PHARMACOLOGICAL EVALUATION OF BAMUSUA ARUNDINACEA (RETZ.) ROXB SEEDS

A THESIS
Submitted by
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DECLARATION BY THE CANDIDATE

I declare that the thesis entitled “PHARMACOLOGICAL EVALUATION OF BAMBUṢA ARUNDINACEA (RETZ.) ROXB SEEDS” submitted by me for the degree of Doctor of Philosophy is a bonafide record of work carried out by me during the period from May 2012 to April 2016 under the guidance of Dr. S. UMAMAHEŚWARI and has not formed the basis for the award of any degree, diploma, associate-ship, fellowship, titles in this or any other University or other similar institution of higher learning and without any plagiarism.

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ABSTRACT

In recent years, focus on plant research has increased all over the world and evidence show immense potential of medicinal plants used in various traditional systems. Herbal drugs have got tremendous momentum in global health care system. Many plants have been found to have therapeutic potential and are being used since time immemorial. The beneficial therapeutic effect of these medicinal herbs is seen in their continued use and proven scientifically. Bamboo is one of the precious plant resources of the earth. The mention of the traditional use of bamboo in Asian medicine dates back to the 6th century. Popular among the common people as “Poor man’s timber” since ancient times, because of its innumerable applications has attained the status of “Green Gold of forests”. Today bamboo constitutes one of the most important renewable natural resources of India. The use of bamboo is not only restricted as structural and building materials but with the advent of time has become an important ingredient of traditional Asian Medicines in general and Chinese medicine in particular. Bambusa arundinacea (Retz.) Roxb belongs to a family Gramineae (also called Poaceae) a graceful spinous bamboo, distributed throughout the moist parts of India. Several species like rice, wheat, oats, barley and maize belong to this grass family. Bamboos are, however, perennial grasses classified under the sub-family bambusoideae. Traditionally bambusa leaves, stem and root were used as astringent, laxative, diuretics and also it has anti-inflammatory, anti microbial, antifertility, antispasmodic, antidiabetic and antiulcer activity. Leaf buds are used to treat menstrual problems. Roots are used to treat cirrhosis and tumors of liver, spleen and abdomen. Tribal women around Salem in Tamil nadu chew leaves of Bambusa arundinacea in the morning and evening for 1-3 days to induce abortion of an early conception. A decoction of bamboo joints is said to increase the flow of lochia after delivery.

Bambusa arundinacea seeds-An Unexploited part of the Bambusa tree: Bamboo blossom is a natural phenomenon in which the bamboos in a location blossom and become hung with bamboo seeds. In China and India, "bamboo blossom" was traditionally seen as a curse or an indication of a starvation coming. It flowers gregariously once in the life
time. Bamboos usually have a life-cycle of around 40 to 80 years, varying among species. At infrequent intervals for most species, they will start to blossom. After blossom, flowers produce fruit (called "bamboo rice" in parts of India and China). Following this, the bamboo forest dies out. Since a bamboo forest usually grows from a single bamboo, the death of bamboos occurs in a large area. This belief is older than the Indian epic Mahabharata, written 5000 years ago. In the story, the evil king Jayadrath forcibly abducted Draupadi, the Pandavas wife, and trampled through the forest in his chariot. She cursed the king that he would be destroyed, just as bamboos bring forth instant ruin by their blooming. Flowers in large panicles, sometimes occupying the whole culm; caryopsis oblong 5-8mm long, grooved on one side. The kani tribes of kanyakumari district used the seeds as food and they believe that the seeds of Bambusa arundinacea enhance the fertility. However there is lack of information about the use of bamboo seed. Hence, an attempt has been made to explore the indigenous knowledge about the use of this bamboo seed. Genus bambusa comprising of around 120 species, however, only few species have been investigated systematically. The information on the phytochemicals and pharmacology of bambusa arundinacea seed is limited. No such pharmacological and phytochemical data are available on the plant Bambusa arundinacea seeds. So, the main intention of the current study is to investigate the unexploited plant, Bambusa arundinacea for its ethno medical claims as well as pharmacological activities based on chemotaxonomic tracing and invitro experimental models.

