, and polyhydroxylalkanoates.

Microorganisms are useful for microbe biodegradation or bioremediation of
domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments. The flexibility of every being to degrade waste matter depends on the character of every material. Since sites generally have multiple waste material sorts, the foremost effective approach to microbic biodegradation is to use a mix of microorganism and plant species and strains, every specific to the biodegradation of 1 or a lot of varieties of contaminants.

There are three types of microbial communities
1. Symbiotic
2. Probiotics
3. Prebiotics

Symbiotic microorganism communities confer advantages to their human and animal host’s health together with aiding digestion, manufacturing useful vitamins and amino acids, and suppressing morbific microbes. Some profit is also reaped by intake hard foods, Probiotics (bacteria probably useful to the organic process system) or Prebiotics (substances consumed to market the expansion of Probiotics microorganisms).

AN Antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines are sorted in keeping with the microorganisms against that they act. E.g. antibiotics are used against bacterium and antifungal are used against fungi. They’re classified in keeping with their operate. Agents that kill microbe’s are known as microbicidal, whereas those that inhibit their growth are known as biostatic. the applying of antimicrobial medicines to treat infection is thought as antimicrobial therapy, whereas the utilization of antimicrobial medicines to forestall infection is thought as antimicrobial bar.

Anti-microorganism agents are classified into
1. Disinfectant is the opposed microorganism that kill a good vary of microbes on non-living surfaces to forestall the unfold of ill health.
2. Antiseptics is the opposed microorganism that ar applied to living tissue and facilitate scale back infection throughout surgery, and
3. Antibiotics the opposed microorganism that destroy
microorganisms inside the body. Bactericide agents are more divided into antiseptic agents, that kill bacteria, and organic process agents, that hamper or stall microorganism growth.

4. Antioxidant phenomenon has been classified into Antifungal agents accustomed kill or stop more growth of fungi.

5. Antiviral medicine is a category of medication used specifically for treating infective agent infections.

6. Antiparasitics are a category of medicines indicated for the treatment of infection by parasites, like nematodes, cestodes, trematodes, infectious protozoa, and amoebae.

7. Antimicrobial medicine is used since last a few years for microorganism pathogenicity and different infectious diseases. Antibiotics play a crucial role to resist the microorganism pathogens. Since artificial medicine don't seem to be solely pricey and inadequate for the treatment of diseases however additionally typically with adulteration and facet effects, thus there's AN imperative have to be compelled to realize new infection fighting ways to regulate microorganism infections.

8. There are several healthful plants that have bactericide properties and that are employed in Ayurveda and ancient healthful system for treatment of symptoms caused by microorganisms. Healthful plants have bioactive compounds that are used for natural process numerous human ailments and additionally play a crucial role in healing. These bioactive compounds are referred to as phytochemical. Healthful plants are those plants that possess therapeutic properties or exert useful medicine effects within the animal body. They need antifungal, bactericide and anti-inflammatory activities phytochemical ar present in healthful plants, leaves, roots and vegetables that have defence mechanism to safeguard human population from numerous diseases. 2 kinds of phytochemical i.e. primary and secondary constituents are gift in healthful plants. Primary constituents contain pigment, proteins, sugars and amino acids. Secondary constituents contain terpenoids and alkaloids that effectively facilitate in natural process the diseases like infective agent fever, dengue, malaria, swine flu. The medicines ready from these vegetal medicine are referred to
as flavouring medicines. About 300,000 plant species exist around the world, out of which only 15% have been explored pharmacologically.

7.1.1 Microbiological analysis

Active chemical constituents are present in any part, like root, stem, leaves, flower, seed, and fruit of medicinal plants. Microbes infect the metabolic system of animals and human through the active chemical compounds present in these parts. The medicinal properties of plants are based on the anti-bacterial, antifungal and anti-oxidant effect of the phytochemical present in them which produce a definite physiological action on human body. The important bioactive constituents present in medicinal plants are flavonoids, tannins, alkaloids and phenolic compounds. The increase in the number of infections and antibiotic resistance effect has become a therapeutic problem. Natural products of the medicinal plants may possess some anti-microbial agents which are effective for the treatment of some of the infectious diseases.

