Phytochemical and biological Investigation of Leaf of *Musa sapientum* (Musaceae)

A dissertation submitted to the Department of Pharmacy, East West University in the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B. Pharm.)

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Department of Pharmacy

East West University

DEDICATED

TO

MY PARENTS

Declaration by the Research candidate

I, Md. Saidul Islam , hereby declare that the dissertation entitled **"Phytochemical and Biological Investigation of leaf of *Musa sapientum (Musaceae)*.."**, submitted by me to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the award of the degree of Bachelor of Pharmacy (B.PHRM) is a complete record of original research work carried out by me during the period 2011-2012 under the supervision and guidance of **Dr. Repon Kumer Saha,** Assistant professor, Department of Pharmacy, East West University and it has not formed the basis for the award of any other Degree/Diploma/Fellowship or other similar title to any candidate of any University.

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Signature of the candidate

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Certificate

This is to certify that the research work on "Phytochemical and biological Investigation of *Musa sapientum* leaf" submitted to the Department of Pharmacy, East West University in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by Md. Saidul Islam (ID: 2008-3-70-022) under or guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and facilities availed of this connection are duly acknowledged.

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Abstract

Musa sapientum (leaf) belonging to the Musacaeae family has been investigated for the evaluation of biological activities of the crude extract with special emphasis to the antimicrobial activity, antioxidant activity, haemaggulitation and antihaemolytic activity. *Musa sapientum* leaf was also investigated with thin layer chromatography (TLC), sensitivity test and Preliminary phytochemical investigations. The antimicrobial assays of *Musa sapientum* leaf determine the zone of inhibition by disc diffusion method and minimum inhibtory concentration (MIC). The anioxidant activity include DPPH test, total phenolic assay, Hydrogen peroxide (H_2O_2) radical scavenging assay, Reducing power activity test and total flavanoid assy . *Musa sapientum* leaf show good antimicrobial activity as the zone of inhibtion is alomst same as the standard. The antioxidant property of *Musa sapientum* leaf is moderate.

Key words: *M. Sapientum*, antimicrobial, antioxodant, MIC, haemaggulitation, zone of inhibition. Antihaemolytic, TLC.

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INTRODUCTION

1. Introduction

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim. Clinical trials directed towards understanding the pharmacokinetics, bioavailability, efficacy, safety and drug interactions of newly developed bioactive compounds and their formulations (extracts) require a careful evaluation. Clinical trials are carefully planned to safeguard the health of the participants as well as answer specific research questions by evaluating for both immediate and long-term side effects and their outcomes are measured before the drug is widely applied to patients. According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. The premier steps to utilize the

biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation. **[1]**

1.1. Medicinal Plant

Medicinal plants are plants which have a recognized medical use. They range from plants which are used in the production of mainstream pharmaceutical products to plants used in herbal medicine preparations. Herbal medicine is one of the oldest forms of medical treatment in human history, and could be considered one of the forerunners of the modern pharmaceutical trade. Medicinal plants can be found growing in numerous settings all over the world.

1.2. Importance and Scope of Medicinal Plant

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It is estimated that world market for plant derived drugs may account for about USD 2, 00,000. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China, India and Bangladesh, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India, Bangladesh than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health

care system of rural population depend on indigenous systems of medicine. Of the 2, 50,000 higher plant species on earth, more than 80,000 are medicinal. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. Even the Allopathic system of medicine has adopted a number of plant-derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants (e.g. diosgenin, solasodine). Not only, that plant-derived drug offers a stable market worldwide, but also plants continue to be an important source for new drugs. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. This fact is well documented in the inventory of medicinal plants, listing over 20,000 species. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future. [2]

1.3 Classification of Medicinal Plants

Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. They are classified according to the part used, habit, habitat, therapeutic value etc, besides the usual botanical classification.

A. Based on part used

i) Whole plant*: Boerhaavia diffusa, Phyllanthus neruri*

ii) Root*: Dasamula*

iii) Stem*:Tinospora cordifolia, Acorus calamus*

iv) Bark*: Saraca asoca*

v) Leaf: *Indigofera tinctoria, Lawsonia inermis, Aloe vera*

vi) Flower*:Biophytum sensityvum, Mimusops elenji*

vii) Fruit*: Solanum species*

viii) Seed*: Datura stramonium*

B. Based on habit

i) Grasses*: Cynodon dactylon*

ii) Sedges*: Cyperus rotundus*

iii) Herbs *: Vernonia cineria*

iv) Shrubs*: Solanum species*

v) Climbers*: Asparagus racemosus*

vi) Trees: *Azadirachta indica*

C. Based on habitat

i) Tropical*: Andrographis paniculata*

ii) Sub-tropical*: Mentha arvensis*

iii) Temperate*: Atropa belladona*

D. Based on therapeutic value

Antimalarial*: Cinchona officinalis, Artemisia annua*

Anticancer*: Catharanthus roseus, Taxus baccata*

Antiulcer*: Azadirachta indica, Glycyrrhiza glabra*

Antidiabetic*: Catharanthus roseus, Momordica charantia*

Anticholesterol*: Allium sativum*

Antiinflammatory*: Curcuma domestica, Desmodium gangeticum*

Antiviral*: Acacia catechu*

Antibacterial*: Plumbago indica*

Antifungal*: Allium sativum*

Antiprotozoal*: Ailanthus sp., Cephaelis ipecacuanha*

Antidiarrhoeal*: Psidium gujava, Curcuma domestica*

Hypotensive*: Coleus forskohlii, Alium sativum*

Tranquilizing*: Rauvolfia serpentina*

Anaesthetic*: Erythroxylum coca*

Spasmolytic*: Atropa belladona, Hyoscyamus niger*

Diuretic*: Phyllanthus niruri, Centella asiatica*

Astringent*: Piper betle, Abrus precatorius*

Anthelmentic*: Quisqualis indica, Punica granatum*

Cardiotonic*: Digitalis sp., Thevetia sp.*

Antiallergic*: Nandina domestica, Scutellaria baicalensis*

Hepatoprotective*: Silybum marianum, Andrographis paniculata*

1.3. Cultivation of Medicinal Plants

Most of medicinal plants, even today, are collected from wild. The continued commercial exploitation of these plants has resulted in receding the population of many species in their

natural habitat. Vacuum is likely to occur in the supply of raw plant materials that are used extensively by the pharmaceutical industry as well as the traditional practitioners. Consequently, cultivation of these plants is urgently needed to ensure their availability to the industry as well as to people associated with traditional system of medicine. If timely steps are not taken for their conservation, cultivation and mass propagation, they may be lost from the natural vegetation forever. In situ conservation of these resources alone cannot meet the ever increasing demand of pharmaceutical industry. It is, therefore, inevitable to develop cultural practices and propagate these plants in suitable agroclimatic regions. Commercial cultivation will put a check on the continued exploitation from wild sources and serve as an effective means to conserve the rare floristic wealth and genetic diversity. **[2]**

It is necessary to initiate systematic cultivation of medicinal plants in order to conserve biodiversity and protect endangered species. In the pharmaceutical industry, where the active medicinal principle cannot be synthesized economically, the product must be obtained from the cultivation of plants. Systematic conservation and large scale cultivation of the concerned medicinal plants are thus of great importance. Efforts are also required to suggest appropriate cropping patterns for the incorporation of these plants into the conventional agricultural and forestry cropping systems. Cultivation of this type of plants could only be promoted if there is a continuous demand for the raw materials. It is also necessary to develop genetically superior planting material for assured uniformity and desired quality and resort to organised cultivation to ensure the supply of raw material at growers end. Hence, small scale processing units too have to be established in order that the farmer is assured of the sale of raw material. Thus, cultivation and processing should go hand in hand in rural areas. **[2]**

In order to initiate systematic cultivation of medicinal and aromatic plants high yielding varieties have to be selected. In the case of wild plants, their demonstration would require careful development work. Sometimes high yielding varieties have also to be developed by

selective breeding or clonal micro propagation. The selected propagation materials have to be distributed to the farmer either through nurseries or seed banks. Systematic cultivation needs specific cultural practices and agronomical requirements. These are species specific and are dependent on soil, water and climatic conditions. Hence research and development work has to be done to formulate Good Agricultural Practices (GAP) which should include proper cultivation techniques, harvesting methods, safe use of fertilizers and pestisides and waste disposal. **[2]**

1.5. Processing and Utilization

Medicinal principles are present in different parts of the plant like root, stem, bark, heartwood, leaf, flower, fruit or plant exudates. These medicinal principles are separated by different processes; the most common being extraction. Extraction is the separation of the required constituents from plant materials using a solvent. In the case of medicinal plants, the extraction procedure falls into two categories.

a) Where it is sufficient to achieve within set limits equilibrium of concentration between drug components and the solution. E.g. Tinctures, decoction, teas, etc.

b) Where it is necessary to extract the drug to exhaustion, i.e., until all solvent extractable are removed by the solvent.

Both the methods are employed depending on the requirement although in industry the latter method is mostly used. In all industrial procedures, the raw material is pre-treated with solvent outside the extractor before changing the latter. This prevents sudden bulk volume changes (which are the main cause of channeling during extraction) and facilitates the breaking up of the cell walls to release the extractables. To facilitate the extraction, the solvent should diffuse inside the cell and the substance must be sufficiently soluble in the solvent. The ideal solvent for complete extraction is one that is most selective, has the best capacity for extraction and is compatible with the properties of the material to be extracted.

These parameters are predetermined experimentally. The cost and availability of the solvent are also taken into account. Alcohol, though widely used, because of its great extractive power it is often the least selective, in that it extracts all soluble constituents. Alcohol in various ratios is used to minimize selectivity. The ideal alcohol ratio for woody or bark material is 75%. For leafy material, it is often less than 50% thus avoiding extraction of the chlorophyll which makes purification difficult.

Some materials such as alkaloids being soluble in acids, their extraction is facilitated by adjusting the pH in the acidic range. A number of alkaloids can be extracted easily with hydrocarbons after they have been released from combination with organic acids by grinding with alkali. It is first ground with moist calcium oxide and extracted with chloroform. A large number of alkaloids can be extracted directly with aqueous acids, organic or inorganic acids, and the alkalized extracts counter extracted with hydrocarbons or other polar solvents. **[2]**

Experiment used for extraction with solvents usually comprise an extraction vessel with a heating jacket for steam heating or fitted with electrical devices, a condenser in reflux position, a solvent reservoir, a facility to convert to reboiler position or a separate reboiler and a short column for solvent recovery. Sometimes, sophisticated and costly equipment like the Carousel or the Inoxa extractor is employed.

Technology for the manufacture of standardized extracts and phytochemicals is available and there are many extracts already in the international market as drugs. A drug such as an extract of *Centella asiatica* can be manufactured as an extract containing a standard quantity of asiaticoside. Similarly for senna a standardised extract of which, containing a standard quantity of sennosides a and b could easily be produced with equipment that can be designed and constructed in most developing countries.

The promotion and development of processing of medicinal and aromatic plants have gained momentum recently in many developing countries. Green consumerism and resurgence of

interest for plant based products, liberalized and free market economy, increasing awareness about biodiversity conservation and sustainable use of natural resources coupled with poor socio-economic conditions of native populations are ground realities for planning and harnessing the low-cost and purpose oriented process technologies.

UNIDO has developed a Polyvalent Pilot Plant with a view to enabling developing countries to upgrade their technology for the processing of medicinal and aromatic plants. This plant incorporates all salient features of a low cost, efficient, small capacity factory which can carry out solvent extraction, solvent percolation, and concentration of miscella, solvent recovery, steam distillation and oil separation. The design and fabrication of the process equipment need not be over emphasized, as even if a good design is available for adaptation, it must be done to fit the given situation.

The polyvalent plant is characterized by simplicity of design, installation, operation, maintenance and repair. Some of its features are

1. Modular construction so as to permit increase in capacity and function by duplicating or adding modules.

2. Simultaneous processing for more than one product, such as extraction at one end, production of solid extract or oleoresins at the other.

3. Standardized or optimized process control and measuring units, pumps and other ancillaries can be easily replaced.

4. All plumbing and electrical wiring are simple and easily accessible.

5. Multipurpose uses. E.g. Solvent/aqueous extraction, continuous extraction, preparation of solid extract and oleoresins, essential oil distillation, fractionation of essential oils and production of absolutes and concretes or even processing of other phytoproducts. **[2]**

1.6. Formulation and Industrial Utilization

Medicinal plants are used as raw materials for extraction of active constituents in pure form (e.g. alkaloids like quinine and quinidine from cinchona bark, emetine from ipecacuanha root, glycosides from digitalis leaves, sennosides from senna leaves), as precursors for synthetic vitamins or steroids, and as preparations for herbal and indigenous medicines. Products such as ginseng, valerian and liquorice roots are part of the herbal and health food market, as well as the food flavours, fragrance and cosmetic industries. Certain plant products are industrially exploited like liquorice in confectionery and tobacco, papaine as meat tenderiser, quinine as soft drink tonic and cinchona as wine flavour. A large quantity of medicinal plant material is used in the preparation of herbal and medicinal teas, e.g. chamomile. These herbal and food uses are of great importance, also to the exporters from developing countries. Hundreds of medicinal plants are items of commerce, however relatively small countries are used in formulated herbal remedies.

Several formulations like herbal teas, extracts, decoctions, infusions, tinctures, etc are prepared from medicinal plants.

1. Herbal teas, Herbal remedies: herbal tea or infusion mixtures are mixture of ungrounded or suitably ground medicinal plants to which drug plant extracts, ethereal oils or medicinal substances can be added. Infusion mixtures should be as homogenous as possible.

2. Drug extracts: They are preparations obtained by extracting drugs of a certain particle size with suitable extraction agents (menstrua). The extract obtained after separation of the liquid from the drug residue is called miscella. It may already represent the final liquid dose form e.g. as a so called fluid extract, or be used as an intermediary product which is to be further processed as quickly as possible.

3. Aqueous drug extracts: The following degrees of comminution are used for the extract depending on the type of plant parts. Leaves, flowers and herbs shredded (4000mm); woods,

barks and roots shredded (2800mm); fruits and seeds (2000mm). Alkaloid containing drugs powdered (700mm).

3.1. Decoctions: The drug in the prescribed comminution is put in to water at a temperature above 90°C. The container is suspended in a water bath and maintained at this temperature for 30 minutes, with repeated stirring. The mixture is then strained while still hot.

3.2. Infusions: One part of the comminuted drug is kneaded several times in a mortar with 3-5 parts of water and left to stand for 15 minutes. The rest of the boiling water is then poured on to the mixture, which is suspended in a container in a water bath and kept for 5 minutes, with repeated stirring at a temperature above 90°C. The mixture is covered and left to stand until cool.

3.3. Macerates: The comminuted drug is left to stand, with occasional stirring, for 30 minutes after the required quantity of water has been poured on to it at room temperature. The extract is then strained and made up to the prescribed weight with rinsings.

3.4. Tinctures: Tinctures are extracts from drug plants prepared with ethanol of varying concentration, ether or mixtures of these, perhaps with certain additives, in such a way that one part of drug is extracted with more than two parts, but at most ten parts, of extraction liquid.

3.5. Fluid extracts**:** Like tinctures, they are liquid preparations, the difference being that they are more concentrated.

3.6. Dry extracts**:** They are usually very hygroscopic and should therefore be ground and mixed under conditions which exclude moisture as much as possible. Intermediate and end product must also be stored under dry conditions. There are also liquid, semisolid, solid and controlled release formulations or preparations. The other dose forms are injections, implants, ocular preparations, inhalations and transdermal systems. **[2]**

1.7. Selection, Collection, and Identification of Plant Material

1.7.1. Selection and Identification of Plant

As per WHO guidelines (WHO 2003), the plant selected for collection should be taxonomically same as recommended by the national pharmacopoeia or other related documents. If a new plant is being selected for collection then it should be properly identified and documented. The botanical identity, scientific name including genus, species, subspecies or variety and family of the plant should be recorded. If available, the local name should also be verified. Complete taxonomical identification is an important factor during selection as taxonomy of the plant species can play an important role in their biological activity.

In general, the search for the medicinal plants can follow three main routes: random, ethno (including ethnobotanical, ethnomedical and ethnopharmacological) and ecological search. Random search is extremely laborious and the success rate could be very low. Nevertheless, important drugs such as taxol, derivatives of camptothecin and homoharringtonine have been discovered by the National Cancer Institute (NCI) in collaborations with the United States Department of Agriculture (USDA) using this method.

The ethnobotanical, ethnomedical or ethnopharmacological approach uses information obtained from ethnobotanical survey such as the geographical distribution of the plant, its abundance, whether it is threatened or endangered, shrub/fast growing tree, easily cultivable, easily identifiable (with minimum varieties) etc. Information such as the season of collection, parts that are used and whether those parts are seasonal/replenishable and if there is any reported toxicity, are also required. The information can be obtained from traditional medical practitioners and other people such as village elders and local women who are traditional users of medicinal plants. **[3]**

1.7.2. Collection of Plant

Medicinal plant materials should be collected in the proper season so as to ensure the best possible quality of both the starting material as well as the finished product. Seasonal variations can affect the chemical composition of the plants and thus its biological activity. In most cases, maximum accumulation of chemical constituents occurs at the time of flowering which then declines at the beginning of the fruiting stage. The time of harvest should also depend on the plant part to be used since it is well known that depending on the plant species the level of biologically active constituents can vary in different parts at different stages of the plant growth and development. Younger leaves of tropical rainforest plants contained secondary metabolites that were either present in very little quantities or totally absent in matured leaves. The extracts from these younger leaves showed better biological activity when tested for anticancer activity or activity against *Bacillus subtilis* and *Artemia salina* (brine shrimp). It also applies to other components in the plant material such as the toxic components. Climatic conditions, e.g. light, rainfall, and temperature (including daytime and nighttime temperature differences) also influence the physical, chemical and biological qualities of medicinal plants. The water and temperature stress related increase in the content of active constituents. Hence the best time of collection should be determined according to the levels of the biologically active constituents rather than the vegetative yield. **[3]**

1.8. Drying and Grinding

After collection the plant material must first be preserved so that the active compound will remain unchanged. The most common method for preserving plant material is drying. Drying also decreases the risk of external attack by moulds. In general, plant materials should be dried at temperature below 30°C to avoid decomposition of thermolabile compound. When air-dried, the plant material has to be spread out with good ventilation to facilitate drying. **[4]**

Plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms, or in buildings; by direct sunlight, if appropriate; in drying ovens/rooms and solar dryers; by indirect fire; baking; lyophilization; microwave; or infrared devices. Where possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials. For example, shade drying is preferred to maintain or minimize loss of color of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances. The drying conditions should be recorded. In the case of natural drying in the open air, medicinal plant materials should be spread out in thin layers on drying frames and stirred or turned frequently. In order to secure adequate air circulation, the drying frames should be located at a sufficient height above the ground. Efforts should be made to achieve uniform drying of medicinal plant materials to avoid mold formation.

Drying medicinal plant material directly on bare ground should be avoided. If a concrete or cement surface is used, the plant materials should be laid on a tarpaulin or other appropriate cloth or sheeting. Insects, rodents, birds and other pests, and livestock and domestic animals should be kept away from drying sites. For indoor drying, the duration of drying, drying temperature, humidity and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and any volatile natural constituents, such as essential oils. If possible, the source of heat for directs drying (fire) should be limited to butane, propane or natural gas, and temperatures should be kept below 30 °C. If other sources of fire are used, contact between those materials, smoke, and the medicinal plant material should be avoided.

Grinding improves efficiency of extraction by increasing the surface area of plant material. This decreases the amount of solvent needed for extraction as it allows the plant material to pack more densely. Therefore, it is essential to grind samples into finer size for better extraction results. **[5]**

1.9. Extraction of plant material

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts. Such preparations popularly have been called galenicals, named after Galen, the second century Greek physician. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as menstruum. The extract thus obtained may be ready for use as a medicinal agent in the form of tinctures and fluid extracts, it may be further processed to be incorporated in any dosage form such as tablets or capsules, or it may be fractionated to isolate individual chemical entities such as hyoscine and vincristine, which are modem drugs. Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug. General methods of extraction of medicinal plants are-

1.9.1. Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the

marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

1.9.2. Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

1.9.3. Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstrum is thereby increased.

1.9.4. Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat-stable constituents. This process is typically used in preparation of Ayurvedic extracts called “quath” or “kawath”. The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further.