The specific objectives of the investigation are: To exploit and evaluate the physiochemistry, pharmacognosy, qualitative and quantitative analysis of phytochemicals and secondary metabolites, vitamins and minerals of Bambusa arundinacea seeds. Further to exploit various extract of Bambusa arundinacea seeds for their antioxidant, antidiabetic, anticaner, anti-inflammatory, antidiabetic, anti-arthritic, anti-microbial, antihelminthic activity (Invitro).

The powdered (100gm) was extracted three times by cold percolation method with 300 ml of Hexane, acetone and hydro ethanol at room temperature for 72 hrs the filtrates were concentrated under reduced pressure at 40ºC and stored in refrigerator at 2-8ºC for use in subsequent experiments. Phytochemical examinations were carried out for the extract as per the standard methods. The amount of total phenol content was determined by
Folin-Ciocalteu reagent method. The amount of total flavonoid content was determined by using HPLC method. The amount of fatty acid were quantified using gas chromatography and estimation of calcium by atomic spectrometry. The Antimicrobial activity was tested against both Gram +ve and Gram -ve micro organism and Antifungal activity using disc diffusion method. The anthelmintic activity was performed according to the standard method. The Anti-Inflammatory was evaluated by invtro method by using standard drug for the invtro, (albumin denaturation technique) method. The Anti-oxidant was evaluated by invtro method by using hydrogen peroxide scavenging method, Nitric oxide radical scavenging activity, DPPH free radical scavenging activity and Reducing assay method. The Antidiabetic was evaluated by invtro method by using α amylase and α glucosidase inhibition activity based on colorimetric method. Anticancer activity of the extracts were evaluated using MTT assay is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product.

In the present investigation preliminary phytochemical screening of the H.ethanol, acetone and hexane extracts of Bambusa arundinacea shows the presence of flavonoids, glycosides, phenol, tannin, coumarins, quinones and phytosteroids. In all these extracts alkaloid, saponins and terpenoids were found to be absent. The analysis of seeds of B. arundinacea has shown its potential significance. It has revealed that B. arundinacea seeds are a rich source of carbohydrate, protein, lipid and fibre. The mineral content showed the presence of calcium, magnesium, zinc, Iron, copper, sodium and potassium. Low content of pb, cd, As and Hg showed that the seed is free from toxic metals. From the present study it can be concluded that, contamination of heavy metals and microorganisms did not observed in the extract of Bambusa arundinacea. Various pharmacognostic standards like botanical description, microscopy, ash values, extractive values, microscopic characteristics of powder, heavy metals, pH, solubility, HPTLC and preliminary phytochemical study of Bambusa arundinacea could be useful for the compilation of a suitable monograph for its proper identification. The hydroethanol extract obtained from Bambusa arundinace exhibit an excellent in-vitro anti-oxidant activity, anti-diabetic, anti-inflammatory, anti cancer, antiarthritis, antibacterial, antifungal and antihelmintic activity. Bambusa arundinacea Retz., by inhibiting α amylase and α glucosidase reduces the digestion of carbohydrates which in turn reduces the blood glucose level. The presence of flavonoids in Bambusa arundinacea Retz., acts as an insulin mimetic to stimulates the
peripheral tissues for increased glucose uptake and regulates the rate limiting enzymes which may be responsible for the antidiabetic activity. In our studies the hydroethanol extracts of Bambusa arundinacea showed moderate and strong antibacterial activity against the pathogen. Tannins were found to be a component of plants that showed antibacterial activity. It could be one of the components responsible for the antibacterial activity. The seeds possess good amount of bioflavonoid compounds such as quercetin and rutin. May be the presence of quercetin and rutin the seed extracts exhibits good anticancer, antioxidant and antiarthritic activity. Both quercetin and rutin are used as medication for various diseases such as cancers, diabetes, heart diseases, arthritis, hemorrhoids, varicosis, micro angiopathy and are ingredients of numerous multivitamin preparations. As the seeds of Bambusa arundinacea possess all the essentials phytoconstituents, amino acids, oils, vitamins and minerals it exhibits great medicinal value. Being non-toxic, having high nutraceutical values when compared with rice and wheat we can develop the seeds of Bambusa arundinacea as an alternative food. However, treatment with plant extracts although may be have some unpredictability in the effectiveness, side effect less alternative, purified plant extracts and their isolated phytoconstituents can be very useful against many diseases.