Microbiological analysis is often applied to diseases causing spoilage microorganisms; End product testing remains a vital part of any food manufacturing control strategy. In products in which microorganisms can survive and grow, routine microbiological analysis is important to confirm that manufacturing control mechanism is effective. Microbiological analytical procedure determines the concentration of bacteria in a given sample.

Microbiology means the study of microbes or microorganisms.

There are different types of microorganisms present on earth as discussed below.

Microbes or microorganisms are microscopic organism that exists as unicellular, multicellular or cell clusters, no cell at all (a cellular). Microbiology testing covers a wide range of studied subject including but not limited to fungi, parasites, algae, viruses.

Microorganisms are widespread in nature, are beneficial to life but some can cause serious harm. They are of six types.

- Bacteria
- Archaea
- Fungi
- Protozoa
These six types of microbes or microorganisms are discussed as below.

7.1.2 Bacteria:

Bacteria are terribly tiny organisms and that they are prokaryotic microorganisms. Microorganism cells don't have a nucleus, and most haven't any organelles with membranes around them. Most have a semi permeable membrane, they contain deoxyribonucleic acid, and their biochemistry is same as different living things. They're amongst the simplest and conjointly the oldest organisms. They perform as freelance organisms. Almost all microorganisms are therefore little they will solely be seen through a magnifier. Microorganism are created of one cell, so that they ar a form of animate thing organism. They among the best one-celled organisms on Earth, and were one among the earliest styles of life. They embrace variety of extremophiles that board extreme habitats. There are in all probability additional individuals microorganismsthan the other variety of organism on the earth. Most microorganismboard the bottom or in water, however several live within or on the skin of alternative organisms, as well as humans. There are concerning 10 times as several microorganism cells as human cells in every of our bodies. Some microorganism will cause diseases, however others facilitate U.S.A. in
everyday activities like digesting food (gut flora). Some even work for U.S.A. in factories, manufacturing cheese and yoghurt.

They are of different four shapes.
1. Rod shape – Bacillus
2. Spherical shape – Coccus
3. Spiral shape – Spirilla
4. Curved shape - Vabrio

These organisms are differing in their cell wall structure and therefore they can be classified by their cell wall structures.

Gram positive and Gram negative organisms

Fig. 7.2 Gram Positive Bacteria

Fig. 7.3 Gram Positive Bacteria
Archaea:
Archaea area unit small, straightforward organisms. They were originally
discovered in extreme environments (extremophiles), however area unit currently thought to be common to a lot of average conditions. Several will survive at terribly high (over 80°C) or terribly low temperatures, or extremely salty, acidic or alkaline water. Some are found in geysers, black smokers, oil wells, and hot vents within the deep ocean. Recent analysis has found ammonia-eating archaea in soil and saltwater. In the past that they had been classed with bacterium as prokaryotes and named Archaea bacterium, however this classification was a slip-up. The Archaea have associate degree freelance history and show several variations in their organic chemistry from alternative type’s of life. They’re currently classified as a separate domain within the three-domain system. During this system, the 3 distinct branches of biological process descent area unit the Archaea, bacterium and eucaryote.

Archaea bacteria are the oldest living microorganisms found on the earth. They are unicellular prokaryotes. They are different from bacteria and eukaryotes. They are found in extreme climatic conditions.

![Fig 7.6 Structure of Archaea](image)

They are different from the bacteria. They are prokaryotic cells based on their habitat they are divided into following types

1. Methanogens (methane producing organisms)
2. Halophiles (lives in salty environment)
3. Thermophiles (lives in extremely hot temperature)
4. **Psychrophilrs (lives in cold temperature)**

The energy sources required for archaea are $\text{H}_2$, $\text{CO}_2$, and S and some sunlight.