1.9.5. Aqueous Alcoholic Extraction by Fermentation

Some medicinal preparations of Ayurveda (like asava and arista) adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (kasaya), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the extraction of the active constituents contained in the plant material. The alcohol thus generated also serves as a preservative. If the fermentation is to be carried out in an earthen vessel, it should not be new: water should first be boiled in the vessel. In large-scale

manufacture, wooden vats, porcelain jars or metal vessels are used in place of earthen vessels. Some examples of such preparations are karpurasava, kanakasava, dasmularista. In Ayurveda, this method is not yet standardized but, with the extraordinarily high degree of advancement in fermentation technology, it should not be difficult to standardize this technique of extraction for the production of herbal drug extracts.

1.9.6. Ultrasound Extraction (Sonication)

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwoli a root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules.**[6]**

1.10. Fractionation of Crude Extract

Chromatographic procedures are the most diverse and the most widely used techniques in the fractionation of crude extracts. There is no doubt that they have made possible the isolation of many naturally occurring compounds. A crude extract is a mixture of thousands compounds. It is difficult to isolate a single compound from the crude extracts by a single separation technique. Hence the crude extract is initially separated into various discrete fractions containing compounds of similar polarities or molecular sizes. These fractions may be contiguous elute from a chromatography column, e.g. vacuum liquid chromatography (VLC), column chromatography (CC), size-exclusion chromatography (SPE), liquid chromatography (LC), etc.

For initial fractionation of any crude extract, it is advisable that do not generate too many fractions, because it may spread the target compounds over so many fractions. As a result,

amount of target compound will be very small that will render the detection. For finer fractionation, often newly discovered detection technique, e.g. ultraviolet (UV), modern preparative, or semi preparative high performance liquid chromatography (HPLC) can be used**.[7]**

1.11. Isolation

The most important factor that has to be considered before isolation is the nature of the target compounds present in the crude extracts or fractions. The general features of the molecule that are helpful to ascertain the isolation process include solubility (hydrophobicity or hydrophilicity), acid-base properties, charge, stability, and molecular size. If isolating a known compound from the same or a new source, it is easy to obtain literature information on the chromatographic behavior of the target compound, and one can choose the most appropriate method for isolation without any major difficulty. However it is more difficult to design an isolation protocol for a crude extract where the types of compound present are totally unknown. In this situation it is advisable to carry out qualitative tests for the presence of various types of compounds, e.g., phenolics, steroids, alkaloids, flavonoids, etc., as well as analytical thin-layer chromatography (TLC) or HPLC. The nature of the extract can also be helpful for choosing the right isolation protocol. For example, a MeOH extract or fractions containing polar compounds are better deals with using reversed-phase HPLC (RP-HPLC). Various physical properties of the extracts can also be determined with a small portion of the crude extract in a series of small batch-wise experiment. Some of these experiments are summarized below.

1. Hydrophobicity or hydrophilicity: An indication of the polarity of the extract as well as the compounds present in the extract can be determined by drying an aliquot of the mixture and trying to redissolve it in various solvents covering the range of polarities, e.g., water, MeOH, acetonitrile (ACN), EtOAc, DCM, $CHCl_3$, petroleum ether, n-hexane, etc. the same

information can be obtained by carrying out a range of solvent portioning, usually between water and EtOAc, $CHCl_3$, DCM, or n-hexane, followed by an assay to determine the distribution of compounds in solvent fractions.

2. Acid-base properties: Carrying out partitioning in aqueous solvents at a range of pH values, typically 3, 7, and 10, can help to determine the acid-base properties of the compounds in an extract. It is necessary to adjust the aqueous solution or suspension with a drop or two of mineral acid or alkali, followed by the addition of organic solvent and solvent extraction. Organic and aqueous phases are assessed, preferably by TLC, for the presence of compounds. This experiment can also provide information on the stability of compounds at various pH values.

3. Charge: Information on the charge properties of the compound can be obtained by testing under batch conditions, the effect of adding various ion exchangers to the mixture. This information is particularly useful for designing any isolation protocol involving ion exchange chromatography.

4. Heat stability: A typical heat stability test involves incubation of the sample at 90°C for 10 min in a water bath followed by an assay for unaffected compounds. It is particularly important for bioassay-guided isolation, where breakdown of active compounds often leads to the loss or reduction of biological activity. If the initial extraction of natural product is carried out at a high temperature, the test for heat stability becomes irrelevant.

5. Size: Dialysis tubing can be used to test whether there are any macromolecules, e.g., proteins, present in the extract. Macromolecules are retained within the tubing, allowing small (<2000 amu) secondary metabolites to pass through it. The necessity of the use of any SEC in the isolation protocol can be ascertained in this way.

6. Chromatographic techniques used in the isolation: The chromatographic techniques used in the isolation of various types of natural products can be broadly classified into two categories:

A. Classical or older chromatographic techniques include-

1. Thin-layer chromatography (TLC)
2. Preparative thin-layer chromatography (PTLC)
3. Open column chromatography (CC)
4. Flash chromatography (FC)

B. Modern chromatographic techniques are-

1. High-performance thin-layer chromatography (HPTLC)
2. Multiflash chromatography (e.g. Biotage)
3. Vacuum liquid chromatography (VLC)
4. Chromatotron
5. Solid-phase extraction
6. Droplet countercurrent chromatography (DCCC)
7. High performance liquid chromatography (HPLC)
8. Hyphenated techniques (e.g., HPLC-PDA, LC-MS, LC-NMR, LC-MS-NMR)

1.12. Structure Elucidation

In the early years, structure elucidation posed a big problem to many researchers as the major spectroscopic equipment, i.e. nuclear magnetic resonance (NMR) and mass spectrometer (MS) were not easily available. Many researches had to send samples to institutions of developed countries for these services. The availability of this expensive spectroscopic instrument in many laboratories towards the late nineties facilitated and hastened elucidation of structures. Low fields 1H and 13C NMR (proton noise decoupling, off-resonance technique, DEPT), MS, IR, UV spectra and elemental analysis data were in most cases

sufficient to obtain the structures of compounds. The acquisition of new techniques such as two dimensional high field NMR, liquid chromatography–mass spectrometry (LCMS), chemical ionization and fast atom bombardment mass spectrometry (CI and FAB-MS), Fourier Transform Infra Red spectrophotometry (FTIR) and X-ray crystallography in the early nineties, enabled natural products chemists to confidently characterized and identify structures of large and complicated molecules at submilligram quantities. **[9]**

1.13. Assay

An assay is an investigative (analytic) procedure in laboratory medicine, pharmacology, environmental biology, and molecular biology for qualitatively assessing or quantitatively measuring the presence or amount or the functional activity of a target entity (the analyte) which can be a drug or biochemical substance or a cell in an organism or organic sample. Chemical, biological, or physical assays are necessary to pinpoint the target compounds from a complex natural product extract.

1.13.1. Physical assay

Physical assays may involve the comparison of various chromatographic method and spectroscopic behavior, e.g., HPLC, TLC, LC-MS, LC-NMR, and so on, of the target compounds with a known standard.

1.13.2. Chemical assay

Chemical assay involves in the separation, identification, and quantification of the chemical components of natural and artificial materials. There are two branches in chemical assay: qualitative assay and quantitative assay. Qualitative assay is the determination of those elements and compounds that are present in a sample of unknown material. Quantitative assay is the determination of the amount by weight of each element or compound present.

1.13.3. Bioassay

Bioassays are typically conducted to measure the effects of a substance on a living organism and are essential in the development of new drugs. It involves a procedure by which the potency or the nature of a substance is estimated by studying its effects on living matter. Bioassays could involve the use of in vivo systems, ex vivo systems or in vitro systems.

In vitro refers to studies in experimental biology that are conducted using components of an organism that have been isolated from their usual biological context in order to permit a more detailed or more convenient analysis than can be done with whole organisms. In contrast, the term in vivo refers to work that is conducted with living organisms in their normal, intact state, while ex vivo refers to studies on functional organs that have been removed from the intact organism.

Plant Review

CHAPTER 2

Chapter

2

PLANT REVIEW

2.1 Overview of family *Musaceae*

*Musaceae***,** the banana family of plants, consisting of 2 genera, *Musa* and *Ensete*, with about 50 species native to Africa, Asia, and Australia. **[11]**

a) Musa - Type genus of the *Musaceae*: bananas

b) Ensete - Old World tropical herbs: Abyssinian bananas **[10]**

Musaceae is in the major group *Angiosperms* (Flowering plants). The common banana (*M. sapientum*) is a subspecies of the plantain (*M. paradisiaca*). Both are important food plants.

Figure 2.1: Plant and fruit

The slender or conical false trunk of *Musaceae* herbs may rise to 15 meters (50 feet). **[11]**

The "trunk" is formed by the leaf sheaths of the spirally arranged leaves, which form a crown at the top. The large leaves may be up to three meters long and half a meter wide. The prominent midrib of the leaf is joined at right or slightly oblique angles with parallel veins. When the plant grows in an unsheltered place, wind and rain easily tear the leaves between

the veins, giving the leaves a fringed or ragged appearance. The large, leathery bracts (leaf like structures) are red to purple. The yellow flowers have five fertile stamens and are rich in nectar.

Some species of wild bananas, such as *M. coccinea*, have ornamental scarlet flowers but inedible fruit. *M. textilis* from the Philippines furnishes Manila hemp, also called abaca fiber. The genus *Ensete* of Africa produces no edible bananas, but the flower stalk of one species, *E. ventricosa*, is edible after cooking. Species of *Ensete* are distinguished from those of *Musa* by their larger seeds. *See also* abaca; banana; plantain.

Figure 2.2: Bunch of Banana

2.1.1 Statistics

Species of *Musaceae* contained within The Plant List belong to 2 plant genera.

The Plant List includes 235 scientific plant names of species rank for the family *Musaceae*. Of these 74 are accepted species names.

The Plant List includes a further 182 scientific plant names of intraspecific rank for the family *Musaceae*. **[12]**

2.1.2. Common names of *Musaceae* family :

Table 2.1: Some plant with common names of *Musaceae* family [13]

Plant	**Common name**
Musa cheesmanii	Cheesman's Banana
Musa nana	Dwarf Banana
Musa bauensis	Bau Banana
Musa borneensis var. flavida	*Angkadan Banana*
Musa campestris var. campestris	Swamp Banana
Ensete glaucum	Snow Banana
Musella lasiocarpa	Chinese Yellow Banana, Golden Lotus Banana
Musa textilis	Abaca
Ensete superbum	Banana
Ensete perrieri	Madagascar Banana
Musa acuminata subsp. microcarpa	Monkey Fingers Banana
Musa sapientum	French plantain banana
Musa acuminata	Dwarf Brazilian Banana 'Dwarf Brazilian'
Ensete ventricosum	False Banana, Red Abyssinian Banana, Wild Banana 'Maurelii'
Musa siamensis	Banana 'Thai Gold'

2.1.3. Size

Banana (*Musaceae*) is a large, perennial, monocotyledonous herb 2–9 m (6.6–30 ft) in height that arises from large, subterranean rhizomes (usually called corms).**[14]**

2.1.4. Flowers

Upon flowering, the true stem or growing point emerges from the center of the tightly rolled bunch of leaves. This odd-looking "flower cluster" is actually an elongated, plump, purple to green "bud" (sometimes called the "bell" or "heart"), which at first displays large female flowers (whose ovaries ripen into fruit). As the "bud" elongates, it exposes semicircular layers of female flowers, then neutral flowers, and finally small, generally non-functional (with no viable pollen) male flowers. Each group of flowers is arranged radially on the stem in nodal clusters. Each flower cluster is borne on a prominence on the stem bearing the fruit (peduncle) and covered by a bract.About 12–20 flowers are produced per cluster. Collectively, the flowering parts and fruit are referred to as the bunch.Individual clusters of fruits are known as hands, and individual fruits are known as fingers. **[14]**

2.1.5. Leaves

The entire above-ground portion of the plant is not a true woody trunk, as in other trees, but a "false trunk" or "false stem" that consists of leaves and their fused petiole bases, referred to as a pseudostem. The pseudostem supports a canopy consisting of 6–20 (or more) leaves. **[14]**

2.1.6. Fruit

Musa fruits are variable in size, shape, and color. They are generally elongate-cylindrical, straight to strongly curved, 3–40 cm (1.2–16 in) long, and 2–8 cm (0.8–3 in) in diameter.The fruit apex is important in variety identification; it may be tapered, rounded, or blunt. The skin is thin and tender to thick and leathery, and silver, yellow, green, or red in color. Inside the ripe fruit, the flesh ranges from starchy to sweet, and in color from white, cream, yellow, or yellow-orange to orange. Bananas also vary in

peel thickness. Some variet-ies have a thin peel and are more susceptible to damage in transport, where as others have a comparably thicker peel. **[14]**

2.1.7. Seeds

Cultivated varieties are typically seedless. When seeds are present, they vary among species in shape and morphology. Seeds of *Musa balbisiana*, parent of many commercial edible banana varieties, are dark brown, ovoid, about 4mm (0.2 in) long, with a conspicuous white, powdery endosperm. **[14]**

2.2. Common name of *Musa sapientum* [14]

ASSAMESE :	Kala
BENGALI :	Kala
BURMESE :	Taw nget byaw .
CHINESE :	Da jiao, Fen ba jiao.
ENGLISH :	Dessert banana, Sweet-fruited banana.
FRENCH :	Banane cultivée, Bananier des sages, Bananier commun.
GERMAN :	Adamsfeige, Dessertbanane, Jamaicabanane, Obstbanane.
ITALIAN:	Banana comune, Banano comune, Fico d'Adamo.
JAPANESE :	Banana.
LAOTIAN :	Kwàyz khauz.
MALAY :	Biu (Bali), Cau (Sunda), Gedang (Java), Puti (Lampung), Kulo (Ambon), Pisang.
SPANISH :	Banana, Bananeira, Guineo, Plátano (Mexico).
TAGALOG:	Saging.
TAMIL :	Vaazhai, Vaazhaipoo (flower).
TELUGU :	Arati, Artipandu, Kadala.
THAI :	Kluai.

PREPARATION OF PLANT EXTRACT FOR **EXPERIMENT**

CHAPTER

3

Chapter 3

PREPARATION OF PLANT EXTRACT FOR EXPERIMENT

3.1 Plant Selection

There are thousands of medicinal plants in Bangladesh and in this Indian subcontinent. Among these plants it was not easy to select a few plants for the research purpose. The selection of plant greatly affects the research work if there is carelessness takes place. Plant secondary metabolites often accumulate in specific plant parts. Thus, unless it is already known which parts contain the highest level of the compounds of interest, it is important to collect multiple plant parts, or the whole plant to ensure the extracts prepared representative of the range of secondary metabolites. For drug discovery from plants, sample may be selected using a number following criteria by which the research work will run smoothly.

From the literature review it is seen that there are lots of work on the plant *Musa sapientum* Linn about the pharmacological activity of the plant. But there is a least of work has been found about chemical investigation of this plant, especially about the leaves of this plant. So I got a chance to select the leaves of *Musa sapientum* Linn for my research work to see whether the leaves have antipyretic and analgesic activity or not.

3.2 Plant Collection

After selection of plant it is must to collect the plant parts for the research purpose. Throughout Bangladesh the plant *Musa sapientum* Linn is available. The plant leaves were collected from the rural area near Dhaka, Bangladesh.

3.3 Plant Identification

It is advisable to attempt field identification of sample collected. To aid taxonomic experts in confirming the field identification and to get a permanent scientific record (accession number) a voucher specimen was prepared on 20th December, 2011. In the voucher specimen the dried leaves of sample plant were attached and some information like local name,

medicinal use, location of the sample plant were also written on that voucher specimen. Finally, from BNH (Bangladesh National Herbarium) I got the identification or accession number of collected sample on 12th May, 2012, and the accession number are 36560 with *Musa sapientum* Linn and *Musaceae* scientific name and family name of the plant respectively.

3.4 Drying of Plant Sample

After the collection of sample it needs to be dried to make the sample extract. In general the plant material should be dried at temperature below 30^0C to avoid the decomposition of thermolabile compounds. So sun drying can be very effective but drawback is sometimes water molecules are absorbed by the sample and hence microbial growth can affect the phytochemical study. The leaves were dried in the sun light thus chemical decomposition cannot take place.

3.5 Grinding of Dried Sample

Small amount of plant material can be milled using grinder or blender. But if the sample is in high amount then it is easier to get powdered sample by grinding from a spice mill. Grinding improves the efficiency of extraction by increasing surface area. It also decreases the amount of solvent required for the extraction. The dried samples were ground to coarse powder with a mechanical grinder (Blender) and powdered samples were kept in clean closed glass containers pending extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign matter deposited on the grinder.

3.6 Maceration of Dried Powdered Sample

3.6.1 Principle

The choice of extraction procedure depends on the nature of the plant material and the components to be isolated. The principle of solid-liquid extraction is that when a solid material comes in contact with a solvent, the soluble components in the solid material move

to the solvent. Thus, solvent extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent, and this takes place in a concentration gradient. The rate of mass transfer decreases as the concentration of active principle in the solvent increases, until equilibrium is reached, i.e. the concentrations of active principle in the solid material and the solvent are the same. Thereafter, there will no longer be a mass transfer of the active principle from plant material to the solvent. Since mass transfer of the active principle also depends on its solubility in the solvent, heating the solvent can enhances the mass transfer. Moreover, if the solvent in equilibrium with the plant material is replaced with fresh solvent, the concentration gradient is changed.

3.6.2 Procedure

After getting the sample as dried powdered, the sample (500 Gram) was then soaked in 1000mL of Ethanol for five days, the process is known as maceration technique. A glass made jar with plastic cover was taken and washed thoroughly with ethanol and dried. Then the dried powder sample was taken in the jar. After that Ethanol (10000mL) was poured into the jar up to 1-inch height above the sample surface as it can sufficiently cover the sample surface. The plastic cover with aluminum foil was closed properly to resist the entrance of air into the jar. This process was performed for five days. The jar was shacked in several times during the process to get better extraction.

3.7 Filtration of the extract

After the extraction process the plant extracts was filtered with sterilized cotton filter. The cotton was rinsed with ethanol and fitted in a funnel. The filtrate was collected in a beaker. Then again it was filtered and this time What man's filter was used for getting more clear extract which would be useful making the sample more concentrate in Rotary Evaporation Technique. Then the filtrate was taken into a volumetric flask and covered with alumina foil paper and was prepared for rotary evaporation.

3.8 Concentrated sample by rotary evaporation

3.8.1 Principle

A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts. A rotary evaporator consists of following parts-

- A motor unit that rotates the evaporation flask or vial containing the user's sample.
- A vapor duct that is the axis for sample rotation, and is a vacuum-tight conduit for the vapor being drawn off of the sample.
- A vacuum system, to substantially reduce the pressure within the evaporator system.
- A heated fluid bath (generally water) to heat the sample.
- A condenser with either a coil passing coolant, or a "cold finger" into which coolant mixtures such as dry ice and acetone are placed.
- A condensate-collecting flask at the bottom of the condenser, to catch the distilling solvent after it re-condenses.
- A mechanical or motorized mechanism to quickly lift the evaporation flask from the heating bath.

3.8.2 Affecting Factors

There are following factors, omission of one of the following factor may interfere the sample concentration procedure and thus which may interfere the phytochemical investigation.

-Remove the flask from the heat bath.

-Opening the stopcock

-Halting the rotor

-Turning off the vacuum/aspirator

-Disconnecting the flask

-Dropping flask in heat bath.

3.8.3 Procedure

After the filtration process two parts were obtained namely ‘residue part’ and ‘filtered part or filtrate’. The filtered part, which contains the substance soluble in ethanol, was putted into a1000ml round bottom flask (BOROSIL), and then the flask was placed in a rotary evaporator. The evaporation was done at 45 degree Celsius temperature. The number of rotation per minute was selected as 130 RPM. The pressure of the vacuum pumper machine (Biometra) was 6 bar. The water flow through the distillation chamber was also provided in a satisfactory flow rate. When the evaporation seemed to be satisfactory, then the ethanolic extract was collected in a 50mL beaker. The evaporator flask was rinsed by diethyl ether. Then the beaker was covered with aluminum foil paper and kept on the water bath for 60 minutes and the temperature of water bath maintained as 50^0C. Finally the concentrated ethanolic plant extract was found and stored in the laboratory refrigerator from which the extract was used for many chemical investigations.