In conclusion the chemical composition of Bambusa arundinacea shows it can be a potential source of nutraceuticals. They were found to be very good source of Phosphorus, Calcium, Magnesium and Iron. Seeds of Bambusa arundinacea are potential source of, phytochemical, tocopherols, essential fatty acids like oleic acids, linoleic acid and linolenic acid. Evidently, the seed is a rich source of bioactive compounds and may be used to develop value added products and other food applications to enhance the health benefits. The obtained extracts have potent antioxidant, anti diabetic, anticancer, antiarthritic properties and may play an important role in drug development and health supplement. Thus, there is enormous scope for future research and further pharmacological investigation on Bambusa arundinacea seeds.
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<td>ADP</td>
<td>Adenosine diphosphate</td>
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<td>ATCC</td>
<td>American Type Culture Collection</td>
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<td>AP-1</td>
<td>Activator protein-1</td>
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<tr>
<td>BCE</td>
<td>Before current era (= B.C.)</td>
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<td>B.P.</td>
<td>British Pharmacopoeia</td>
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<td>CE</td>
<td>Current era (= A.D.)</td>
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<td>COX</td>
<td>Cyclooxygenase</td>
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<td>ED50</td>
<td>Median effective dose</td>
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<td>ELAM-1</td>
<td>Endothelial leukocyte adhesion molecule-1</td>
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<td>EtOH</td>
<td>Ethanol</td>
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<td>g</td>
<td>Gram</td>
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<td>GC</td>
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<td>GC-MS</td>
<td>Gas chromatography – mass spectrometry</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony-stimulating factor</td>
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<td>HDL</td>
<td>High density lipoprotein HETEs</td>
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<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
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<td>LC-MS</td>
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<td>IP-10</td>
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LD50 : Median lethal dose
LOX : Lipoxygenase
LPS : Lipopolysaccharide
LTC4 : Leukotriene C4
MAP : Mitogen-activated protein kinase
MIC : Minimum inhibitory concentration
MCP-1 : Macrophage (monocyte) chemotactic protein-1
MCSF : Macrophage colony-stimulating factor
mg : Milligram
MIP-1α : Monocyte inflammatory protein-1-alpha
mL : Millilitre
MMPs : Metalloproteinases MS
Nuclear factor kappa B NK cells :
Natural killer cells
NMR : Nuclear magnetic resonance
NO : Nitric oxide
NSAIDs : Non-steroidal anti-inflammatory drugs
ORAC : Oxygen radical absorption capacity
PAF : Platelet-activating factor
PG : Prostaglandin
PGD2 : Prostaglandin D2
PGI2 : Prostaglandin I2 (prostacyclin)
PLA2 : Phospholipase A2
PMNs : Polymorphonuclear neutrophils
q.i.d. : quarter in die (four times a day)
SD : Standard deviation
TFA : Trifluoroacetic acid
t.i.d. : ter in die (three times a day)
TLC : Thin layer chromatography
TNF : Tumour necrosis factor
TNF-α : Tumour necrosis factor-alpha
TxA2 : Thromboxane A2
UV-VIS : Ultraviolet-visible
UVB : Ultraviolet B
VLDL : Very low density lipoprotein
μg : Microgram
μL : Microlitre
μM : Micromolar
CHAPTER 1