![Fig7.7 Structure of Archaea](image)

**Fungi:**

![Fig.7.8 Structure of fungi](image)

A fungus is a group of eukaryotic, non-phototrophic organisms with stiff cell walls that contains of mushrooms, molds and yeast. They are eukaryotic cells with true nucleus. They are multicellular. They obtain nutrients by absorbing organic material from their environment i.e. decomposes, plants (symbionts) or host, parasites. Fungi reproduce by releasing spores. They contain large amounts of chitin, a structural component only found in the cell walls of fungi.
The chitin makes the cells walls rigid. They cannot perform photosynthesis. Some of them acquire food from dead microorganisms.

A flora is of an oversized cluster of being organisms that contains microorganisms like yeasts and molds, yet because the additional known mushrooms. The Fungi are classified as that's break free all the plants and animals. The biology dedicated to the study of fungi is thought as mycology that is usually considered a branch of phytology, even if genetic studies have shown that fungi are additional closely associated with animals than to plants. Fungi reproduce via spores that are usually created on specialised structures or in mature bodies, like the top of a mushroom. Abundant worldwide, most fungi are unnoticeable to the ocular attributable to the little size of their structures, and their cryptic lifestyles in soil, on dead matter, and as symbionts of plants, animals, or alternative fungi. Fungi perform a necessary role within the decomposition of organic matter and have basic roles in nutrient athletics and exchange. They need long been used as an on the spot supply of food, like mushrooms and truffles, as a leavening agent for bread, and in fermentation of varied food product, like wine, beer, and condiment. Since the Forties, fungi are used for the assembly of antibiotics and additional recently, numerous enzymes created by fungi are used industrially and in detergents. Fungi also are used as biological agents to manage weeds and pests.

Many species manufacture bioactive compounds known as mycotoxins, like alkaloids and polypeptides that are noxious to animals as well as humans. The mature structures of some species are consumed recreationally or in ancient ceremonies as a supply of psychoactive compounds. Fungi will break down factory-made materials and buildings, and become vital pathogens of humans and alternative animals. Losses of crops because of fungous diseases or food spoilage will have an oversized impact on human food provides and native economies. Despite their importance on human affairs, very little is thought of actuality diverseness of fungus kingdom that has been calculable at around one point five million species, with regarding five hundred of those having been formally classified.
Protozoa:
They are small organisms. They are eukaryotic which feeds on bacteria. Protozoa is old term while protest is new term for junior education protozoa is generally used. It is very convenient term but it divided into different phyla. This term is problematic because it contains different kinds of cells which are found in aquatic nature. There are some species found in soil and algae. Around 30000 protozoan’s species are found in the nature.
Fig. 7.10 Structure of protozoa

They make up the largest group of organisms in the world in terms of numbers of biomass and diversity. Their wall is made up of cellulose. They don't have any tissues or organs. They have one or more nuclei but no nucleus is in charge of special part of cytoplasm. They are classified by their mode of locomotion.

1. Flagellates – Move because whip like structure.
2. Chillates – Move because tiny hair present on body.
3. Amoeboids – Pseudopodia
4. Sporozoans – They do not move.

Algae:
Algae are an outsized and various cluster of plant life organisms. Some have single cell et al. have multi cells. The biggest and complicated marine protoctists are known as sea-weeds. The same like plants, and that they area unit straightforward as a result of they lack the numerous distinct organs found in land plants. For that reason they're not classified as plants. Although the prokaryotic blue-green algae i.e. blue-green protoctist cyanobacteria, eubacteria, eubacterium, true bacteria were enclosed as algae in older textbooks however it's not currently. The protoctist area unit the term that is currently used for organism organisms. The protoctist has a nucleus inside a membrane and chloroplasts within one or additional membranes called true protoctist. Trendy taxonomists propose cacophonous them up into monophyletic teams, however, not everybody agrees a way to try this. Algae don't have a similar structure that land plants do, like leaves, roots, and alternative organs. Nearly all protoctist have elements that do chemical action a similar means as blue-green algae. They create atomic number 8, not like alternative chemical change microorganism like purple and inexperienced microorganism. Some animate thing species use solely external energy sources and have restricted or no chemical change elements. Any of diverse teams of chlorophyll containing, mainly aquatic organism organism’s travel from microscopic single celled forms to multicellular forms a hundred feet (30 meters) or additional long, distinguished from plants by the absence of true roots, stems, and leaves and by a scarcity of no reproductive cells within the reproductive structures.