PHYTOCHEMICAL AND PHARMACOLOGICAL REVIEW (LITERATURE REVIEW) OF *MUSA SAPIENTUM*

CHAPTER 4

Chapter 4

PHYTOCHEMICAL AND PHARMACOLOGICAL REVIEW (LITERATURE REVIEW) OF MUSA SAPIENTUM

4.1 Phytochemical Review

Carbohydrates have been isolated from M. Sapientum. **[21]** Catecholamines such as norepinephrine, serotonin, dopamine **[92] & [89]**, tryptophan, indole compounds **[81]**, pectin have been found in the pulp. Several flavonoids andrelated compounds (Leucocyanidin, quercetin and its 3-O-galactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside) wereisolated from the unripe pulp of plantain **[52] & [53].** Serotonin, nor-epinephrine,tryptophan, indole compounds, tannin, starch, iron, crystallisableand non crystallisable sugars, vitamin C, B-vitamins, albuminoids, fats, mineral salts have been found in the fruit pulp of *M. Paradisiaca* and *M. Sapientum* **[38]**.

Acyl steryl glycosides such as sitoindoside-I,sitoindoside-II, sitoindoside-III, sitoindoside-IV and sterylglycosides such as sitosterol gentiobioside, sitosterol myo -inosityl- β -D-glucoside have been isolated from fruits of M. Paradisiaca a bicyclic diarylheptanoid, rel - (3S, 4aR, 10bR) – 8 - hydroxy-3-(4-hydroxy phenyl) – 9 – methoxy - 4a,5,6,10b – tetra hydro - 3H - naphtho [2,1-b] pyran, and 1,2-dihydro-1,2,3-tri hydroxy -9 - (4 – methoxy phenyl) phenalene, hydroxyl anigorufone, 2 - (4 – hydroxyl phenyl) naphthalic anhydride, 1, 7 – bis (4-hydroxy phenyl) hepta – 4 (E), 6 (E) – dien -3 - one. **[39]**

The isolation of severaltriterpenes such as cyclomusalenol, cyclomusalenone, 24 methylene cycloartanol, stigmast-7-methylene cycloartanol, stigmast-7 en-3-ol, lanosterol and β –amyrin was reported**[76].** An antihypertensive principle, 7,8-dihydroxy-3-methylisochroman-4-one was isolated from the fruit peel of M. Sapientum **[93].** Cycloartanetriterpenes such as 3-epicycloeucalenol, 3-epicyclomusalenol, 24 methylen epollinastanone, 28-norcyclo

musalenone, 24-oxo-29-norcycloartanone have been isolated from the fruit peel of M.sapientum **[17]**. Cellulose, hemicelluloses, arginine, aspartic acid,glutamic acid, leucine, valine, phenylalanine and threonine havebeen isolated from pulp and peel of M. Paradisiaca **[50].** Hemiterpenoid glucoside (1,1-dimethylallyl alcohol), syringin, (**6S**, 9R)-roseoside, benzyl alcohol glucoside,(24R)-4α,l4 α,24-trimethyl-Sacholesta-8,25(27)-dien-3β o1 havebeen isolated from flower of M. Paradisiaca [30].Structures of some important isolatedchemicals-

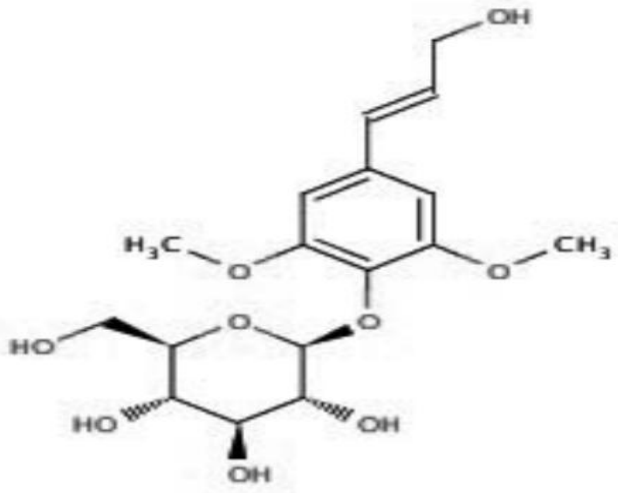


Figure 4.1: Syringin

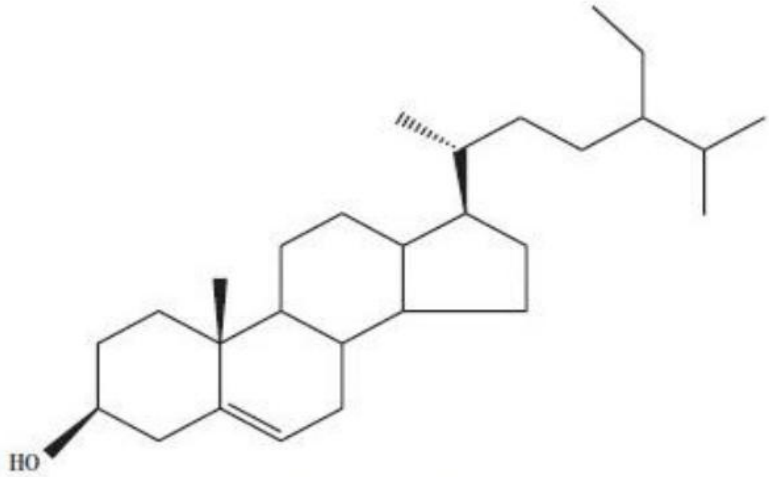


Figure 4.2: β-Sitosterol

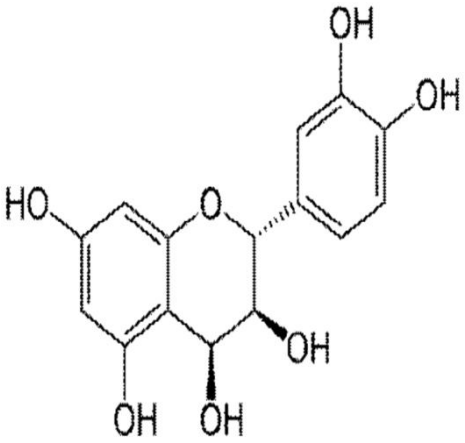


Figure 4.3 : Leucocyanidin

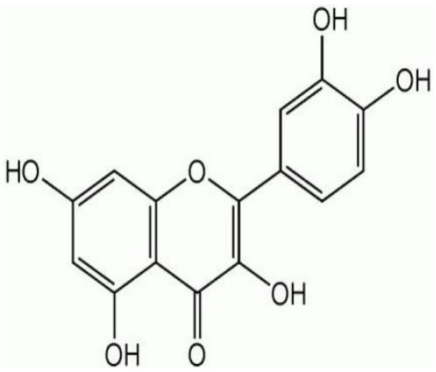


Figure 4.4: Quercetin

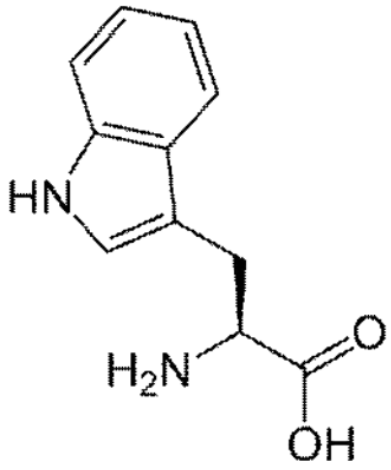


Figure 4.5: L-tryptophan

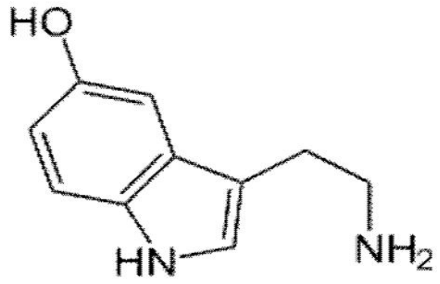


Figure 4.6: 5-Hydroxytryptamine

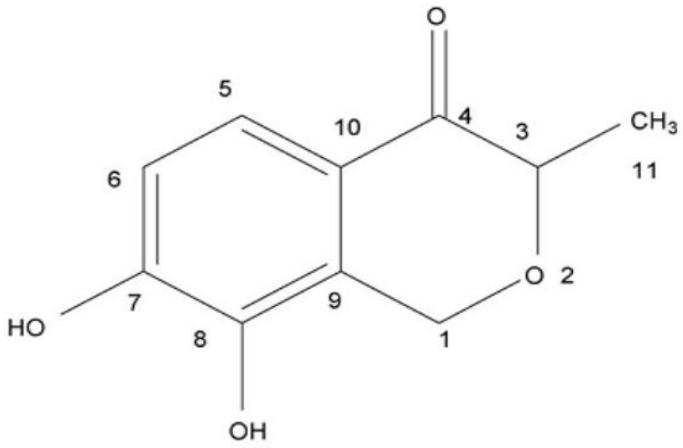


Figure 4.7: 7, 8-dihydroxy-3-methyl isochroman-4-one

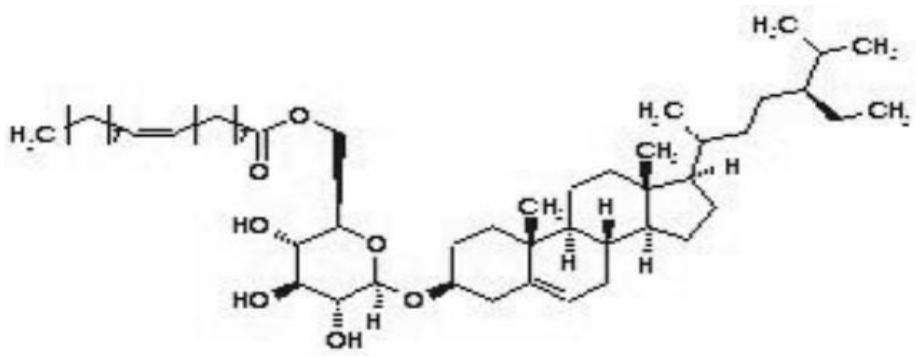


Figure 4.8: Sitoindoside-II

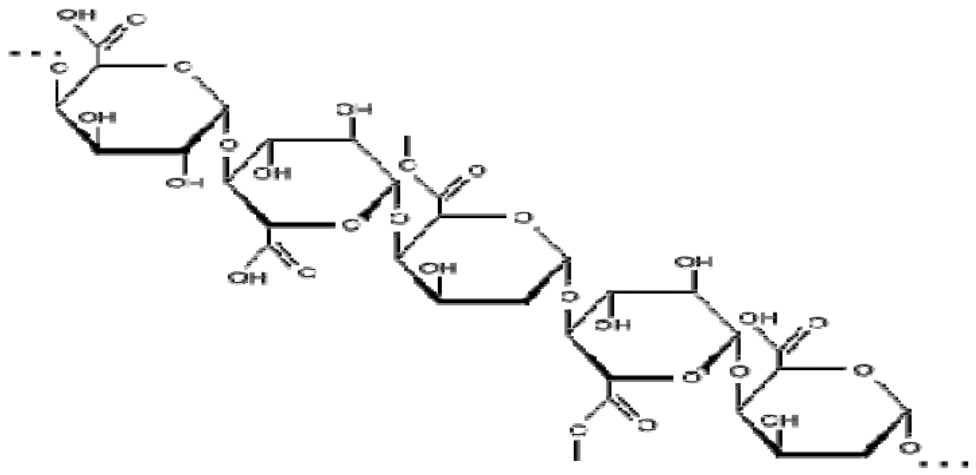


Figure 4.9: Pectin

Structures of some phytochemicals isolated from Musa paradisiaca and Musa sapientum.

4.1.1 Chemical composition of of *Musa sapientum*

Musa sapientum peels were analyzed for minerals, nutritional and anti – nutritional contents. The result of mineral content indicate the concentrations (mg/g) of potassium, Calcium, sodium, iron, manganese, bromine, rubidium, strontium, zirconium and niobium to be 78.10, 19.20, 24.30, 0.61, 76.20, 0.04, 0.21, 0.03, 0.02 and 0.02 respectively. The percentage concentrations of protein, crude lipid, carbohydrate and crude fiber were 0.90, 1.70, 59.00 and 31.70 respectively **[21].** The results indicate that if the peels are properly exploited and process, they could be a high-quality and cheap source of carbohydrates and minerals for livestock.

Table 4.1 Minerals composition of *Musa sapientum* peel

Element	**Concentration (mg/g)**
Potassium	78.10 + 6.58
Calcium	19.20 + 0.00
Sodium	24.30 + 0.12
Iron	0.61 + 0.22

Manganese	76.20 + 0.00
Bromine	0.04 + 0.00
Rubidium	0.21 + 0.05
Strontium	0.03 + 0.01
Zirconium	0.02 + 0.00
Niobium	0.02 + 0.00

Values are Mean ± SE

Table 4.2 Proximate composition and anti - nutritional content of *Musa sapientum* peel

Parameter	**Concentration**
Moisture (%)	6.70 + 02.22
Ash (%)	8.50 +1.52
Organic matter (%)	91.50 + 0.05
Protein (%)	0.90 + 0.25
Crude Lipid (%)	1.70 + 0.10
Carbohydrate (%)	59.00 + 1.36
Crude Fiber (%)	31.70 + 0.25
Hydrogen cyanide (mg/g)	1.33 + 0.10
Oxalate (mg/g)	0.51 + 0.14
Phytate (mg/g)	0.28 + 0.06
Saponins (mg/g)	24.00 + 0.27

Values are Mean ± SE

Plantain sheath (*Musa sapientum*) contained 6.4% dry matter and 3.4% crude protein, 31.4% crude fiber **[85]**, 34.6% cellulose, 15.5% hemicellulose and 6% lignin in dry matter. Five novel cycloartane-type triterpenes were isolated from the nonsaponifiable lipids obtained

from the methanol extract of the fruit peel of Musa sapientum L. **[17]**. Their structures were determined to be 3-epicycloeucalenol, 3-epicyclomusalenol, 24-methylenepollinastanone, 28-norcyclomusalenone and 24-oxo-29-norcycloartanone by spectroscopic and chemical methods.

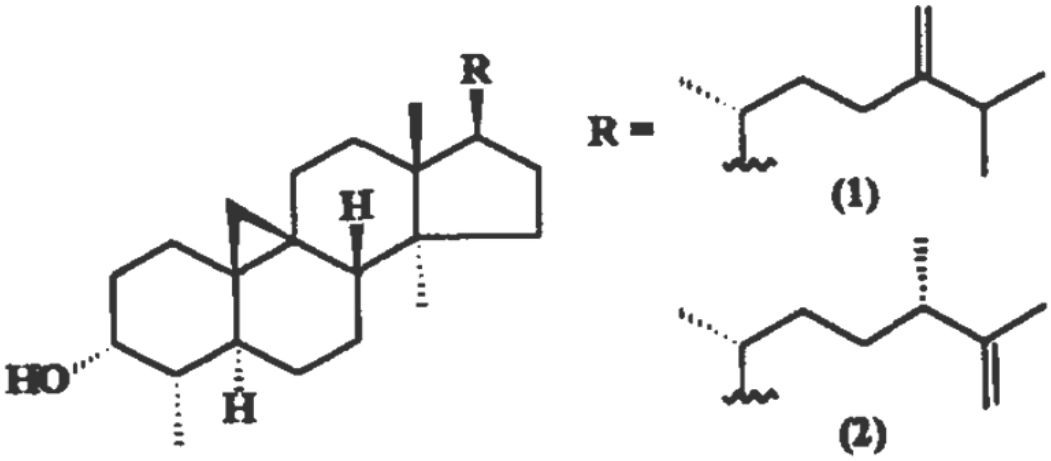


Figure 4.10: (1)3-epicycloeucalenol and (2) 3-epicyclomusalenol

A new isochroman-4-one, 7, 8-dihydroxy-3-methylisochroman-4-one was isolated from water soluble fraction of *Musa sapientum* **[72]**. Its structure was determined by spectroscopic evidences and its total synthesis has also been reported. The compound showed potent antihypertensive activity.

Alkali treatment coupled with high pressure defibrillation and acid treatment have been tried on banana fibers obtained from the pseudo stem of the banana plant *Musa sapientum* **[26]**. The structure and morphology of the fibers have been found to be affected on the basis of the concentration of the alkali and acid and also on the pressure applied. Steam explosion in alkaline medium followed by acidic medium is found to be effective in the depolymerization and defibrillation of the fiber to produce banana nanowhiskers. The chemical constituents of raw and steam exploded fibers were analyzed according to the ASTM standards. Structural analysis of steam exploded fibers was carried out by FTIR and XRD. The fiber diameter and percentage crystallinity of the modified fibers were investigated using X-ray diffraction

studies. Characterization of the fibers by SFM and TEM supports the evidence for the development of nanofibrils of banana fibers.

An enzyme system isolated from the pulp of banana fruit (*Musa sapientum*) was capable of catalyzing the hydroxylation of the monophenol, tyramine, to the diphenol, dopamine (3, 4-dihydroxyphenylethylamine) **[29]**. Unlike some tyrosinases, the reaction was not stimulated by catalytic amounts of diphenolic reaction product. Ascorbic acid, however, reduced the initial lag period in the oxidation of tyramine, stimulated the reaction rate and promoted the accumulation of dopamine during the first few minutes of the reaction. The hydroxylation of tyramine was apparently dependent upon molecular oxygen. On the basis of these observations it is tentatively suggested that the enzyme is a tyramine hydroxylase which may be responsible for the formation of dopamine in the banana.

4.2 Pharmacological Review

4.2.1 Antidiarrhoeal activity

The antidiarrhoeal activity of banana in rats was observed as early as in 1930s. This effect in the intestinal diseases was attributed to the pectin content of banana. Later banana diet was reported to be effective and advantageous in bacillary dysentery in a proctoscopic study on 127 patients of age nine month to forty eight years **[24]**. Banana flakes has also been tested and found effective in the treatment for diarrhoea in critically ill patients receiving enteral feedings[34]. The antidiarrhoeal activity of green banana diet was found very effective in children with diahorea **[74]**.

4.2.2 Antiulcerative activity

Banana is used in herbal medicin to treat peptic ulcer disease. The use of M. Sapientum in peptic ulcer as a component of herbal medicine has been evaluated and found effective [41]. It was reported that pectin and phosphatidylcholine in green banana strengthens the mucous-phospholipid layer that protects the gastric mucosa [32]. They also reported that the gastric mucosa

protective activity of the banana is due to multiple active components. It was reported that a natural flavonoid from the unripe banana (M. sapientumvar. paradisiaca) pulp, leucocyanidin, protects the gastric mucosa fromerosions**[53]**. Leucocyanidin and the synthetic analogues, hydroxyethylated leucocyanidin and tetraallyl leucocyanidin werefound to protect the gastric mucosa in aspirin-induced erosions inrat by increasing gastric mucus thickness **[53]**. Banana pulp powder (*M. Sapientum* vs. *Paradisiaca*) showed significant antiulcerogenic activity inaspirin, indomethacin-, phenylbutazone-, prednisolone-inducedgastric ulcers and cysteamine- and histamine-induced duodenalulcers in rats and guinea-pigs, respectively **[41]**. The authors attributedthe effect to increased mucosal thickness and increased [3H] thymidine incorporation into mucosal DNA that results in mucosal cellular proliferation and healing. Mukhopadhyaya in (1987) also found that the same effects like Goel (1986) in rat after orally administering banana pulp powder as aqueous suspension at 0.5g/kg twice daily dose for 3 days. They also reported a significant decrease in gastric juice DNA content after the treatment. It was reported that the antiulcerative effect of banana may vary depending on different varieties of banana **[65]**. The article showed that the ethanolic extract of both M. Sapientum and M. Paradisiaca have significant gastroprotective effect but only M. Paradisiaca promotes ulcer healing by a similar mechanism like prostaglandins **[65]**. It was reported that acid neutralizing capacity of M. Sapientum fruit peel ash in rats **[47]**.

A group of scientists showed that Musa sapientum contain a monomrtic flavanoid compound leuococyanidin which has anti-ulcerogenic activity by High - Performance Thin Layer Chromatography **[71]**. They used aqueous extract of Musa sapientum as the sample and esomeprazole as the standard drug.