INTRODUCTION

1.1. History of Herbal Medicine

Herbal medicine is the oldest form of healthcare known to mankind. It was an integral part of the development of modern civilization. Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago which was evidenced by a burial site of a Neanderthal man uncovered in 1960. Primitive man observed and appreciated the great diversity of plants available to him. Gradually, each tribe added the medicinal power of herbs in their area to its knowledgebase. All cultures have long folkmedicine histories that include the use of numerous plants. Even in ancient cultures, people methodically and scientifically collected information on herbs and developed well-defined herbal pharmacopoeias. Perhaps one of the earliest pharmacopoeias is the De Materia Medica by the Greco-Roman military physician Dioscorides in the 1st century A.D. Later, in the second century of the present era, Galen shaped pharmaceutical practice for centuries to come. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native people. As mute witness to this fact, marshmallow root, hyacinth, and yarrow have been found carefully tucked around the bones of a Stone age man in Iraq (Cox and Balick 1994). These three medicinal herbs continue to be used today. Marshmallow root is a demulcent herb, soothing to inflamed or irritated mucous membranes, such as sore throat or irritated digestive tract. Hyacinth is a diuretic that encourages tissues to give up excess water. Yarrow is a time-honored cold and fever remedy that may once have been used much as aspirin is used today.
The entire Middle East has a rich history of herbal healing. There are
texts surviving from the ancient cultures of Mesopotamia, Egypt, and India
that describe and illustrate the use of many medicinal plant products. The
Ebers Papyrus, the most important of the preserved Egyptian manuscripts, was
written around 1500 B.C. and includes much earlier information. It contains
876 prescriptions made up of more than 500 different substances, including
many herbs. In 2735 B.C., the Chinese emperor Shen Nong wrote an authoritative
treatise on herbs that is still in use today. Traditional Chinese medicine was brought to
Japan via Korea, and Chinese-influenced Korean medicine was adapted by
the Japanese during the reign of Emperor Ingyo. The records of King Hammurabi
of Babylon include instructions for using medicinal plants and prescribed the use of mint
for digestive disorders. Modern research has confirmed that peppermint does indeed
relieve nausea and vomiting by mildly anesthetizing the lining of the stomach. The
principal storehouse of the Muslim materia medica is the text of Jami of IbnBaiar,
which lists more than 2,000 substances; including many plant products. The principal
Ayurvedic book on internal medicine, the Chark Samhita, describes 582 herbs. The
main book on surgery, the Sushruta Samhita, lists some 600 herbal remedies. Most
experts agree that these books are at least 2,000 years old. By the seventeenth century,
the knowledge of herbal medicine was widely disseminated throughout Europe. The
first U.S. Pharmacopoeia was published in 1820. This volume includes an
authoritative listing of herbal drugs, with descriptions of their properties, uses,
dosages, and tests of purity. It was periodically revised and became the legal standard
for medical compounds in 1906. But as Western medicine evolved from an art to a
science in the nineteenth century, information that had at one time been widely
available became the domain of comparatively few. The use of herbs, which had been
mainstream medical practice, began to be considered unscientific or at least
unconventional, and began to fall into relative obscurity (Dhar et al. 2002).