They have been classified into the six categories

1. Phyla Euglenophyta
2. Crysophyta,
3. Pyrrophyta,
4. Chlorophyta,
5. Phaeophyta,
6. Rhodophyta.
Viruses:

Fig 7.12 Structure of virus

They consist of nucleic acid core (DNA and RNA). Viruses cannot reproduce outside a host cell and also cannot metabolize on their own. Dengue fever is also spread by a dengue virus. It is a single stranded RNA virus and it is the cause of the dengue fever. Dengue found in worldwide mostly in tropical and sub-tropical areas. This disease occurs in those areas where infected mosquitoes get dengue virus mostly in rainy season. The diagram of dengue virus is discussed below
Fig 7.13 Dengue virus

The virus of dengue is spherical in shape. The nucleocapsid is present inside the virus and it is made up of the viral genome and C proteins. Nucleocapsid is surrounded by lipid bilayer taken by the host called as viral envelope, which is lipid bilayer and they control the entry of the virus.

Medicinal microbiology testing is the study of microbial germs such as viruses, bacteria, parasites and fungi. Generally human body offers a natural defence against these microbes in the form of our immune system and helpful bacteria. Pharmaceutical microbiology is related to the testing of antibiotics, enzymes, vitamins and vaccines. Both of them are interrelated.

Since human health is negatively affected by the presence of microorganisms such as pathogens, bacteria, yeast and molds, it becomes all the more important to do the microbiological analysis of the plant assays.

Microbial assay is the method of measuring compounds such as vitamins and amino acids using microorganisms. Plant assays are prepared by washing the selected medicinal plant leaves, roots, bark, fruits or flowers with water several times and drying them in shade. The dried parts are crushed into fine powder and extracted with distilled water. The assay thus prepared is subjected to microbiological analysis.

In the present study the microbial structure of extracts of medicinal plants and the extracts of the plants in combination ratios were doped with the normal and infected blood. These preparations were then studied under light microscope.

1.1.3 Instrumentation:
A microscope may be a device that enlarges objects that are too tiny to envision with the oculus. Magnification is calculated by dividing the image size by the objects size. There are 3 varieties of microscopes:

1. Optical microscope or light microscope
2. Electron microscope

Light microscope was used for the present analysis. Light microscopes are used as primary visualization tools. It uses visible light to detect small objects like microbes, bacteria. Since they do not offer highest magnification therefore they are used to view cells which have limited structure. The object should have higher wavelength than that of the light to produce an image in this type of microscope. The cell components are stained before viewing because they are transparent in nature.
1. **Eyepiece:**
   It is the lens by which the viewer appearance through to visualize placed the specimen. It contains a pair of or a lot of lenses that focus the image. It always contains a ten times magnification.

1. **Dioptre Adjustment:**
   It is wont to modification the main target for vision between our eyes.

2. **Turret**
   This holds a pair of or a lot of objective lenses and may be revolved simply to alter magnification power. Commonly once viewing a slide for instance, it's best to start out the magnification at the bottom and so work your manner upward.
3. **Body tube** (Head)
   It connects the ocular to the target lenses.

4. **Arm**:
   It connects the body tube and also the base of the magnifier.

5. **Coarse adjustment**:
   It brings the specimen or sample into general focus.

6. **Fine adjustment**:
   It focuses the specimen and will increase the detail of the specimen.

7. **Nosepiece**:
   By spinning the nosepiece we are able to choose totally different objective lenses.

8. **Objective lenses**:
   It is one amongst the foremost vital components of a magnifier. It's one or a lot of objective lens to gather lightweight. The lenses square measure typically cylindrical formed tube. The shortest lens has the bottom power i.e. the bottom level of magnification; the longest one is that the lens that has the best magnification power. a regular magnifier has 3, four, or 5 objective lenses that range in power from 4X to 100X.