It was studied that methanolic extract of plantain banana pulp (BE) was evaluated for its (i) antiulcer and antioxidant activities in 2 hr cold restraint stress and (ii) anti-H.pylori activity in vitro [42]. The extract (BE, 50 mg/kg, twice daily for 5 days) showed significant antiulcer

effect and antioxidant activity in gastric mucosal homogenates, where it reversed the increase in ulcer index, lipid peroxidation and super oxide dismutase values induced by stress. However it did not produce any change in catalase values, which was significantly decreased by stress. Further, in the in vitro study. BE (0.32-1,000 µg/ml) did not show any anti-H.pylori activity. The results suggest absence of anti-H. pyloric activity of methanolic extract of banana in vitro and its antioxidant activity may be involved in its ulcer protective activity.

4.2.3 Analgesic activity

The pill extract of Musa sapientum was found to have analgesic property.The investigation was undertaken to study analgesic activity of aqueous (AMS) and ethanolic (EMS) extract of stem of Musa sapientum Linn. using hot plate method and tail immersion method. AMS and EMS (100mg/kg and 200mg/kg, i.p) significantly increased reaction time as compared to vehicle treated group. Maximum analgesic effect was observed at 30 min. interval for 100 mg/kg and 200 mg/kg, i.p. (P = 0.01). The study indicates that AMS & EMS have central analgesic action. **[86]**

4.2.4 Antimicrobial activity

Aqueous extract of unripe fruit peels and leaves of *M. Paradisiaca* vs. *Sapientum* has been reported to show antimicrobial activity against *Staphylococcus* and *Pseudomonas* species in dehydrogenase assay. The IC50 of the aqueous fruit peel extract were 143.5 and 183.1 µg/ml against *Staphylococcus* and *Pseudomonas* species respectively and in case of leaf extract were 401.2 and 594.6µg/ml respectively **[19]**. In this assay the fruit peel extract showed better activity against both the bacteria than leaf extract while the peel extract was more active against *Staphylococcus* (Gram-positive) than *Pseudomonas* species(Gram-negative). However, the alcoholic extract of stem of M. Paradisiaca showed no activity against Staphylococcus aureus,Salmonella paratyphi, Shigella dysenteriae, Escherichia coli, Bacillus subtilis, Candida albicans **[16]**. It has been reported that both ethanolic and aqueous extract of unripe M.

Sapientum fruit showed good activity against *S. Aureus* ATCC 25921, *S. aureus, Salmonella paratyphi, Shigella flexnerii, E. Coli* ATCC 25922, *E. coli, Klebsiella pneumoniae, B. Subtilis* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration(MBC) of unripe banana ranged 2-512 mg/ml and 32-512 mg/mlrespectively considering both the solvents **[37]**.Though both ethanolic and water extracts showed significant activity against the organisms, the activity of ethanolic extract was stronger indicating that ethanol can dissolve the active phytochemicals than water. The aqueous extract of banana pureehas also reported to have bacteriostatic activity against *B. cereus, B. coagulans, B. stearothermophilus,* and *Clostridium sporogenes* **[78]**.

4.2.5 Hypoglycemic activity

The green fruit of *M. Paradisiaca* has been reported to have hypoglycemic effect due to stimulation of insulin production and glucose utilization **[60]**. Its high potassium (K) and sodium (Na) content has been correlated to the glycemic effect **[77]**. Fibers from *M. Paradisiaca* fruit increased glycogenesis in the liver and lowered fasting blood glucose **[88]**. Antihyperglycemic effect of the hydromethanolic extract of *M. Paradisiaca* root has been found significant **[55]**. *Musa sapientum* showed antihyperglycemic effect in hyperglycemic rabbit **[18]**. The chloroform extract of flowers of *M. Sapientum* showed blood glucose and glycosylated haemoglobin reduction and total hemoglobin increase after oral administration in rats. It also controls lipid peroxidation in diabetes **[67]**. However, *M. Paradisiaca* stem juice showed hyperglycemic activity **[82]**. Isolated pectin from the juice of the inflourescence stalk of *M. Sapientum* increases the glycogensynthesis, decreases glycogenolysis and gluconeogenesis**[44]**.

Oral administration of 0.15, 0.20 and 0.25 g/kg body weight of the chloroform extract of the flowers for 30 days resulted in a significant reduction in blood glucose and glycosylated haemoglobin and an increase in total haemoglobin **[67]**. The extract prevented a decrease in body weight, and also resulted in a decrease in free radical formation in the tissues. Thus the

study shows that banana flower extract has an anti hyperglycemic action. The decrease in thiobarbituric acid reactive substances and the increase in reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase clearly show the antioxidant property of banana flower extract. The effect of banana flower extract was more prominently seen in the case of animals given 0.25 g/kg body weight [67]. Banana flower extract was more effective than glibenclamide.

It was found that the antihyperglycemic effect of ethanolic extract of flowers of *Musa sapientum* in alloxan induced diabetic rats **[31]**. Oral administration of the ethanolic extract showed significant ($p < 0.001$) blood glucose lowering effect at 200 mg/kg in alloxan induced diabetic rats (120 mg/kg, i.p.) and the extract was also found to significantly ($p < 0.001$) scavenge oxygen free radicals, viz., superoxide dismutase (SOD), catalase (CAT) and also protein, malondialdehyde and ascorbic acid in vivo. *Musa sapientum* induced blood sugar reduction may be due to possible inhibition of free radicals and subsequent inhibition of tissue damage induced by alloxan **[31]**. The antidiabetic activity observed in this plant may be attributed to the presence of flavonoids, alkaloids, steroid and glycoside principles.

4.2.6 Hypocholesterolaemic activity

Hemicellulose and other neutral detergent fibers (NDF) from the unripe *M. Paradisiaca* fruit showed low absorption of glucose and cholesterol and low serum and tissue levels of cholesterol and triglycerides **[88]**. Flavonoids isolated from unripe fruits showed hypolipidemic activity evidenced by decrease in cholesterol, triglycerides (TG), free fattyacids and phospholipids levels in serum, liver, kidney and brain of rats. The cholesterol lowering effect was attributed to a higherdegradation rate of cholesterol than synthesis **[91**). Methanolic root extract of *M. Paradisiaca* showed total cholesterol (TC), triglyceride (TG), LDLc and VLDLc lowering effect in diabetic rats **[55]**.The pectin content to the juice of the inflorescence stalk of M. Sapientum has also been reported to possess cholesterol and triglyceride lowering activity in rats **[43]** .

A Study Showed that soluble and insoluble components of dietary fibre participate in the hypocholesterolaemic effect of banana pulp **[45]**. The pulp of banana fruit (*Musa sapientum L.* vs. *Cavendishii*) was examined for its cholesterol-lowering effect with male rats fed on a diet containing lard (50 g/kg) and cholesterol (5 g/kg). Freeze-dried banana pulp showed a marked cholesterol-lowering effect when incorporated into a diet at the level of 300 or 500 g/kg, while the banana pulp dried in a hot-air current (65 degrees) did not **[45]**. Starch and tannin prepared from banana pulp were not responsible for the cholesterol-lowering effect. The results also suggest that banana lipids did not affect the concentration of serum cholesterol. Feeding of dopamine, n-epinephrine and serotonin tended to raise the concentration of serum cholesterol. Thus, all the substances tested which were thought to be susceptible to influence by hot-air drying were unlikely to be responsible for the hypocholesterolaemic effect. However, both soluble and insoluble fibers fractionated from banana pulp had a cholesterol-lowering effect, with the exception of cellulose **[45]**. It was assumed that a browning reaction undergone during hot-air drying might be related to the disappearance of the hypocholesterolaemic effect of banana pulp dried in a hot-air current.

4.2.7Antihypertensive activity

The antihypertensive effect of *M. Paradisiaca* in albinorats was reported **[63]**. Later same group reported that banana diet has a mean arterial blood pressure lowering as well as onset preventing effect in rats with elevated blood pressure induced by desoxycorticosterone acetate (DOCA)administration[64]. It was reported that the antihypertensive effect of ripe banana pulp in deoxycorticosteroneenantate-induced hypertensive rats which may be due to the high tryptophan and carbohydrate content of banana that increase esserotonin levels and gives serotonin-mediated natriorexic effect **[70].** However, another group reported that serotonin produced acontraction in place of relaxation in isolated rat aortic rings [62]. The aqueous extract of the ripe *M. Paradisiaca* fruit was found to give aconcentration dependent hypotensive effect in both

noradrenaline-and potassium chloride-contracted aortic rings isolated from rat. The effect was due to the non-specific interference in calcium ion availability needed for the smooth muscle contraction that results in relaxation.

4.2.8 Effect in atherosclerosis

Saraswathi and Gnanam reported that M. Paradisiaca inhibits cholesterol crystallization in vitro which mayhave an effect on atherosclerosis plaque and gallstones in vivo. Parmar and Kar tested the peel extract of M. Paradisiaca inrats with diet-induced atherosclerosis. This study reports the protective role of the extract in atherosclerosis and thyroids function though it was not very effective like other plants tested. Yin X. and Quan J. further studied the effect of banana inhuman and found that plasma oxidative stress was significantly reduced and the resistance to oxidative modification of LDL was enhanced only after a single banana meal. The effect may be due to the presence of dopamine, ascorbic acid and other antioxidants present in banana.

4.2.9 Antioxidant activity

Plasma oxidative stress is significantly reduced only after a single banana meal in healthy human due to the presence of dopamine, ascorbic acid and other antioxidants present in banana **[93].** Antioxidant activity was also reported with aqueous acetone extract of banana peel by β –carotene bleaching method, DPPH free radical scavenging and linoleic acid emulsion method. Glycosides and monosaccharide components are mainly responsible for the antioxidant activity **[57]**. Vijayakumar reported the antioxidant activityof the extracted flavonoids from M. Paradisiaca in rats **[90]**. This group found that the flavonoids from banana stimulated the activities of superoxide dismutase (SOD) and catalase which might beresponsible for the reduced level of peroxidation products such asmalondialdehyde, hydroperoxides and conjugated dienes.

4.2.10 Diuretic activity

Ash of the peel of *M. Sapientum* showed an increase in urine volume and K + as well as other electrolyte excretion than normal saline in a study in rats. Successive ethanolic extract also give this diuretic effect [47] .Phytochemicals such assaponin, flavonoids and terpenoids are known to be responsible for this effect **[79]**

4.2.11 wound healing activity

Agarwal reported the wound healing activityof both methanolic and aqueous extract of plantain banana (*M.sapientum var. paradisiacal*) in rats. Both extracts were found to increase hydroxyproline, hexuronic acid, hexosamine, super oxidedismutase as well the wound breaking strength and reduced glutathione level. They also decreased the wound area, scar area and lipid peroxidation. The effects were attributed to the antioxidant property of the plantain.

4.2.12 Anti-allergic activity

The water extract of pulp of ripe *M. Sapientum* has been reported to have significant anti-allergic activity on antigen-induced degranulation in RBL-2H3 cells with an IC50 value of 13.5±2.4 **[87]**.

4.2.13 Antimalarial activity

The decoction of the leaves of *M. Paradisiaca* added to *Ocimum americanum* and *Ocimum gratissimum* is used as to treat malarial. But in vitro study using Plasmodium falciparum chloroquine-resistant strain proves this plant ineffective in malarial **[49]**.

4.2.14 Effects on Muscle

The stem juice of plantain banana tree (*M. Sapientum vs paradisiaca*) has been found to induce contraction in skeletal muscles by enhancing excitation-contraction coupling and transmembrane Ca 2+ fluxes **[85]**. Later, Benitez reported the trunks juice of *M. Sapientum* vs. *Cavendishi* has muscle paralyzing effect in rat and attributed the effect to monopotassium oxalate present in the juice.

4.2.15 Anti-snake venom activity

Borges reported the in vitro neutralizing capacity of bothrops jararacussu and Bothrops neuwiedi snakevenoms by the stem juice of *M. Paradisiaca* .The phospholypase A 2(PLA 2) and hemorrhagic activities induced by the venom was inhibited by the extract as it forms unspecific complex with the venom protein. However, the in vivo activity of the extract in mice was not significant to protect against the venom **[25]**.

4.2.16 Mutagenecity

A report describes that the mutagenic effect of *M. paradisiaca* fruit peel extract in mice assessed by the single-cell gel electrophoresis (SCGE) and micronucleus assays. The experiments showed DNA damaging property in peripheral blood leukocytes for 1500 and 2000 mg/kg body weight.

4.2.17 Digestion

The digestion and absorption from the small bowel of the carbohydrate of banana has been studied by feeding ileostomy subjects banana from six batches of different ripeness and measuring the amounts excreted in the effluent **[35]**. Starch content of bananas depended on the ripeness being 37% of dry weight in the least ripe and 3% in the most ripe. Excretion of carbohydrate from banana in ileostomy effluent ranged from 4-19 g/day and was directly related to the starch content (r = 0.99). Up to 90% of the starch could be accounted for in the effluent. Complete recovery of non starch polysaccharides NSP (dietary fiber) was obtained. The amount of banana starch not hydrolyzed and absorbed from the human small intestine and therefore passing into the colon may be up to 8 times more than the NSP present in this food and depends on the state of ripeness when the fruit is eaten **[35]**.

METHODS

CHAPTER 5

Chapter

5

METHODS

5.1 Antimicrobial assay

The antimicrobial screening which is the first stage of antimicrobial drug research is performed to as certain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth. This ability may be estimated by any of the following three methods.

i) Disc diffusion method

ii) Serial dilution method

iii) Bioautographic method

But there is no standardized method for expressing the results of antimicrobial screening **[94]**. Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms. However, a great number of factors viz., the extraction methods, inoculum volume, culture medium composition, p^H, and moisture and incubation temperature can influence the results.

Among the above mentioned techniques the disc diffusion is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity. That's why the antimicrobial ability of the crude ethanolic extract of *Musa sapientum* is estimated by Disc diffusion method. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method **[95]**.

5.1.1 Principle of Disc Diffusion Method

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media **[96]**. The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter **[96].**

In the present study the crude extracts, fractions as well as some pure compounds were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required.

5.1.2 Apparatus and Reagents

1. Filter paper discs
2. Autoclave
3. Nutrient Agar Medium
4. Laminar air flow hood
5. Petridishes
6. Spirit burner
7. Sterile cotton
8. Refrigerator
9. Micropipette
10. Incubator
11. Inoculating loop
12. Distilled water
13. Sterile forceps
14. Ethanol
15. Screw cap test tubes
16. Nosemask and Hand gloves

5.1.3 Plate preparation

Five different bacterial strains were used to carry out this assay.These are *V.mimicus, S.typhi, S.dysentry, S.aureus & B.serus.*

Nutrient agar was used as the culture media.

5.1.4 Reviving of Stock cultures

Stocks of are V.mimicus, S.typhi, S.dysentry, S.aureus & B.serus were revived in nutrient agar by incubating at 37^0C for 24 hrs.

5.1.5 Bioassay

A single disk diffusion method was used to assess the presence of antimicrobial activities of the methanolic extract of the banana leaves. Whatman's filter paper was punched, and 6mm disks were collected in a beaker. The beaker was covered with foil paper and autoclaved. Different concentration extracts of the three plants (banana leaves methanolic extract-2mg/ml & 20 mg/ml were loaded of 20 mcl pipette and the 20µl of sample extract was pipette per disk. The revived test organisms were plated onto nutrient agar plates. The disks were then placed equidistant on all plates for all extracts. The plates were incubated at 37^0C for 24 hrs; after which results were observed and recorded in tables.

Figure 5.1 Laminar air flow

Figure 5.2 Incubation of petri dish

5.2 Minimum Inhibitory concentration (MIC)

The disc diffusion method is a semi quantitative method. Determination of MIC values provides a quantitative measure for the level of resistance expressed by the test organism. Next to the agar dilution method, the broth dilution method is one of the most frequently used methods to determine minimum inhibitory concentration (MIC) of the crude extract. The method is based on the inoculation of a standardized liquid culture of the test strain in a dilution series of the extract for which the MIC is determined. The first concentration of the dilution series at which no visual growth of microorganism can be determined is considered as the MIC **[97]**.

5.2.1 Apparatus and Reagents

Nutrient Agar Medium	Autoclave
Nose mask and Hand gloves	Eppendrof tube
Petri dishes	Laminar air flow hood
Sterile cotton	Spirit burner
Micropipette	Refrigerator
Inoculating loop	Incubator
Sterile forceps	Ethanol
Screw cap test tubes	

5.2.2 Plate preparation for MIC

Vibrio mimicus bacterial strains were used to carry out this assay. Nutrient agar was used as the culture media.

5.2.3 Reviving of Stock cultures for MIC

Stocks of are V.mimicus was revived in nutrient agar by incubating at 37^0C for 24 hrs.

5.2.4 Procedure of MIC

The agar diffusion method was employed for the determination of antibacterial activities of *Musa sapientum*. The leave extracts were dissolved in 100% methanol to a final

concentration of 100 mg/ml. The bacterial strains were cultured in a nutriment broth for 24 hours. Then, previously prepared 1ml of suspension bacteria (30 X 1012 CFU estimated) was spread on nutrient Broth agar. Disks were made by using a sterile filter paper and were loaded with 20 µl of each sample extract. Methanol was used as negative control and Azithromycin (30 mcg/disk) as positive reference standard. All the plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition in millimeters. All experiments were done in triplicates.

5.3 Antioxidant test

5.3.1 Mechanism of Antioxidant

Antioxidant may be defined as any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate **[98]**. For convenience, antioxidants have been traditionally divided into two classes, primary or chain breaking antioxidants and secondary or preventative antioxidants. Secondary or preventative antioxidants are compounds that retard the rate of oxidation. This may be achieved in a number of ways including removal of substrate or singlet oxygen quenching. Primary antioxidants, AH, when present in trace amounts, may either delay or inhibit the initiation step by reacting with a lipid radical (L•) or inhibit the propagation step by reacting with peroxyl or alkoxyl radicals:

$$L^{\bullet} + AH \rightarrow LH + A^{\bullet}$$
$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet}$$
$$LO^{\bullet} + AH \rightarrow LOH + A^{\bullet}$$

The antioxidant free radical may further interfere with chain propagation reactions by forming peroxy antioxidant compounds: **[99]**

$$A^{\bullet} + LOO^{\bullet} \rightarrow LOOA$$
$$A^{\bullet} + LO^{\bullet} \rightarrow LOA$$

Chain breaking antioxidants may occur naturally or they may be produced synthetically as in the case of Butylated hydroxyanisole, Butylated hydroxytoluene, *tert*-Butylhydroquinone and the gallates. The use of naturally occurring antioxidants has been promoted because of concerns regarding the safety of synthetic antioxidant, with natural alternatives (*e.g.*, plant biophenols) possessing antioxidant activity similar to or even higher than that of synthetic antioxidants **[100]**.

5.3.2 Methods of evaluating antioxidant activity

Antioxidant property of the various fraction of the plant was determined by following methods-

1. Determination of DPPH radical scavenging assay (Quantitative analysis)
2. Determination of total phenolic content
3. Determination of reducing power ability
4. Determination of total flavonoids content
5. Determination of total antioxidant capacity by phosphomolybdenum method

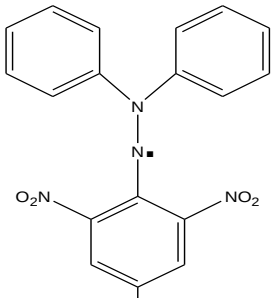
5.3.3 DPPH Test

The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts. Different concentrations of the plant extract (2, 4,6,8 & 10 µg/ml, in methanol) were added at an equal volume (10ml) to methanol solution of DPPH 400µg/ml, 100 ml. Different concentrations of L-Ascorbic acid (2-10 mg/ml) were used as the standard antioxidant. After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the following equation:

$$\text{DPPH antiradical scavenging capacity (\%)} = [1 - (A_{sample}/A_{control}] \times 100.$$

Methanol plus different concentration of plant extract solution was used as a blank, while DPPH solution plus methanol was used as a control. The IC_{50} values were calculated by the sigmoid non-linear regression model using plots, where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of scavenging

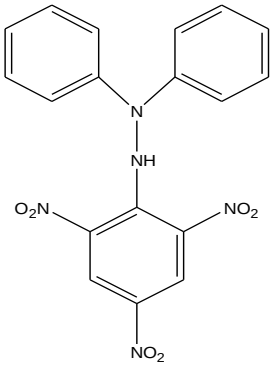
capacity. IC_{50} values denote the concentration of the sample required to scavenge 50% of DPPH radicals.