Plants have provided humans with medicines since time immemorial. The oldest
known document concerning medicinal plants and their uses is the Chinese Pen Ts’ao,
which was written 4800 years ago and describes no less than 360 plants, suggesting
that herbal medicine was already at an advanced stage in China at this time. In
Mesopotamia (part of present-day Iraq), 4600-year old clay tablets inscribed with cuneiform characters have been found that contain references to familiar medicinal plants such as myrrh, licorice and the opium poppy. Another famous early document detailing the use of plants as medicines is the Ebers papyrus from Egypt, which was written about 3500 years ago. Much more ancient, albeit less conclusive, evidence suggests that humans might have employed the pharmacological properties of plants much earlier. At the famous burial site in the Shanidar Cave in the northern part of Iraq, a Neanderthal (Homo neanderthalensis) was laid to rest with bunches of flowers about 60,000 years ago. Of the eight plants identified in the grave from preserved pollen, seven are considered medicinal plants today. There is of course no way of knowing with certainty whether they were placed in the grave because of their medicinal properties, to serve the dead man on his final journey, or whether they simply were used for decorative purposes. The more recent discovery of the „Iceman‘ on the Italian-Austrian border in the Alps provides intriguing evidence of early use of medicinal fungi in Europe. This hunter, who had been lying well preserved in the ice for about 5300 years, was found to be in possession of a fungus, the birch polypore (Piptoporus betulinus), which is known to have purgative and antibiotic properties, and which he might well have been using to treat the whipworm infestation of his intestines (Ghanbari et al. 2012). Plants play a key role in sophisticated ancient traditional medical systems such as traditional Chinese medicine and Ayurveda of India, and have also been central in the Greco-Roman medical tradition, which developed into modern biomedicine. Hippocrates (468-377 BCE), used more than 400 plant species for therapeutic purposes, and it was a Roman army surgeon by the name of Dioscorides who wrote the most influential early European manual of medicinal plants, De Materia Medica, in the first century. This comprehensive work included illustrations and descriptions of about 600 plant species, along with text detailing their uses, doses and potential toxic effects (Phillipon and Anderson 1989).

The writings of Galen, who classified herbs according to their humoral properties, had a profound and almost unimaginable impact on medical thought in Europe for about 1500 years. The English apothecary Nicholas Culpeper wrote many
herbal books, the most famous being The English Physician in which he presented herbal medicine in an astrological framework. Although modern biomedicine to a significant degree employs synthetic drugs as therapeutic agents, plants still occupy a prominent place in contemporary pharmacy, either as sources of pharmaceutical drugs in the form of isolated plant compounds, as sources of precursors to drugs, or as sources of compounds that have served as models for synthetic or semisynthetic drugs. It has been estimated that about one-half of all drugs in current use are natural compounds or derivatives thereof (Singh and Singh 2000). It is however important to realise that despite the many advances of biomedicine, the progress afforded residents of first world countries is beyond the reach of the majority of the world's population. For the majority of people, many of whom live in miserable poverty, crude plants preparations are still the main form of medicine. In acknowledgment of this situation, the World Health Organization (WHO) is actively promoting the development of traditional medicine (WHO 2002).

1.2. Medicinal Plants: Indian Wealth and Heritage

India is a varietal emporium of medicinal plants and is one of the richest countries in the world as regards to genetic resources of medicinal plants. All known types of agroclimatic, ecologic and edaphic conditions are met within India. The biogeographic position of India is unique which makes India rich in all the three levels of biodiversity such as species diversity, genetic diversity and habitat diversity (Natarajan et al. 2005). A survey conducted by the All India Coordinated Research Project on Ethnobiology during the last decade recorded over 8000 species of wild plants used by the tribals and other traditional communities in India for treating various health problems. The Indian subcontinent, with the history of one of the oldest civilizations, harbors many traditional health care systems. One of the ancient classics, -Charak Samhita- is the oldest text available on the complete treatment of diseases which specifies the use of hundreds of herbs in the complete treatment of diseases (Dahanukar and Thatte 2000).