9. **Specimen or slide**:
   It is the article that is being examined. Most specimens' square measure placed on slides, flat thin glass. The specimen is placed on the glass and a canopy slip is placed thereon.

10. **Stage**:
    It is the flat platform on that the slide or specimen is mounted.

11. **Stage clips**:
    It is a metal clips that holds the slide or specimen.

12. **Stage height adjustment (Stage Control)**:
    The moving knobs facilitate to manoeuvre the left stage and right or up and down.

13. **Aperture**:
    It is the outlet within the between the stage that permits lightweight from the illuminator to the specimen.
14. **On/off switch:**
   This switch placed on the bottom of the magnifier that turns the illuminator on and off.

15. **Illumination:**
   It is the sunshine supply for a magnifier. Older microscopes used mirrors to mirror lightweight from Associate in Nursing external supply up through very cheap of the stage; but, most microscopes currently use a low-tension bulb.

16. **Iris diaphragm:**
   It adjusts the quantity of sunshine or light that reaches to the specimen

17. **Condenser:**
   It gathers and focuses the sunshine from the illuminator onto the specimen.

18. **Base:**
   The base supports the magnifier.

19. **Focus wheel:**
   These square measure the wheels that square measure wont to move the stage within the vertical plane. There square measure wheel for focusing eye piece additionally.

20. **Frame**
   The frame consists of the arm, the bottom and is in essence the bodywork of the magnifier. It permits attachment of the main target wheels and also the stage to the magnifier.

21. **Light**
   A light supply utilized in place of a mirror. Most microscopes do enable manual lightweight adjustment via a wheel situated close to the bottom.

22. **Condenser**
   The operate of the condenser lens is to focus the sunshine onto the specimen. To extend the standard the condenser lens can also have filters or a diaphragm.

7.2 **Principle:**
A general biological magnifier primarily consists of Associate in Nursing objective lens, ocular lens, lens tube, stage, and reflector. Associate in nursing object placed on the stage is enlarged through the
target lens. Once the target is targeted, an enlarged image may be curtained through the ocular lens.

7.3 Working:

**The microscope increases the angle of light or cone angle $\theta$**

Fig 7.1.3 Working of Optical Microscope

Fig 7.1.4 Working of Optical Microscope

7.3 Working:
A compound microscope gathers lightweight from a little space and sends this light through the target lens. The target lens magnifies the sample, as do the eyepieces we glance through. So as to focus the image, the coarse focusing is employed. Sample is placed within the correct location to get a transparent image by moving the focusing knob either stage up and down, or moving the pinnacle of the magnifier up and down. Once the coarse focus knob has been accustomed place the sample within the correct location, next the fine focus knob is employed to fine-tune the main focus and build a crisp and clear image.

Light microscopes use a condenser on top of the sunshine supply so as to focus and direct the sunshine up through the specimen (in little beam of light) and into the eyepieces. Each the resistor on the sunshine in addition because the iris on the condenser is employed so as to urge this beam of sunshine centred properly once viewing the sample. The objective lenses may be turned so as to alter the magnification of the magnifier. Most lightweight magnifiers utilize ten eyepieces and this magnification is increased by the target lens price to work out the microscope magnification. as an example, once victimisation the 4x objective lens (4x10=40x) the magnification is so 40x. If the target lens is turned to the 10x objective (10x10=100x) the full magnification becomes 100x. Light microscopes offer magnification up to 1000x. Any microscope that advertises magnification on top of 1000x is going to be providing empty magnification and therefore the pictures won't be clear or focused.

Microscopic examination helps within the higher analysis of compounds. throughout microscopic examination or microstructure analysis, the structure of fabric is studied beneath magnification. The properties of materials confirm however they'll perform beneath a given application, and these properties square measure dependent square measure on the material’s structure.