DPPH (oxidized form)

Diphenyl picrylhydrazyl (λ_{max} 517 nm)

+ RH ⟶



DPPH (reduced form)

+ R.

5.3.4 Reducing power activity test:

5.3.4.1 Principle

Reducing power assay is based on the principle that substances which have reduction potential react with potassium ferricyanide [$K_3Fe(CN)_6$] to form potassium ferrocyanide [$K_4Fe(CN)_6$], which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm (Hemalatha S *et al.,* 2010). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity **[102]**. The presence of reducing agent such as antioxidant substances in the crude extract causes the reduction of the ferricyanide (Fe^{3+}) complex to the ferrous form (Fe^{2+}). The resulting ferrous complex (Fe^{2+}) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicated the increased reducing power of the crude extract. The following reaction occurs in reducing power assay:

$$\mathbf{K_3Fe(CN)_6 + FeCl_3 \xrightarrow{\text{Antioxidant}} K_4Fe(CN)_6 + FeCl_2}$$

5.3.4.2 Materials & Reagents

Test tube	Analytical balance
Pipette	UV- visible spectrophotometer
Spatula	Hot air oven
Vortex mixer	Centrifuge machine
0.2 M Phosphate buffer	1 % Potassium ferricyanide [$K_3Fe(CN)_6$]
10 % Trichloroacetic acid (TCA)	Distilled water
Ferric chloride ($FeCl_3$)	L-ascorbic acid

5.3.4.3 Methods

1 ml of the methanol solution of the crude extract of different concentrations (1, 5, 10, 50, 100 µg/ml) was mixed with 2.5 ml phosphate buffer and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50°C for 20min. 2.5 ml of trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged at 3000rpm for 10min. The upper layer of the solution was separated and mixed with 2.5 ml distilled water and 0.5ml $FeCl_3$. The absorbance was measured against a blank at 700nm. All the tests were carried out in triplicate and average absorption was noted for each time. L-Ascorbic acid was used as positive control. Percentage (%) increase in reducing power was calculated as follows:

$$\% \text{ Increase in reducing power} = (A_{Test} / A_{Blank}) -1 \text{ X } 100$$

Where, A_{Blank} is absorbance of blank (containing all reagents except the test material) and A_{Test} is absorbance of test solution.

5.3.5 Total Phenolics Assay

The antioxidative effect is mainly due to phenolic components, such as flavonoids **[103]**, phenolic acids, and phenolic diterpenes **[108]**. The phenolic compounds exert their antioxidant properties by redox reaction, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

Many phytochemicals possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of cancer in several human populations **[100]**.

5.3.5.1 Principle

The total phenolic concentration of the extract of *Spondias pinnata* fruit was determined by the modified Folin-Ciocalteu method. The process of measuring total phenolic content of the crude extract of *Spondias pinnata* fruit involves the use of Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. It measures the amount of substance being tested needed to inhibit the oxidation of the Folin-Ciocalteu reagent **[105]** .The reagent does not contain phenol. Rather, the reagent will react with phenols and nonphenolic reducing substances to form chromogens that can be detected spectrophotometrically. The color development is due to the transfer of electrons at basic pH to reduce the phosphomolybdic-phosphotungstic acid complexes to form chromogens in which the metals have lower valence. The generated chromogens give a strong absorption maximum at 760 nm **[96]**.

Alkaline condition

Phenols ⟶ Ionized Phenols

Complete ionization

Oxidation

Ionized Phenols + Folin-Ciocalteu reagent ⟶ Folin-Ciocalteu reagent complex

(Yellow) (Blue)

5.3.5.2 Materials & Reagents

Test tube	Analytical balance
Pipette	UV- visible spectrophotometer
Spatula	Vortex mixer
Folin-Ciocalteu reagent	Distilled water
Sodium carbonate (Na_2CO_3)	Methanol
Gallic acid	Aluminium foil

5.3.2.3 Composition of Folin-Ciocalteu reagent

SL. No.	Component	Percent
1	Water	57.5
2	Lithium Sulfate	15.0
3	Sodium Tungstate Dihydrate	10.0
4	Hydrochloric Acid ≥25%	10.0
5	Phosphoric Acid 85 % solution in water	5.0
6	Molybdic Acid Sodium Dihydrate	2.5

5.3.5.4 Methods

0.5 ml of a methanol solution of the crude extract of concentration of 1 mg/ml was mixed with 5 ml Folin ciocalteu reagent (1:10 v/v distilled water) and 4 ml (75g/L) of Sodium carbonate. The mixture was vortexed for 15 second and allowed to stand for 30min at room temperature in dark place for color development and the absorbance was measured at 760 nm against methanol as blank by using a UV- visible spetrophotometer. The total phenolics was expressed as mg of GAE (gallic acid equivalent) per gm of the dried extract using the following equation obtained from a standard Gallic acid calibration curve: $y = 0.0162x + 0.0215$, $R^2=0.9985$.

5.3.6 Total Flavonoids Concentration

5.3.6.1 Principle

Aluminium chloride ($AlCl_3$) colorimetric method is incorporated to determine the total flavonoid contents of the crude plant extract. The basic principle of the assay method is that

aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols of the crude extract. In addition aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. The formed flavonoid-aluminium complex between flavonoid of the crude extract and aluminium chloride has an absorptivity maximum at 415 nm. Therefore, the amount of flavonoid in the crude extract can be quantified by measuring the absorbance of reaction mixture at 415 nm using a UV-visible spectrophotometer against a blank containing all reagents except the extracts. Quercetin at various concentrations was used as standard **[107]**.

Flavonoid (Extract) + $AlCl_3$ (reagent) = Formation of flavonoid-aluminium complex (λ_{max} 415 nm)

5.3.6.2 Materials & Reagents

Methanol	UV- visible spectrophotometer
10% aluminum chloride ($AlCl_3$)	Test tube
1M potassium acetate (CH_3COOK)	Aluminium foil
Distilled water	Spatula
Pipette (5ml)	Analytical balance

5.3.6.3 Methods

The total flavonoid compounds in each extract was determined by partial modification of Ismail et al. An aliquot (1.5 ml) of methanolic extract was added to 6ml of deionized water and then 0.45 ml 5% (w/v) $NaNO_2$ and incubated for 6 min. 0.45 ml 10% (w/v) $AlCl_3$ and 6 ml 4%(w/v) NaOH was added and the total volume was made up to 15 ml with distilled water. The absorbance was measured at 510 nm by using visible spectrophotometer. The results were expressed as mg rutin equivalents/g DW. The analyses were done in three replications.

5.3.7 Hydrogen peroxide radical scavenging (H2O2) assay

A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50mMpH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (4 - 20 □g/ml) in phosphate buffer is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. L-ascorbic acid was used for comparison. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ Scavenged } (H_2O_2) = [1 - (A_{sample} / A_{control}) \text{ X } 100$$

Where; $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of test. Ascorbic acid is used as a positive control.

5.4 Thin Layer Chromatography (TLC)

5.4.1 Principle

The extracts were analyzed by performing TLC to determine the composition of each extract. TLC was done under three solvent systems. Successively the polarity of the solvent systems was increased to get a clear graph of all the possible compounds present in the extract. Most organic solvents are nonpolar in nature and so organic compounds such as benzene was used to elute the non polar compounds present in the extract. Again, since Alkaloid and terpenoid type compounds are basic in nature, ammonium base is used in the solvent system to help them run over TLC plate.

Table 5.1: The compositions of various solvent systems for TLC

Nonpolar Basic solvent	**Intermediate polar Basic Solvent**	**Polar Basic solvent**
Benzene 9mL Ethanol 1mL Ammonium hydroxide 0.1mL	Chloroform 5Ml Ethyl acetate 4mL Formic acid 1mL	Ethyl acetate 8mL Ethanol 1.2mL Water 0.8mL

5.4.2 Apparatus

1. TLC tank 2.Pencil

3. Scale 4.UV-lamp

5 .Spray bottle 6.Heat gun

7. Petri dish 8.Capillary tube

5.4.3 Reagents

1. Benzene 2.Ethanol

3. Ammonium hydrooxide 4.CHCl3

5. Formic acid 6.Ethyl acetate

7. EtOH 8.Water

9. Nutrient agar 10.H2SO4

11. DPPH 12.Folin & ciocalteu solution

13.1-butanol 14.Glacial acetic acid

15.2% ninhydrine solution 16.Acetone

17. Phosphate Buffer 18.Methanol

19. N-Hexane 20.Standard flavonoid solution

5.4.4 Procedure

Firstly, above three solvent systems were prepared.

In the next step, TLC plates were prepared. For this nine TLC plates were prepared, three for each solvent system to run with. The TLC plates were labeled in the following maner:

1-C, 1-D, 1-F 2-C, 2-D, 2-F 3-C, 3-D, 3-F

Where,

1denoted run with nonpolar solvent system, 2 denoted run with intermediate polar solvent system, 3 denoted run with polar solvent system and C denoted treatment with 10% Sulphuric

acid,D denoted treatment with 0.04%DPPH solution, F denoted treatment with 10% Folin & ciocalteu solution.

On each plate four spots were spotted. The spotting patterns were as follows:

1^{st} spot was spotted with Solvent; in this case methanol was used.

2^{nd} & 3^{rd} spots were spotted with the extracts of banana leaf.

4^{th} spot was the control, spotted with standard flavonoid solution

After spotting the respective TLC plate was exposed to the respective solvent system. Like all plates that were marked 1 were run with nonpolar solvent system, those that were marked 2 were run with intermediate polar solvent system and the 3 marked plates run with polar solvent system.

Upon completion of TLC, the plates were exposed to reagent for compound detection and identification. For this the C marked plates were exposed to 10% sulphuric acid solution, dried and then heated to $80\text{-}90^0$ C for charring purpose. This helped in the fixation of spot and allowed it to be prominently visible.

Likewise, the D marked plates were dipped into 0.04% DPPH solution and dried while keeping in a dark place. The F marked plates were washed with Folin & ciocalteu reagent and dried

5.5 Biomedical test

5.5.1 Haemagglutination activity

5.5.1.1 Principle

Many viruses attach to molecules present on the surface of RBCs. A consequence of this is that at certain concentrations, a viral suspension may bind together (agglutinate) the RBCs, thus preventing them from settling out of suspension. Since agglutination is rarely linked to infectivity, attenuated viruses can therefore be used in assays. By serially diluting a virus suspension into an assay tray (a series of wells of uniform volume) and adding a standard

amount of blood cells, an estimation of the number of virus particles can be made. While less accurate than a plaque assay, it is cheaper and quicker (taking just 30 minutes). This assay may be modified to include the addition of an antiserum. By using a standard amount of virus, a standard amount of blood cells, and serially diluting the antiserum, one can identify the concentration of the antiserum (the greatest dilution which inhibits hemagglutination).

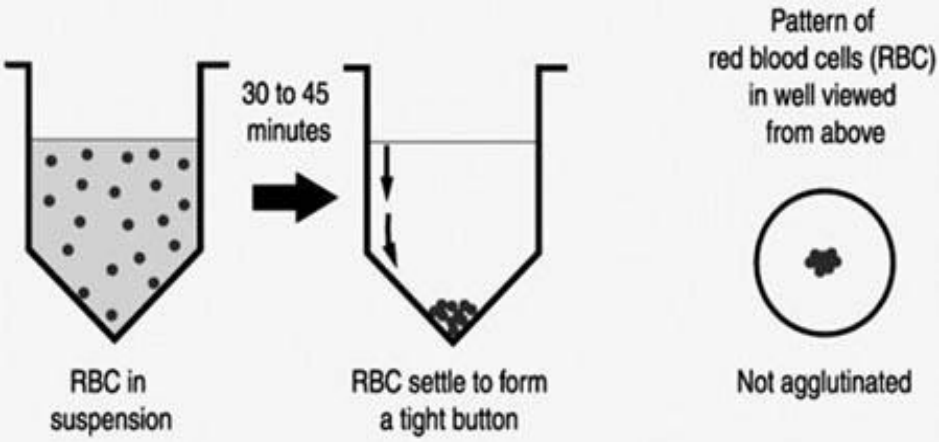


Figure 5.3: Formation of tight button with no agglutination

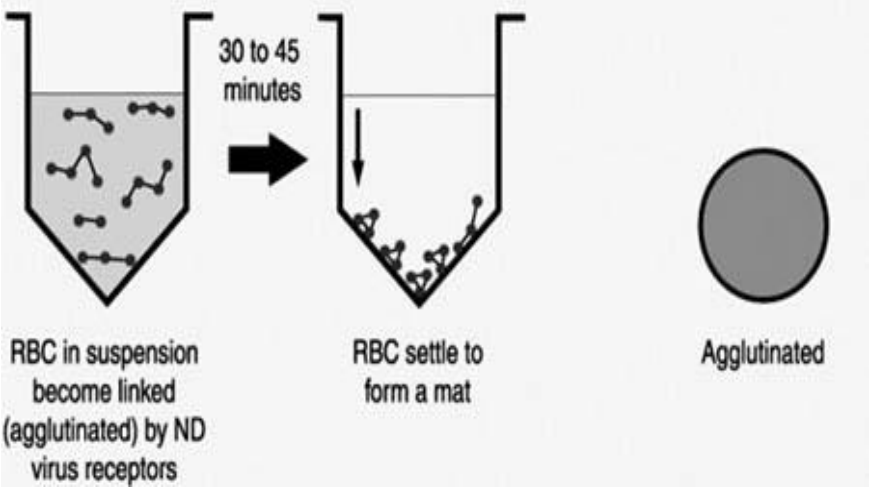


Figure 5.4*:* Formation of agglutination

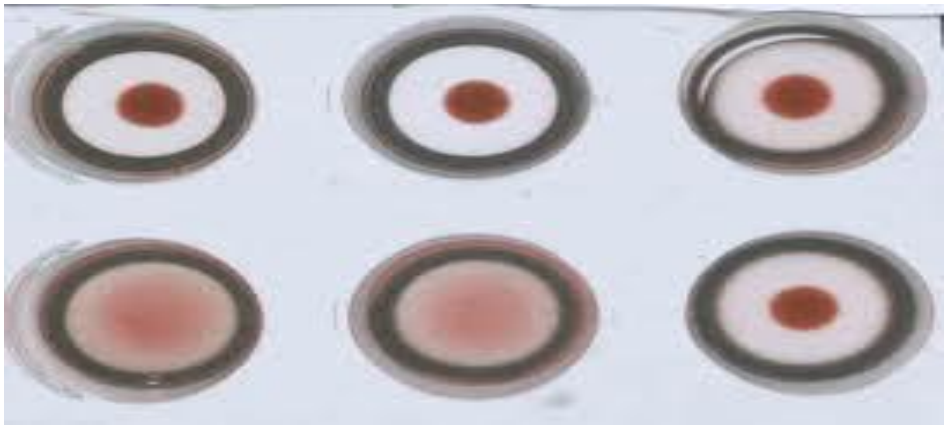


Figure 5.5*:* Overview of HA and not HA

5.5.1.2 Apparatus

Centrifuge machine	Refrigerator
Ephindorf tube	Test tube
Micro pipette	Eppendorf tube box
Syringe	CD marker
Cotton	

5.5.1.3 Reagents

Isotonic phosphate buffer	Blood

5.5.1.4 Procedure

Stock solution of the test sample was prepared at concentration of 5 mg/ml and each solution was serially diluted. Fresh blood from healthy person was collected only for the test of haemagglutination Assay (HA). The blood group A^+, B^+, AB^+, O^+ were collected from healthy person for this test of east West University students. Then the all bloods were centrifuged and the erythrocytes were separated. 4% erythrocyte suspension was prepared in phosphate buffer (pH 7.4) of all blood groups. 1 ml of the test sample dilution was taken with

1 ml of 4% erythrocyte and incubated at 25°C. After incubation, the results were noted. Smooth button formation in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of haemagglutination was determined from the extent of deposition.

5.5.2 Anti-Hemolytic Activity

5.5.2.1 Apparatus

Centrifuge machine	Refrigerator
Micro pipette	Test tube
Syringe	CD marker
Cotton	Uv-spectrophotometer

5.5.2.2 Reagents

H_2O_2

Isotonic sodium phosphate buffer

Blood samples

5.5.2.3 Procedure

The erythrocytes from man blood were separated by centrifugation and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant was colorless. The erythrocytes were then diluted with saline or phosphate buffer to give a 4% suspension. Varying amounts of sample (1000,500,250,125,62.5 μg/ml) with saline or buffer were added to 2 ml of the suspension of erythrocytes and the volume was made up to 3.5 ml with saline or buffer. This mixture was pre-incubated for 120 min and then 0.5 ml H_20_2 solutions of appropriate concentration in saline or buffer were added. The concentration of H_20_2 in the reaction mixture was adjusted so as to bring 90% hemolysis of blood cells after 120 min incubation. About 80-90% hemolysis of rat erythrocytes was obtained after 4-6 h. Incubation

was concluded after these time intervals by centrifugation during 5 min at Xl000 g and the extent of hemolysis was determined by measurement of the absorbance at 540 nm corresponding to hemoglobin liberation. Antihemolytic activity was expressed as the inhibition percentage and was calculated using the following formula:

Antihemolytic activity (%) = [1 – (Sample $_{540\ nm}$ /Control $_{540\ nm}$)X 100]

where, Sample $_{540\ nm}$ was the absorbance of the sample and Control $_{540\ nm}$ was the absorbance of the control.

5.6 Preliminary phytochemical investigations

5.6.1 Principle

The secondary metabolites in the plant sample are the main concern of research work. There are many tests available for this purpose. In the following table these tests are shown.

Table 5.2: Preliminary phytochemical investigations of secondary metabolites

Secondary metabolite	Name of test	Methodology	Observation
Alkaloid	Wagner test	Add 2ml filtrate with 1% HCl + steam. Then add 1ml of the solution with 6 drops of Wagner's reagent	Brownish-red precipitate
Anthraquinone	Borntrager's test	Add 1 ml of dilute (10 %) ammonia to 2 ml of chloroform extract	A pink-red color in the ammoniacal (lower) layer
Cardiac glycosides	Kellar - Kiliani test	Add 2ml filtrate with 1ml of glacial acetic acid, 1ml ferric chloride and 1ml concentrated sulphuric acid	Green-blue coloration of solution
Flavonoid	NaOH test	Treat the extract with dilute NaOH,	A yellow solution

		followed by addition of dilute HCl	with NaOH, turns colorless with dilute HCl
Reducing sugar	Fehling test	Add 25ml of diluted sulphuric acid (H_2SO_4) to 5ml of water extract in a test tube and boil for 15mins. Then cool it and neutralize with 10% sodium hydroxide to pH 7 and 5ml of Fehling solution	Brick red precipitate
Saponin	Frothing test/ Foam test	Add 0.5ml of filtrate with 5ml of distilled water and shake well	Persistence of frothing
Steroid	Liebermann-Burchardt test	To 1ml of methanolic extract, add 1ml of chloroform, 2–3ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid	Dark green coloration
		To 1 ml of extract, add 2 ml acetic anhydride and 2 ml concentrated sulphuric acid	Color change to blue or green
Terpenoid	Liebermann-Burchardt test	To 1ml of methanolic extract, add 1ml of chloroform, 2–3ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid	Pink or red coloration

5.6.2 Apparatus

Test tubes	Beaker
Conical flask	Filter paper
Pipette	Dropper
Electric balance	Hot plate
Shaker	Hot water bath

5.6.3 Reagents

1% HCl	Dilute sulphuric acid
Wagner's reagent	Water
Dilute (10 %) ammonia	Fehling solution
Glacial acetic acid	Chloroform
Ferric chloride	Acetic anhydride
Concentrated sulphuric acid	10% alcoholic ferric chloride
Dilute NaOH	Dilute HCl
Phoshomolybdic acid reagent	

5.6.4 Procedure

Extract of different solvents were prepared. According to above table the tests were done. Detection of various secondary metabolites present in the plant was done according to above principle.