In the present work we've allotted the microscopic studies of the pure extracts of healthful plants, their combos in numerous ratios and conjointly the study of combos doped with infected blood

7.3 Procedure:
1. The selected medicinal plants were washed and dried in air under shade.

2. The dried leaves were crushed and extracted with distilled water to prepare a solution of the medicinal plants.

3. These extracts were applied on glass slides observed under light microscope.

4. Their structures were then analysed. The analysis results revealed different microscopic structure in case of each preparation, as shown below.

Epidermal fragment  Stone cell

Fig 7.1.5 Microscopic structure of Basil extract

The extract of basil under light microscope shows epidermal fragment which contains short conical hair and it also shows stone like cell.
The above diagram shows microscopic structure of Azadirachta Indica. The Microscopic examination shows stone like structure which is without cell wall.

Stone like cell

Fig 7.1.6 Microscopic structure of AzadirachtaIndica

Stone like structure

Fig 7.1 Carica papaya.

Pollen grain like structure
The carica papaya under microscope shows lots of pollen grain like structures. And little stone like structure.

Stone like structure
Fig 7.1.8 Zingiber
Zingiberene under microscope shows lots of stone like and mesh like structures.
Pollen grain like structure
Fig 7.1.9 Allium sanctum

Allium sanctum and curcuma longa shows lots of pollen grain like structures. But curcuma longa shows little small stone like structures too.

Each plant shows different microscopic structure as shown in the above pictures. Each of the plant shows different structures with different shapes.

7.4 Phytochemical study of medicinal plants:
Phytochemical are naturally occurring chemical in the medicinal plants i.e. in leaves, roots etc. Each phytochemical have definite mechanism to protect from various diseases. For the present study the selected medicinal plants i.e. basil, AzadirachtaIndica, Carica papaya, Allium sativum, Zingiber and Curcuma longa gave positive results when subjected to some phytochemical tests.

Saponins: Saponins are nothing but steroids or tri terpenoids glycosides. They are characterised by their bitter taste as well as foaming properties. Their haemolytic effect on red blood cells is also one characteristic of Saponins. Plant produce Saponins to fight different infections by parasites
and also it helps to immune system in human and also protects against different viruses and bacteria.

The name saponides is because they form foam like structure when comes in contact with the water. As said before, chemically they are located on a Steroid or Tri-terpene fat-soluble base attached to the sugar molecule which is water soluble, and produces a detergent. They also show in the digestive tract of the body the emulsification of fat-soluble molecules. The saponins in the plants are especially found in skins of the plants. They produce a waxy covering on body for protection. For plants the saponis are the natural antibiotics and they are the active immune system parts of the plants. Steroidal saponins are chemically structured as hormones of the body. They are proved as the hormonal activities of estrogen and cortisol. They consist of a portion called aglycone and it is known as sapogenin. Their aglycone portion is referred to as the sapogenin. Triterpenoid saponins contain very low hormonal activity. Number of times they are medicine for cough.

From all the chemical properties of saponins, the polarity, hydrophobicity and nature of the reactive groups appear valuable object of their biological characteristics, and therefore they are difficult to isolate as well as research.

Activities of saponins in human body:

The saponins consists of properties are more & different. They can also include alterative, diuretic, expectorant, anti-catarrhal, anti-inflammatory, antispasmodic, aphrodisiac, antioxidant, emmenagogue, cardiac stimulant, hormone modulating, hepatoprotective, and adrenal adaptogenic effects. The main property of saponins is the capacity to absorb different active compounds.

For detoxification kupffer cells are very supportive and they are provided by hepatoprotective. They lower down the cholesterol by binding with the cholesterol and therefore it should not be re-absorbed within the body. The digestive fluid acids make mixed micelles with cholesterol, facilitating their absorption. By preventing the biological process the Saponins cause a depletion of body cholesterol, and therefore increasing its excretion, in a lot of similar means of alternative cholesterol lowering medicine (sequestrants). The binding of digestive fluid acids by saponins have alternative necessary implications as primary digestive fluid acids area unit metabolized.
by microorganism within the colon manufacturing secondary the digestive fluid acids which may be promoters of carcinoma. Anti-cancer – The planned mechanism of anti-carcinogenic properties of saponins embody inhibitor impact, direct and choose toxicity of cancer cells, immune-modulation, acid and neutral alcohol metabolism and regulation of cell proliferation. Cancer cells even have a lot of cholesterol-type compounds in their membranes than traditional cells, so as saponins will bind steroid alcohol and that they might interfere with cell growth and division of cancer cells.

Toxicity Effects

Saponins will have an irritating impact on secretion membranes of the metabolic process and digestive tube, probably inflicting innate reflex, bloating, stomach flu, nausea, diarrhoea, and ejection. Saponins have conjointly been noted for his or her haemolytic properties as they will effectively “dissolve” the cell walls of red blood cells and disrupt them once taken endogenous or intramuscularly. Once take orally but they are relatively harmless or they’re not absorbed in any respect.

Flavonoids: flavonoids are the water soluble phenols of polyphenolic molecules. They are having activities like antioxidants and more health benefits such as anti-allergic, anti-cancer anti-oxidant, anti-inflammatory, anti-viral etc. The flavonoids gives health advantages by cell signalling pathways and antioxidant effects as they belongs to major group of metabolites present in plants. Many of fruits and vegetables contain these molecules. The flavonoids are 15 carbon atoms containing polyphenolic compounds. The two benzene rings are connected by a short three carbon chain. One of the benzene rings is connected with the carbon from the carbon chains directly or by oxygen chain, and it gives a third middle ring. The flavonoids are divided into six major subtypes,

- Chalcones
- flavones
- Iso-flavonoids
- Flavanones
- Anthoxanthins
• **Anthocyanins**
  Yellow colour of petals, red colour of buds as well as purple red colour of autumn is responsible just because of anthoxanthins molecules. The large number of flavonoids presents in the plants, and therefore they perform different roles and functions too. The flavonoids produce the colour by the pigments present in it to attract the insects for pollination. The flavonoids are also useful for UV filtration, nitrogen fixation, cell cycle inhibition, as well as chemical messengers in high order plants. The plants roots of some vegetables secrets the flavonoids to aid the cooperative relation between rhizobia. Example, peas, clover and beans. In the presence of flavonoids the rhizobia from soil make Nod factors. This is very useful for plants to recognize some reactions like ion fluxes as well as formation of root nodules. By preventing some cells, the flavonoids helps in protect different plant diseases. The plants contain flavonoids all over their parts. Human contains some common polyphenolic compounds in their diet. Many animals including human ingest plants and plant compounds which are the great source of flavonoids together with low toxic in nature. Onions, parsley, blueberries, and bananas, dark chocolate and red wine are the best example of rich flavonoids they are the best and very essential antioxidants for the health benefits. Other than these properties they give following useful effects.
  
  • Anti-viral
  • Anti-cancer
  • Anti-inflammatory
  • Anti-allergic
  Eczema, sinusitis, asthma, and hay fever are aid by the flavonoid known as quercetin. It is said by many researchers that, heart disease and flavonoid are inversely proportional to each other. It is very beneficial to reduce the oxidation of low-density lipoproteins and help to reduce atherosclerosis developing risk. Red wine is the rich source of flavonoid. By many studies it is proved that the French people have low number of heart disease as the people consume more red wine compare to other Europeans. One to two glasses of wine per day also help to prevent heart disease is said
by many researchers. The triglycerides and cholesterol in the blood can be lowered by consumption of some type of tea which is high in flavonoids.

Tannins: All plants produce Tannins in higher or lower concentration by the tissues like leaves, fruits and bark which are lost by them. Tannins are polyphenolic compounds and contain astringent characteristics like healing of wound. The tannins in plants help in healing of wound various ulcers and cancer. Tannins can be used for many treatments like, tissues tightening i.e. varicose veins, diarrhoea, protect skin, heavy menstrual flow and also check the infection. They also act as anti-inflammatory, antimicrobials & keratolytics. Tannins are phenols located on the benzene ring with a hydroxyl group combine to it. These compounds are water soluble. When glycosides combined with herbs in tincture form they precipitate alkaloids, nitrogenous bases and some. In the ingested tannins some are getting absorbed in kidneys while some of remain unabsorbed.