RESULTS & DISCUSSION

CHAPTER 6

Chapter

6

RESULTS & DISCUSSION

The aim of this chapter is to illustrate the results and discussions of crude methanolic extract of *Musa sapientum* (leaf). This chapter will include the following results and discussions of the test samples of *Musa sapientum* (leaf):

1. In vitro antimicrobial screening 2. Minimum inhibitory concentration (MIC)

3. In vitro antioxidant activity

    3.1. Total phenolic content

    3.2. DPPH radical scavenging assay (Quantitative analysis) and IC_{50}

    3.3. Reducing power assay

    3.4. Hydrogen Peroxide radical scavenging assay and IC_{50}

    3.5. Total flavonoids content

4. Haemagglutination test 5. Antihaemolytic Activity

6. Sensityvity test 7. Preliminary phytochemical investigations

8. Thin Layer Chromatography (TLC)

Besides the results of various tests are represented through suitable graphical representation methods. All the data are expressed as mean ± standard deviation (n=3).

6.1 In vitro antimicrobial screening

The antimicrobial activities of extracts were examined in the present study. The crude extract produced strong activity against a number of the test organisms. The results are given in table 6.1. The zones of inhibitions produced by the crude methanolic extract of the fruit of *Musa sapientum* (leaf) were ranged from 6-11 mm at a concentration of 40µg/disc. While the zones

of inhibition produced by the crude methanolic extract of the fruit of *Musa sapientum* (leaf) were ranged from 7-17 mm at a concentration of 400µg/disc. The methanolic extract showed greatest activity against *Staphylococcus aureus* having the zone of inhibition of 11 mm (40µg/disc) and 16 mm (400µg/disc). Besides the extract showed strong activity against *Shigella dysentery* (7mm for 2mg/disc and 16 mm for 20mg/disc), *Salmonella typhi* (7 mm for 40µg/disc and 16 mm for 400µg/disc), *Vibrio mimicus* (7 mm for 40µg/disc and 17 mm for 400µg/disc). Moderate activity was found against *Bacillus cereus* (6 mm for 40µg/disc and 7 mm for 400µg/disc). Among the tested Gram positive bacteria, the extract showed greatest activity against *Staphylococcus aureus* having the zone of inhibition of 11 mm (40µg/disc) and 16 mm (400µg/disc). Among the tested Gram negative bacteria, the extract showed strong activity against *Shigella dysentery* (7mm for 40µg/disc and 16 mm for 400µg/disc), *Salmonella typhi* (7 mm for 40µg/disc and 16 mm for 400µg/disc) and, *Vibrio mimicus* (7 mm for 40µg/disc and 17 mm for 400µg/disc). In brief, the mehanolic extract of *Spondias pinnata* fruit showed a potent inhibitory activity against *Staphylococcus aureus*.

Table 6.1 Antimicrobial activity of crude methanolic extract *Musa sapientum* (leaf)

Test microorganisms	Diameter of zone of inhibition (mm)		
	Crude extract (Dose 40µg/disc)	Crude extract (Dose 400µg/disc)	Control Azithromycin (30 µg/disc)
Gram positive bacteria			
Bacillus cereus	6	7	23
Staphylococcus aureus	11	16	25
Gram negative bacteria			
Shigella dysentery	7	16	23
Salmonella typhi	7	16	21
Vibrio mimicus	7	17	22

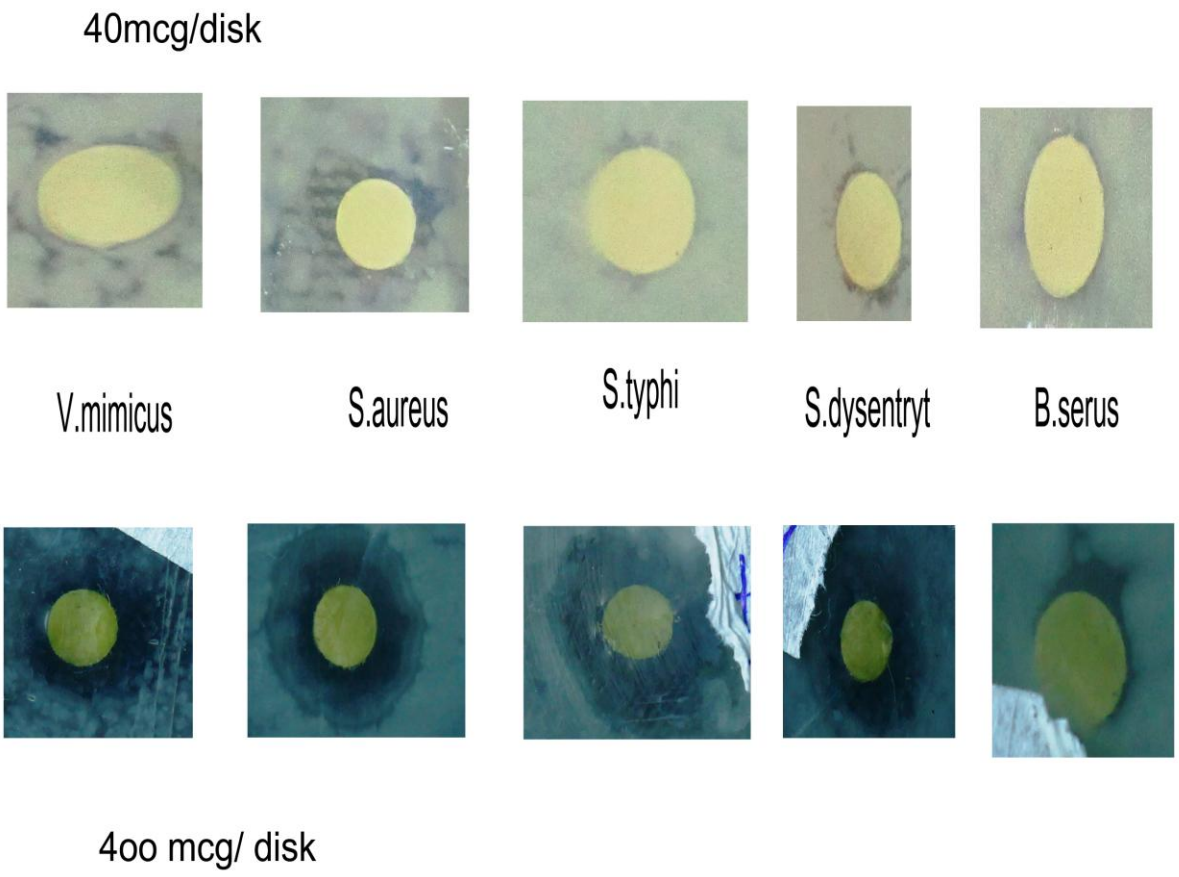


Figure 6.1 Antimicrobial activity of crude methanolic extract *Musa sapientum* (leaf)

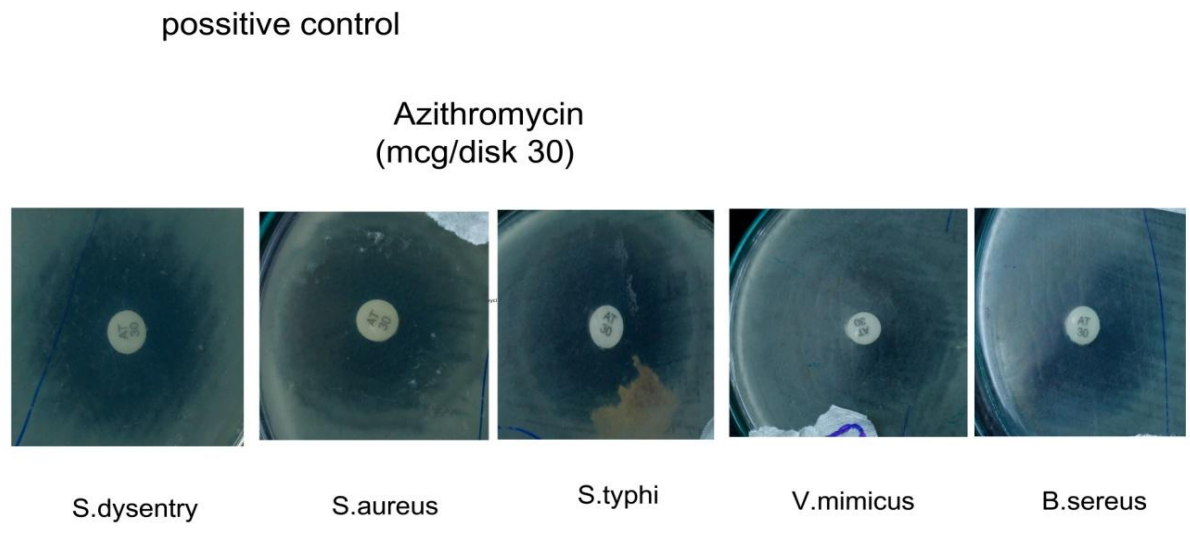


Figure 6.2 Positive control test against microorganisms

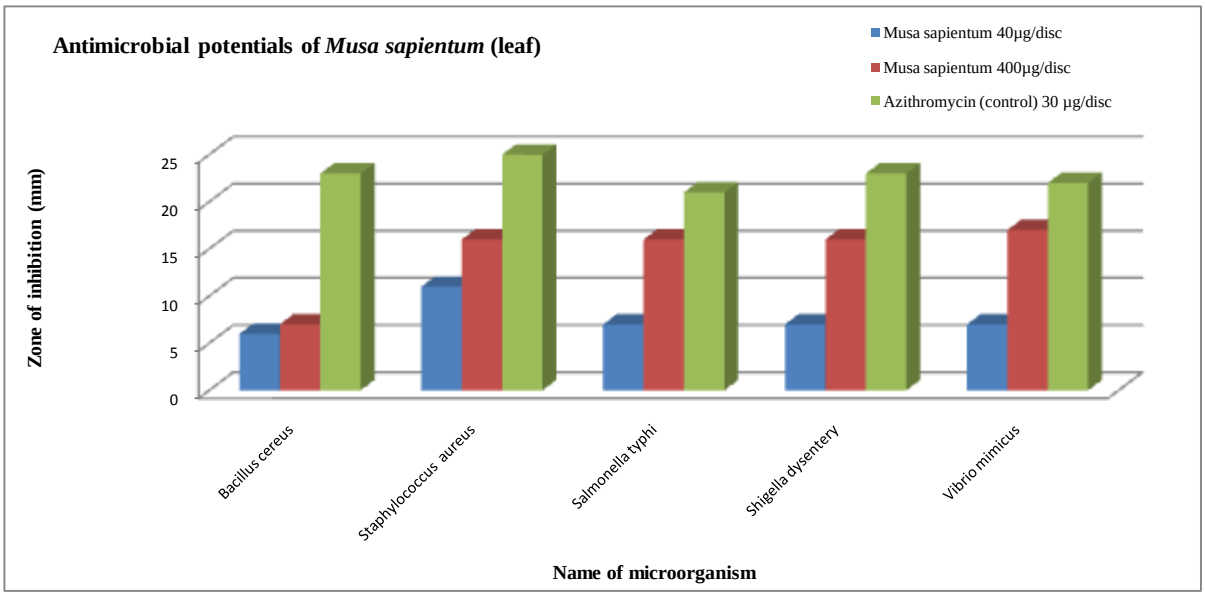


Figure 6.3: Comparison between the antimicrobial activities (zone of inhibition) of the crude methanolic extract of *Musa sapientum* (leaf) and positive control Azithromycin

6.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the crude extract was determined for *Vibrio mimicus* against which the crude methanolic extract of *Musa sapientum* (leaf) showed promising and potent antimicrobial activity in the preliminary in vitro antimicrobial screening test. The minimum inhibitory concentration (MIC) of crude methanolic extract of *Musa sapientum* (leaf) for the test microorganisms is expressed in table 6.2 and is shown by figures (Figure 6.4).

Table 6.2 Result of MIC

Concentration (µg/disc)	Zone of Inhibition (mm)
0.2	10
2	11
20	14
200	14
2000	16

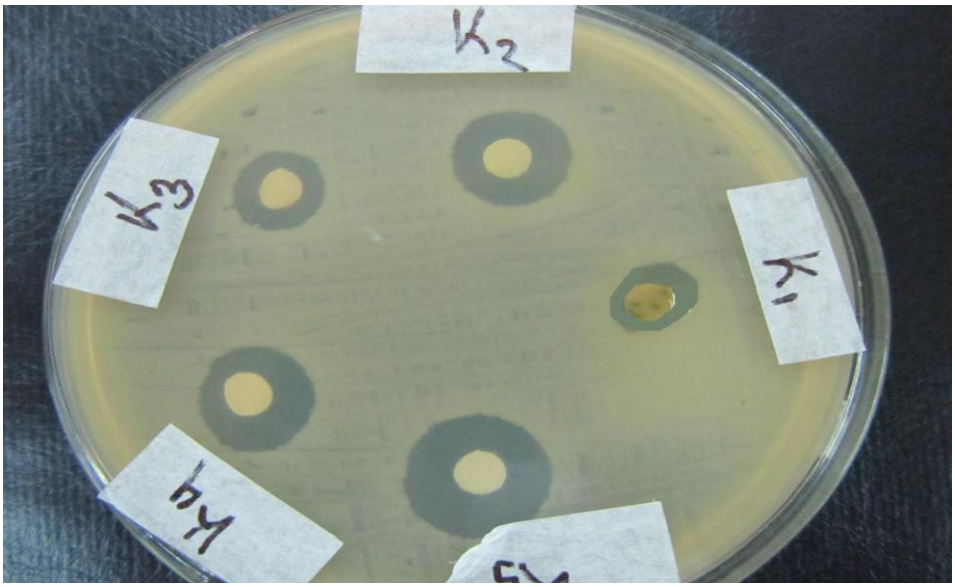


Figure 6.4: Zone of inhibition of *Musa sapientum* (leaf) against *vibrio mimicus* by MIC

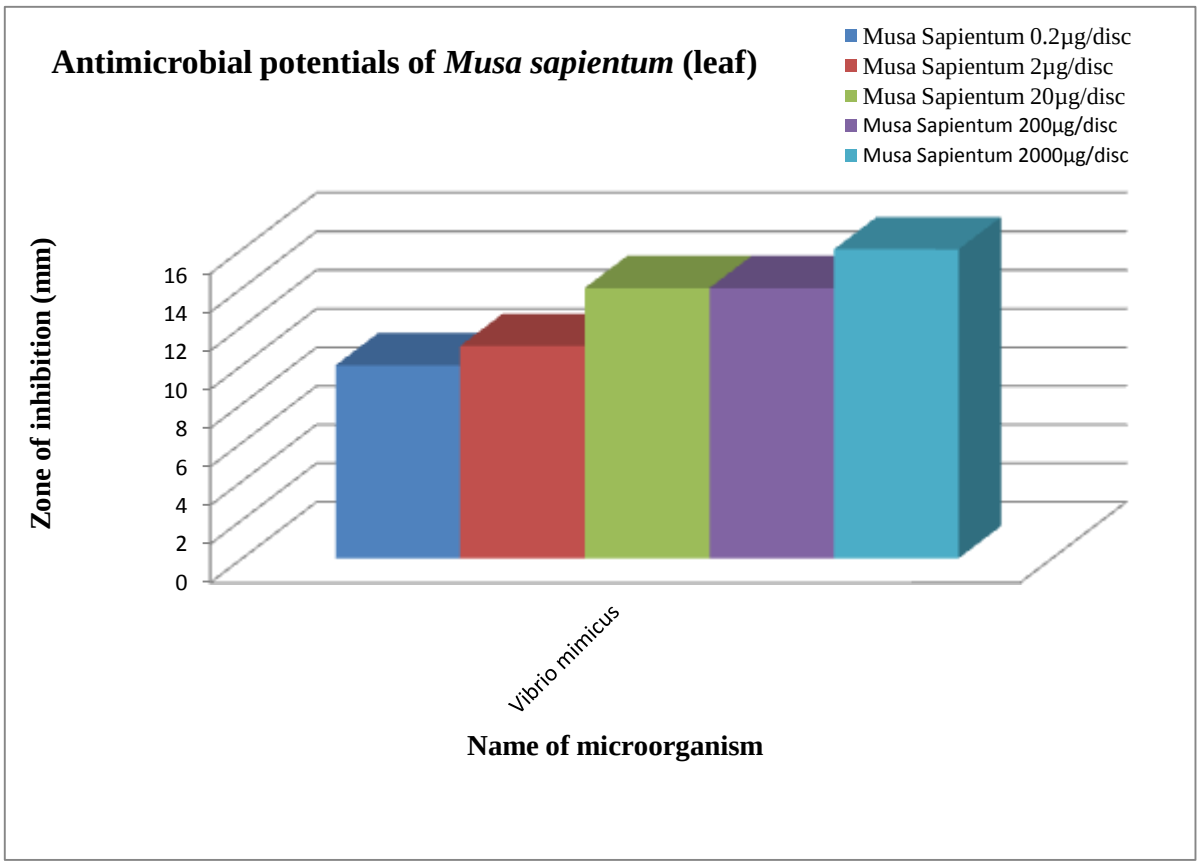


Figure 6.5: MIC (zone of inhibition) of the crude methanolic extract of *Musa sapientum* (leaf)

6.3 Antioxidant Test

6.3.1 Total phenolic content

The crude methanolic extract of *Musa sapientum* (leaf) was subjected to total phenolic content determination. Based on the absorbance values of the extract solution, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid (table 6.3) equivalents, result of the colorimetric analysis of the total phenolics are given in table 6.4. Total phenolic content of the samples are expressed as mg of GAE (gallic acid equivalent) per gm of dried extract. The phenolic content found in the crude ethanolic extract of *Musa sapientum* (leaf) was 8.564±0.716 mg of gallic acid (GAE) per gm of dried extract. Absorbance Values are expressed as average ± SD (n=4).

Table 6.3 Standard curve preparation by using gallic acid

SL. No.	Concentration (µg /ml)	Absorbance	Regression line	R^2
1	100	1.620	y = 0.0162x + 0.0215	0.9985
2	50	0.866		
3	25	0.450		
4	12.5	0.253		
5	6.25	0.120		
6	3.125	0.059		
7	1.5625	0.034		
8	0.78125	0.022		
9	0.3906	0.020		
10	0	0.011		

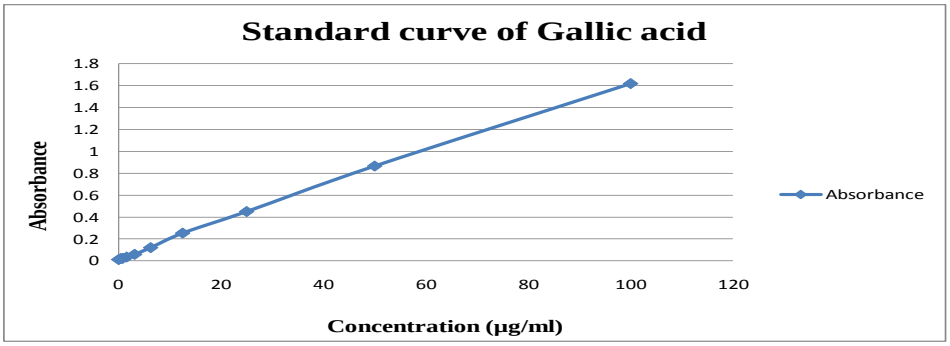


Figure 6.6: Standard curve for gallic acid

Table 6.4 Total phenolic content assay

Sample	Conc (mg/ml)	Absorbance	mg of Gallic acid equivalent (GAE) per gm of dried extract)
Crude ethanolic extract of *Musa sapientum* (leaf)	20mg/ml	0.115±0.011	8.564±0.716

6.3.2 DPPH radical scavenging assay (Quantitative analysis) and IC_{50}

The crude methanolic extract of *Musa sapientum* (leaf) was subjected to free radical scavenging activity and it showed significant free radical scavenging activity with an IC_{50} value of 10.527µg/ml. The percentage inhibition of free radical DPPH and the IC_{50} value of crude ethanolic extract of *Musa sapientum* (leaf) are given in table 6.5 and figure 6.6. Absorbance Values are expressed as average ± SD (n=3).

Table 6.5 IC_{50} value of crude methanolic extract of *Musa sapientum (leaf)*

SL no	A_{Blank}	Concentration (µg/ml)	A_{Sample}	% inhibition of free radical DPPH = $(1 - A_{Sample}/A_{Blank}) \times 100$	IC_{50} µg/ml
1	0.689	2	0.489±0.480	29.028	10.527
2		4	0.434±0.381	36.961	
3		6	0.395±0.327	42.622	
4		8	0.340±0.246	40.605	
5		10	0.314±0.231	54.378	

Here, A_{Blank} and A_{Sample} are the absorbance of blank and sample respectively.