Alkaloids: These are naturally occurring chemical compounds basically containing nitrogen. They have wide range of pharmacological properties like anti-malarial, anti-asthma, anti-cancer, anti-bacterial, analgesic, etc. The plants having alkaloids have anti-inflammatory, psychotropic and stimulant effects which helps the human to develop resistance against diseases.

The major groups of alkaloids are as follows:

- Tropane (or Pyrrolidine)
- Isoquinoline
- Pyridine
- Pyrrolizidine
- Quinoline
- Indole
- Purine

The above groups are well known drugs and can be recognized by medicinal use. The Vincristine is used to treat the cancer cells. Another alkaloids such as atropine has a direct effect on the body such as reducing spasms, pain relief, diarhoea, and also pupils dilating during eye procedure.
Around 20 families of flowering plants contain alkaloids, the following are the most important alkaloids, Apocynaceae

- Asteraceae,
- Berberidaceae,
- Boraginaceae,
- Fabaceae,
- Papaveraceae
- Ranunculaceae,
- Rubiaceae,
- Rutaceae
- Solanaceae.

The Alkaloids in plants show various effects such as storage and transport of acids, antioxidant protection, and anti-predation effects etc. The Free alkaloids are normally soluble in ethanol as well as other organic solvents, but partially soluble in water. Tannins are responsible for alkaloids readily and irreversible precipitation.

In this present study, as we have selected all the medicinal plants with their different parts we tried to find out these phytochemicals from their extracts. By following tests we found some phytochemical properties of our selected plant materials.

1.5 Phytochemical Tests
A. Test for Alkaloids:
   Plant extract was treated with 2ml. Methanol. Few drops of 1% HCl were added to the mixture. On heating the mixture a white precipitate appears which confirm the presence of alkaloids.

B. Test for Tannin:
   Addition of 1-3 drops of ferric chloride solution to 1 ml of plant extract gives green colour confirms the presence of tannin.

C. Test for Glycosides:
   Acidifying 2ml of plant extract with glacial acetic acid and adding 1-2 drops of ferric chloride to the acidified solution with 0.5 ml concentrated H₂SO₄ gives brown ring at the junction confirms the presence of glycosides.
D. Test for Saponins:
Formation of foam by addition of 3 ml of plant extract to olive oil confirms the presence of Saponins.

E. Test for Flavonoids:
2ml of plant extract when treated with0.5 ml dil ammonia and acidified with conc. H$_2$SO$_4$ gives yellow colour which confirms the presence of flavonoids.

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Phytochemical tests.</th>
<th>Basil Indica</th>
<th>Azadirachta Indica</th>
<th>Carica papaya</th>
<th>Allium sativum</th>
<th>Zingiber</th>
<th>Curcuma longa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 7.5 Phytochemical tests for medicinal plants

The above table shows the presence of alkaloids in the selected medicinal plants. Thus the analyses of all the phytochemical activities of the aqueous extract of the medicinal plants selected for the formulation shows the presence of important and active phytochemical compounds present in these...
medicinal plants. This justifies the various therapeutic uses attributed to it by all the medicine.
PART B
ANALYSIS OF EXTRACTS AND THEIR COMBINATIONS DOPED WITH INFECTED BLOOD

Analysis of medicinal plants and their extracts were carried out by using different instruments which gave different results in each case. These results provided the information which was helpful for selection of the plants for further studies. Basil, Azadirachta Indica, zingiber, Carica Papaya, Curcuma Longa are the plants which have been selected for the final studies. The different combination ratios of this plants are (1:2:2:3:3), (2:2:1:2:3), (2:2:2:3:1) respectively. This sequence was maintained during further analysis of the extracts.

For further analysis the pure medicinal plant extracts and their finalized ratio combinations were doped with the infected blood samples. The blood samples used for this purpose are dengue infected blood sample and viral infected blood samples. The doped samples were subjected to spectroscopic analysis, GC-MS analysis, Microbial analysis after the determination of their physical parameters.