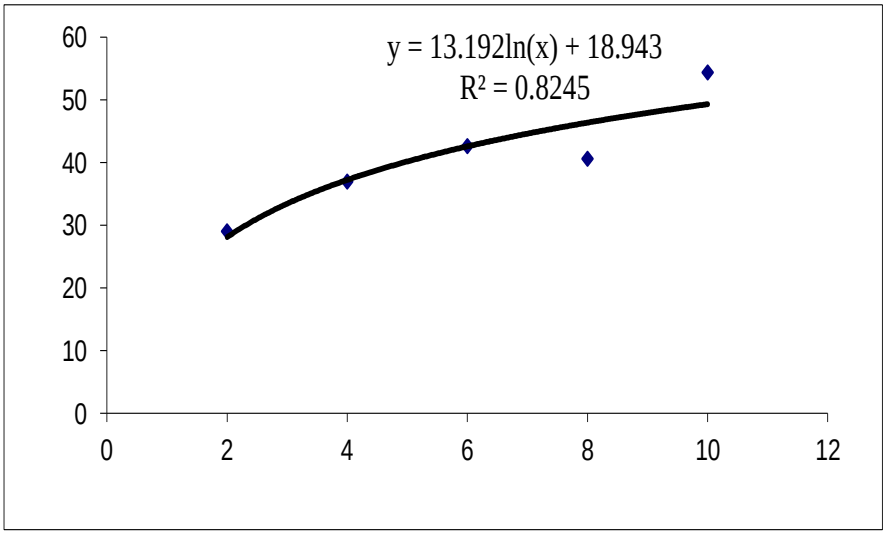


Figure 6.7 DPPH scavenging potential & IC_{50} value of crude ethanolic extract of *Musa sapientum* (leaf)

6.3.3 Reducing power assay

The reducing properties are generally associated with the presence of reductanes which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Duh *et al.*, 1999). The reducing power ability of crude ethanolic extract of *Musa sapientum* (leaf) was determined using L-ascorbic acid as positive control. In case of reducing power, the higher the concentration of the test samples, the higher the absorbance. The higher the absorbance, the higher the inhibition. The reducing power of various concentrations of crude ethanolic extract of *Musa sapientum* (leaf) are given in table 6.6 & figure 6.7. The percentage increase in reducing power of various concentrations of test material and EC_{50} value of the extract is represented in figure 6.8. The highest % increase in reducing power was observed for 84 mg/ml concentration of crude ethanolic extract and it was 63.433%. The EC_{50} value of the extract was found to be 42.52 mg/ml for crude ethanolic extract of *Musa sapientum* (leaf).

Table 6.6 EC_{50} value of crude ethanolic extract of *Musa sapientum (leaf)*

SL no	Absorbance of blank, A_{Blank}	Concentration (mg/ml)	Absorbance of test, A_{Test}	% increase in reducing power = (A_{Test} / A_{Blank}) -1 X 100	EC_{50} mg/ml
1	0.402	12	0.475	18.159	42.52
2		36	0.594	47.761	
3		56	0.64	59.204	
4		84	0.657	63.433	

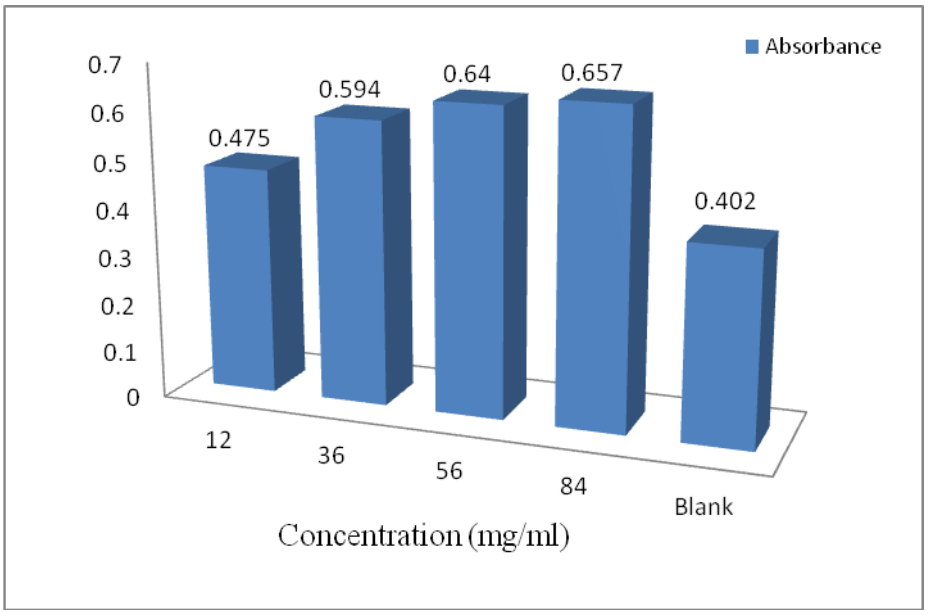


Figure 6.8 Bar diagram of Reducing power assay of various concentration of crude extract of *Musa sapientum* (leaf)

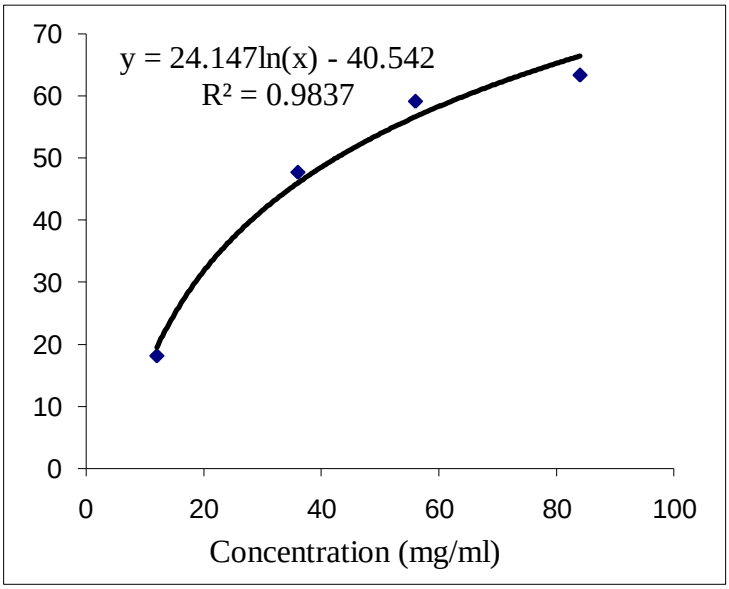


Figure 6.9 :% reducing power & EC_{50} value of crude extract of *Musa sapientum* (leaf)

6.3.4 Hydrogen Peroxide radical scavenging assay and IC_{50}

The crude ethanolic extract of *Musa sapientum* (leaf) was subjected to free radical scavenging activity and it showed significant free radical scavenging activity with an IC_{50} value of 311.06µg/ml. The percentage inhibition of free radical Hydrogen peroxide and the IC_{50} value of crude ethanolic extract of *Musa sapientum* (leaf) are given in table 6.7 and figure 6.9.

Table 6.7 IC_{50} value of crude ethanolic extract of *Musa sapientum (leaf)*

SL no	A_{Blank}	Concentration (µg/ml)	A_{Sample}	% inhibition of free radical DPPH = $(1 - A_{Sample}/A_{Blank}) \times 100$	IC_{50} µg/ml
1	1.615	4	1.13	30.031	311.06
2		8	1.11	31.269	
3		12	1.07	33.746	
4		16	1.039	35.666	
5		20	1.003	37.895	

Here, A_{Blank} and A_{Sample} are the absorbance of blank and sample respectively.

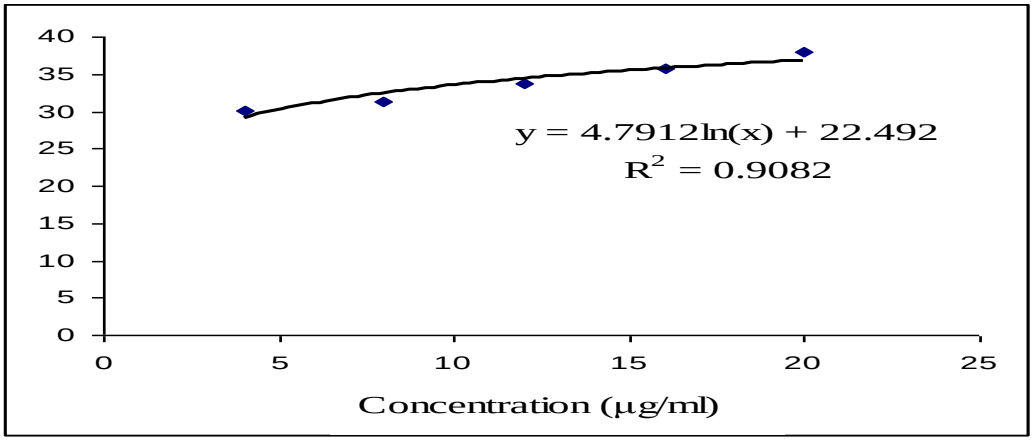


Figure 6.10: Hydrogen Peroxide scavenging potential & IC_{50} value of crude ethanolic extract of *Musa sapientum* (leaf)

6.3.5 Total flavonoids content

To determine the total flavonoids content of crude ethanolic extract of *Musa sapientum* (leaf) by using partially modified method of Ismail et al., a standard curve is needed which is obtained from a series of different rutin concentrations (table 6.8). The total flavonoids content of the sample is expressed as mg of rutin per gm of dried extract in table 6.9 by using the standard curve equation of rutin ($y = 0.002x + 0.0318$, $R^2 = 0.9989$). Where y is absorbance at 510 nm and x is flavonoid content of crude plant extract. The total flavonoids content found in the crude ethanolic extract of *Musa sapientum* (leaf) was 1431±65.57 mg of rutin per gm of dried extract. Absorbance Values are expressed as average ± SD (n=3).

Table 6.8 Standard curve preparation by using rutin

SL. No.	Concentration (µg /ml)	Absorbance	Regression line	R^2
1	2.5	0.0365	y = 0.002x + 0.0318	0.9989
2	5	0.0417		
3	10	0.0521		
4	20	0.0735		
5	30	0.0906		
6	40	0.1127		

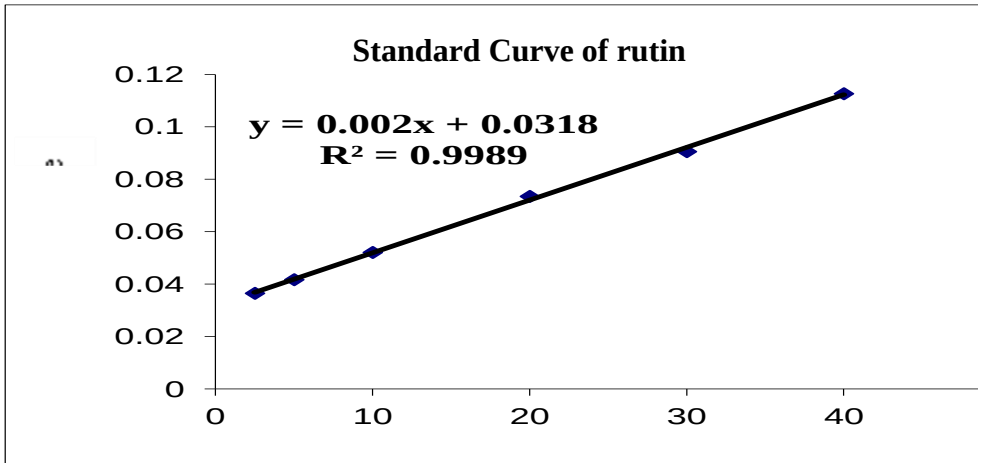


Figure 6.11 Standard curve for rutin

Table 6.9 Total flavonoids content assay

Sample	Conc (mg/ml)	Absorbance	mg of rutin equivalent per gm of dried extract
Crude methanolic extract of *Musa sapientum* (leaf)	1 mg/ml	0.318±0.013	1431±65.57

6.4 Haemagglutination test for methanolic extract of *Musa sapientum* (leaf)

From the table 6.10 it is seen that in case of A^+, B^+ blood group maximum Haemagglutination activity was found of 5mg/ml extract. In case of AB^+ blood group no activity was found. From the table it is also seen that increase the activity with the increased concentration thus in case of concentration 2.5mg/ml moderate activity found

Table 6.10 Haemagglutination test for methanolic extract of *Musa sapientum* (leaf)

Blood group	Banana leaves		
	K1	**K2**	**K3**
	5mg/ml	**2.5mg/ml**	**1.25mg/ml**
A+	+++	+	+
B+	+++	++	+
O+	++	++	+
AB+	+	+	-

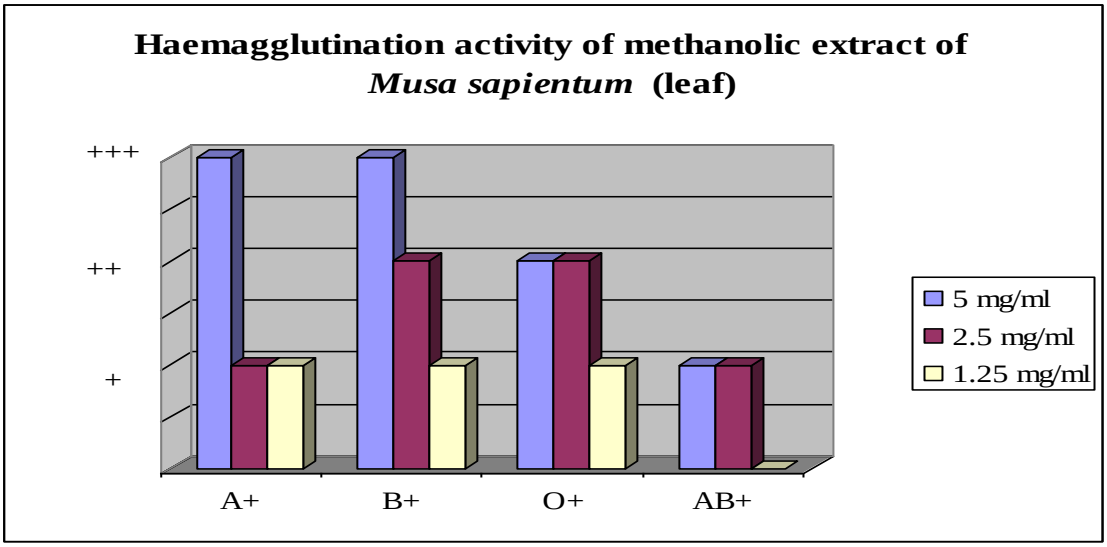


Figure 6.12 : Bar diagram of Haemagglutination test of *Musa sapientum* (leaf)

6.5 Antihaemolytic test for methanolic extract of *Musa sapientum* (leaf)

The crude methanolic extract of *Musa sapientum* (leaf) was subjected to antihaemolytic activity and it showed significant antihaemolytic activity with an IC_{50} value of 3.107mg/ml. The percentage inhibition of haemolysis and the IC_{50} value of crude methanolic extract of *Musa sapientum* (leaf) are given in table 6.11 and figure 6.13.

Table 6.11 Antihaemolytic test for methanolic extract of *Musa sapientum* (leaf)

Sample	concetration	Absorbance	Sample	concetration	Absorbance	% Antihemolytic activity
Musa sapientum (leaf) crude extract	0.3125mg/ml	0.8615	Positive control	0.3125mg/ml	0.896	3.850446
	0.625mg/ml	0.537		0.625mg/ml	0.853	37.04572
	1.25mg/ml	0.3675		1.25mg/ml	0.753	51.19522
	2.5mg/ml	0.275		2.5mg/ml	0.683	59.73646
	5mg/ml	0.2225		5mg/ml	0.629	64.62639

IC50 = 3.107 mg/ml

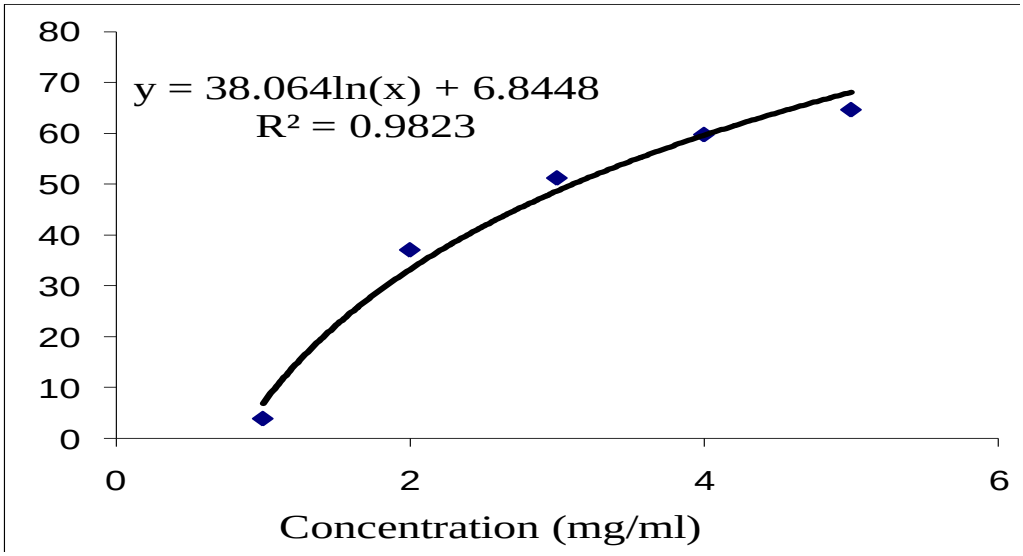


Figure 6.13 : Concetration Vs % Antihemolytic activity

6.6 Sensitivity test

From the table 6.12 it is seen that the crude methanolic extract of *Musa sapientum* (leaf) (200 µg/disc) show sensityvity against *Shigella dysentery*. But the crude methanolic extract of *Musa sapientum* (leaf) (200 µg/disc) show no sensityvity against *Sacharomyces cerevacae* and *Escherichia coli*. Figure 6.14 show the Sensitivity test for methanolic extract of *Musa sapientum* (leaf).

Table 6.12 Sensitivity test for methanolic extract of *Musa sapientum* (leaf)

Sample & concentration	Name of Microorganism	Zone of Inhibition (mm)
Musa sapientum (leaf) crude extract (200 µg/disc)	*Sacharomyces cerevacae*	0
	Escherichia coli	0
	Shigella dysentery	6

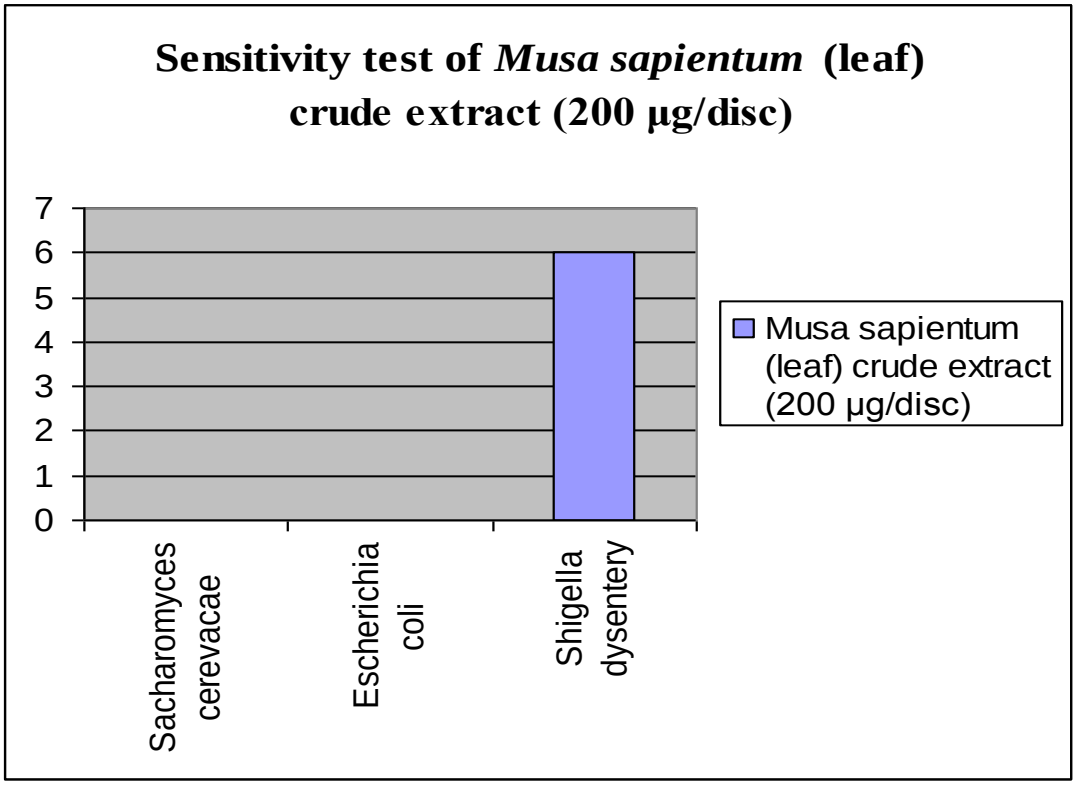


Figure 6.14: Sensitivity test for methanolic extract of *Musa sapientum* (leaf)

6.7 Preliminary phytochemical investigations

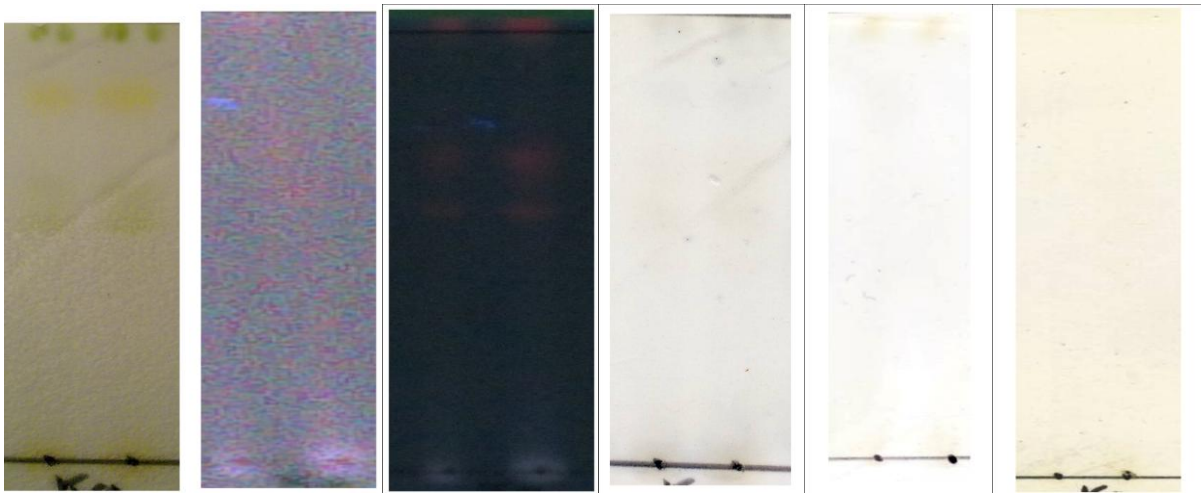
Secondary metabolites are very important for the plant. In this test I searched for whether my plant contains some kind of secondary metabolites like alkaloid, flavanoid, steroid, rducing sugar etc. Simply by following standard methods and by the observation of different color change of the solution the following results were made. From the tests it was concluded that there might be alkaloid, flavanoid, tanin, steroid, cardiac glycoside present in the sample because of test of these compound has given the positive result by showing specific color change or making specific precipitation. In addition, some of the secondary metabolites like anthra-quinone, terpe-noid, reducing sugar, saponin were absent as their test showed negative result.

Table 6.13 Result of preliminary phytochemical investigation of secondary metabolites

Sample name	Phytochemical composition	Result
Musa sapientum (leaf)	Alkaloid	+
	Flavonoid	+
	Anthraquinone	-
	Terpe-noid	-
	Reducing sugar	-
	Saponin	-
	tanin	+
	Steroid	+
	Cardiac Glycolside	+
	Phenol	-
	Volatile oil	-

6.8 Thin Layer Chromatography (TLC)

The results obtained after TLC of the methanolic extract of the *Musa sapientum* (leaf) in solvent system 1 is given below-

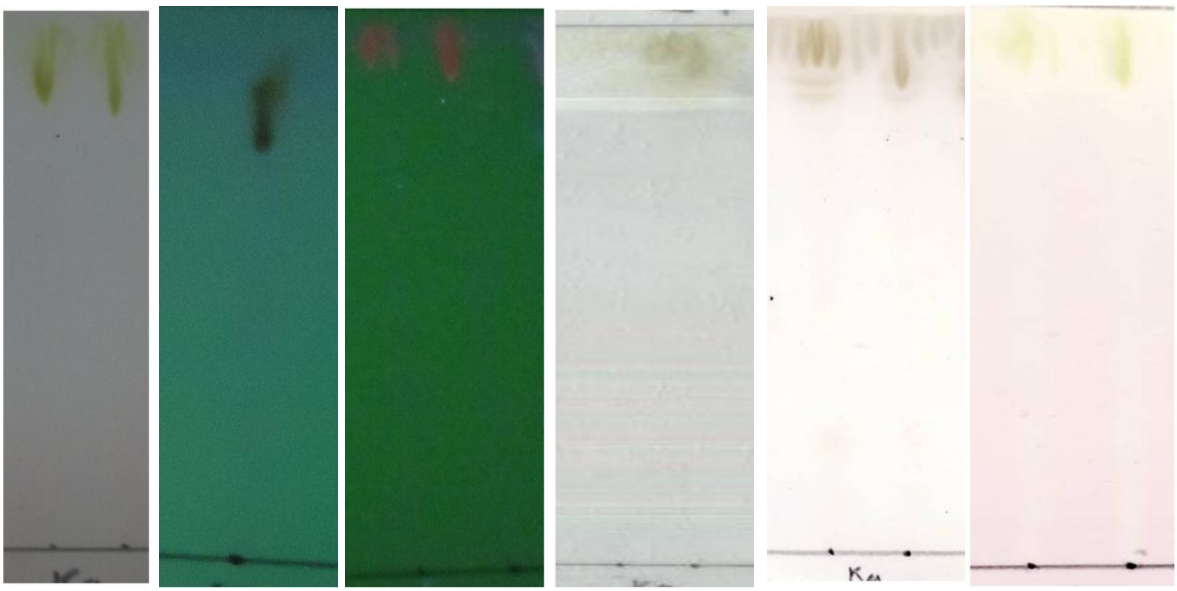


1 2 3 4 5 6

Figure 6.15*:* Results for TLC in nonpolar basic solvent (1= naked eye view; 2 & 3 = UV light view; 4= after application of FC reagent; 5= after charing; 6= after application of DPPH)

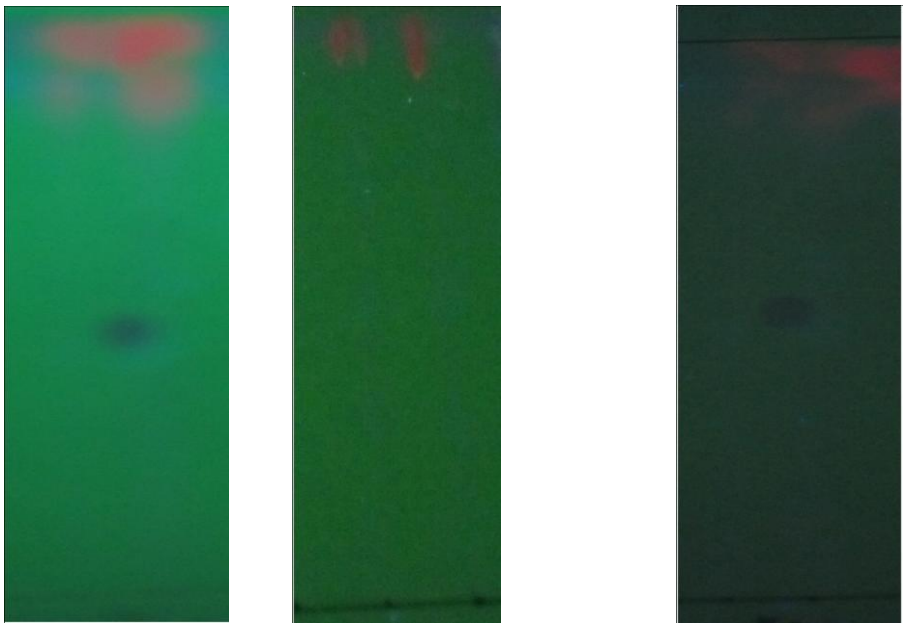
The naked eye view of the TLC was mentioned in the plate 1 which did not show any clear spot. Then the plate was observed under UV which is shown in the plate 2 and 3. It showed some spots which indicate the presence of different compounds in that sample. Folin & ciocalteu solution was used to determine the presence of the phenolic compouns in the sample. The formation of intense violet color after application of the Folin & ciocalteu solution in the sample indicates the presence of phenolic compound in the sample. Spraying of the Folin & ciocalteu solution in the TLC plate showed moderate violet color which indicates the presence of phenolic compound in that fraction (plate 4).

After charing of the TLC plate with sulfuric acid has showed (plate 5) two spots at the bottom as well as at the top of the TLC plate. Spraying of DPPH solution on the TLC plate did not show any significant color changes which indicate the less free radical scavenging property of that fraction



1 2 3 4 5 6

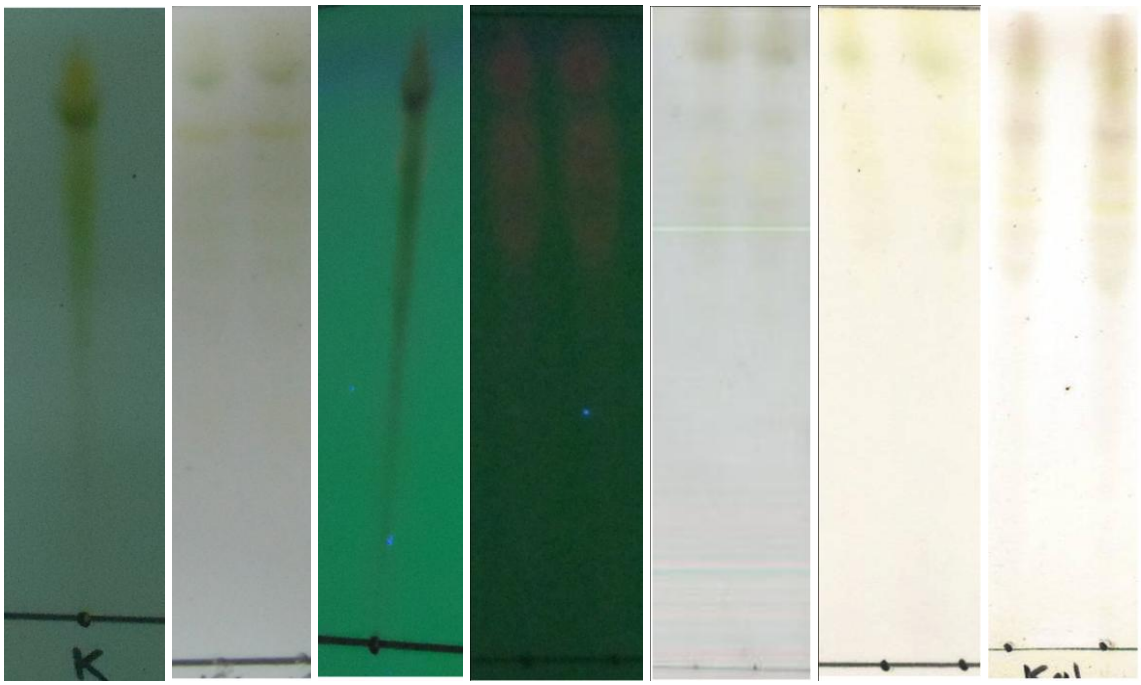
Figure 6.16: Results for TLC in intermediate polar basic solvent (1= naked eye view; 2 & 3 = UV light view; 4= FC reagent view; 5= after charing view 6= after application of DPPH solution



1 2 3

Figure 6.17*:* Before charing (1 and 2) and after charing (3) with $ALCl_3$

From the figure 6.16 we see that the naked eye view of the TLC is mentioned in the plate 1 showed two clear spots at the top of the plate. Then the plate was observed under UV which is shown in the plate 2 and 3. It showed some additional spots which indicate the presence of different compounds in that sample. Folin & ciocalteu solution was used to determine the presence of the phenolic compouns in the sample. The formation of intense violet color after application of the Folin & ciocalteu solution in the sample indicates the presence of phenolic compound in the sample. Spraying of the Folin&ciocalteu solution in the TLC plate shows formation of intense violet color which indicates the presence of phenolic compound in that fraction (plate 4). After charing of the TLC plate with sulfuric acid (plate 5) has revealed many spots in the TLC plate. Spraying of DPPH solution on the TLC plate have shown significant formation of pale yellow color (plate 6) in the place of the spots which indicates significant free radical scavenging property of that fraction.



1 2 3 4 5 6 7

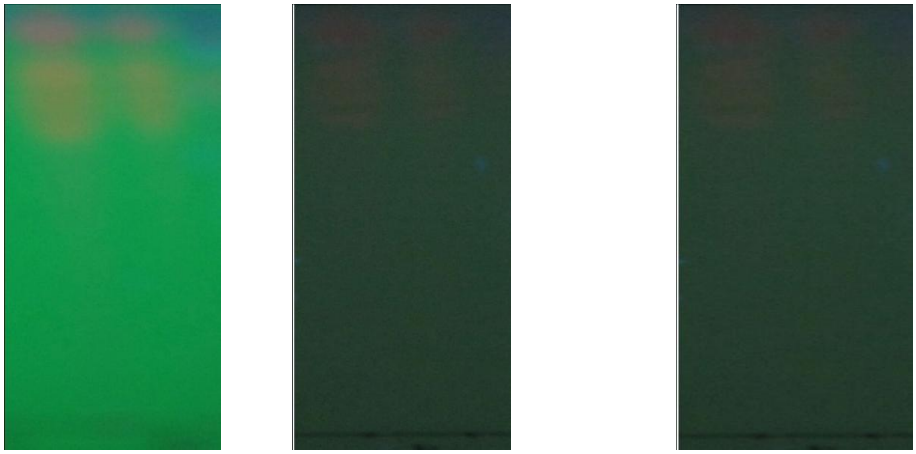
Figure 6.18 Results for TLC in polar basic solvent (1 & 2= naked eye view; 3 & 4 = UV light view; 5= after application of FC solution), 6= after application of DPPH solution; 7= after charing;

From the figure 6.18 we see that the naked eye view of the TLC was mentioned in the plate 1 which showed clear spot. Then the plate was observed under UV which is shown in the plate 3 and 4. It showed some spots which indicate the presence of different compounds in that sample. After charing of the TLC plate with sulfuric acid (plate 7) has revealed many spots in the TLC plate.

Spraying of DPPH solution on the TLC plate did not show significant formation of pale yellow color (plate 6) in the place of the spots which indicates less significant free radical scavenging property of that fraction.

Folin & ciocalteu solution was used to determine the presence of the phenolic compouns in the sample. The formation of intense violet color after application of the Folin & ciocalteu

solution in the sample indicates the presence of phenolic compound in the sample. Spraying of the Folin&ciocalteu solution in the TLC plate shows moderate violet color which indicates the presence of phenolic compound in that fraction (plate 5)



1 2 3

Figure 6.5*:* Before charing (1 and 2) and after charing (3) with $ALCl_3$

Discussion

Discussion

The crude methanolic extract of *Musa sapientum* produced strong activity against a number of the test organisms. The results are given in table 6.1. The zones of inhibitions produced by the crude methanolic extract of the fruit of *Musa sapientum* (leaf) were ranged from 6-11 mm at a concentration of 40µg/disc. While the zones of inhibition produced by the crude methanolic extract of the fruit of *Musa sapientum* (leaf) were ranged from 7-17 mm at a concentration of 400µg/disc. The methanolic extract showed greatest activity against *Staphylococcus aureus* having the zone of inhibition of 11 mm (40µg/disc) and 16 mm (400µg/disc). Besides the extract showed strong activity against *Shigella dysentery* (7mm for 2mg/disc and 16 mm for 20mg/disc), *Salmonella typhi* (7 mm for 40µg/disc and 16 mm for 400µg/disc), *Vibrio mimicus* (7 mm for 40µg/disc and 17 mm for 400µg/disc). Moderate activity was found against *Bacillus cereus* (6 mm for 40µg/disc and 7 mm for 400µg/disc). Among the tested Gram positive bacteria, the extract showed greatest activity against *Staphylococcus aureus* having the zone of inhibition of 11 mm (40µg/disc) and 16 mm (400µg/disc). Among the tested Gram negative bacteria, the extract showed strong activity against *Shigella dysentery* (7mm for 40µg/disc and 16 mm for 400µg/disc), *Salmonella typhi* (7 mm for 40µg/disc and 16 mm for 400µg/disc) and, *Vibrio mimicus* (7 mm for 40µg/disc and 17 mm for 400µg/disc). In brief, the mehanolic extract of *Spondias pinnata* fruit showed a potent inhibitory activity against *Staphylococcus aureus.*

The minimum inhibitory concentration (MIC) of the crude extract was determined for *Vibrio mimicus* against which the crude methanolic extract of *Musa sapientum* (leaf) showed promising and potent antimicrobial activity in the preliminary in vitro antimicrobial screening test.

The phenolic content found in the crude methanolic extract of *Musa sapientum* (leaf) was 8.564±0.716 mg of gallic acid (GAE) per gm of dried extract. Absorbance Values are expressed as average ± SD (n=4). The crude methanolic extract of *Musa sapientum* (leaf) has not good antioxidant property.

The percentage inhibition of free radical DPPH and the IC_{50} value of crude methanolic extract of *Musa sapientum* (leaf) are given in table 6.5 and figure 6.6. Absorbance Values are expressed as average ± SD (n=3). The crude methanolic extract of *Musa sapientum* (leaf) has not good antioxidant property.

The highest % increase in reducing power was observed for 84 mg/ml concentration of crude methanolic extract and it was 63.433%. The EC_{50} value of the extract was found to be 42.52 mg/ml for crude methanolic extract of *Musa sapientum* (leaf).

The crude methanolic extract of *Musa sapientum* (leaf) was subjected to free radical scavenging activity and it showed significant free radical scavenging activity with an IC_{50} value of 311.06µg/ml.

The total flavonoids content found in the crude methanolic extract of *Musa sapientum* (leaf) was 1431±65.57 mg of rutin per gm of dried extract. Absorbance Values are expressed as average ± SD (n=3).

From the haemagglutination test it is seen that in case of A^+, B^+ blood group maximum haemagglutination activity was found of 5mg/ml extract. In case of AB^+ blood group no activity was found. It is also seen that increase the activity with the increased concentration thus in case of concentration 2.5mg/ml moderate activity found

The crude methanolic extract of *Musa sapientum* (leaf) was subjected to antihaemolytic activity and it showed significant antihaemolytic activity with an IC_{50} value of 3.107mg/ml.

From the sensitivity test it is seen that the crude methanolic extract of *Musa sapientum* (leaf) (200 µg/disc) show sensityvity against *Shigella dysentery*. But the crude methanolic extract of

Musa sapientum (leaf) (200 μg/disc) show no sensityvity against *Sacharomyces cerevacae* and *Escherichia coli*.

From the phytochemical tests it was concluded that there might be alkaloid, flavanoid, tanin, steroid, cardiac glycoside present in the sample because of test of these compound has given the positive result by showing specific color change or making specific precipitation. In addition, some of the secondary metabolites like anthra-quinone, terpe-noid, reducing sugar, saponin were absent as their test showed negative result.

From TLC it is seen that there have small presence of flavanoid and phenolic compound in the methanolic extract of *musa saientum* (leaf). Various spots are alsoo seen in TLC plate but the spots are not clear.

Conclusion

Conclusion

The crude methanolic extract of *Musa sapientum* showed significant antioxidant, antimicrobial and haemagglutination, some of which supports the traditional use of this plant in various diseases.

The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates. Very few compounds are isolated from the *Musa sapientum* leaf. Therefore there is huge potential to find active principles which could be beneficial for mankind for targeting various diseases.

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