The Ayurveda, whose history goes back to 500 B.C., is one of the ancient health care systems, which is a potential source of indigenous drugs. A large number
of such herbs are mentioned in -Bhavprakash- as well as "Aryavaidhya Kalanidhil. -Indian Materia Medical also gives a large number of medicinal plants for the treatment of various diseases. In rural areas, 75 percent of the population is dependent on herbal medicines for healthcare. In the last few decades, herbal medicine has been found to have some impressive credentials. In India, over 2600 plant species have been considered useful in the traditional system of medicine like Ayurveda, Unani, Siddha and Home remedies. Number of herbal drugs and their compositions are recommended for combating human ailments in the ancient texts as well as in modern medicine. The Mankind's discovery of antibiotics ushered in a new age of medicine during the 19 century, an age wherein many predicted an end to diseases that had plagued themankind for centuries with the appearance of penicillin during World War II as the first miracle drug. From 1940s to almost 1980s many classes of antibiotics discovered have helped tame many of the terrors of human health. The use of these "wonder drugs", combined with improvements in sanitation, housing, nutrition, and the advent of widespread immunization programmes, led to a dramatic drop in deaths from diseases that were previously widespread, untreatable, and frequently fatal. Over the years, antimicrobials have saved the lives and eased the suffering of millions of people. Advances in synthetic chemistry for identification of many key chemical molecules offered more opportunities to develop novel compounds. Numerous drugs like sulphonamides, isoniazid, anti-psychotics, anti-histamines and penicillin were developed from thousands of chemicals. Emergence of modern pharmaceutical industry is an outcome of all these different activities that developed potent single molecules with highly selective activity for a wide variety of ailments. These successes resulted in reduced interest in natural products drug discovery. Thus, herbal medicines became the domain of 'old wives tales'. It was not until the 1970s that antibiotic resistance was considered to be a real threat (Gesler 1992).

In the past, medicine and science were able to stay ahead of this natural phenomenon through the discovery of potent new classes of antimicrobials, a process that flourished from 1930-1970 and has since slowed to a virtual standstill, partly because of misplaced confidence that infectious diseases had been conquered, at least in the industrialized world. In just the past few decades, the development of resistant
microbes has been greatly accelerated by several concurrent trends like urbanization, pollution, AIDS epidemic, etc (Napolean et al. 2009). These have worked to increase the number of infections and thus expand both the need for antimicrobials and the opportunities for their misuse. Recently, infections have become the leading cause of death world-wide which has led to an increase in antibacterial resistance, making it a global growing-problem. More and more bacteria are developing a resistance to antibiotics conferred by randomly mutated genes. Each year infectious diseases cause 14 million deaths worldwide, with mortality increasing even in the United States at an annual rate of 4.8 percent. In 2000, the World Health Organization (WHO) estimated that pneumonia, diarrhoeal disease, and tuberculosis accounted for more than half the deaths due to infectious disease worldwide. The problem is worsened by antibiotic resistance, as well as the emergence of new pathogens with the potential for rapid global spread. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. The alarming incidence of antibiotic resistance forced scientists for developing new and effective therapeutic agents with new principles from botanical medicine with novel modes of action that render them impervious to existing resistance mechanisms. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action. Contrary to the synthetic drugs, antimicrobials of plant origin are affordable and are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. The study of natural products has advantages over synthetic drug design as it leads optimally to materials having new structural features with novel biological activity. Not only do higher plants continue to serve as important source of new drugs, but phytochemicals derived from them are also extremely useful as lead structures for synthetic modification and optimization of bioactivity. The starting materials for about one-half of the medicines used today come from natural sources. Virtually every pharmacological class of drugs includes a natural product prototype. Undisputedly, the history of herbal medicine is inextricably intertwined with that of modern medicine (Farooq et al. 2007). Many drugs listed as conventional medications were originally derived from plants. Salicin, a precursor of aspirin, was originally derived from Salix alba (white willow bark) and flowers of Filipendula ulmaria
(meadowsweet plant) are used as analgesic. Digoxin derived from Digitalis lantana (foxglove) is included in a drug class cardiotonic. Cinchona ledgeriana bark is the source of malaria-fighting quinine. Vincristine, used to treat certain types of cancer, comes from Catharanthus roseus. Ephedrine, a bronchodilator used to decrease respiratory congestion, comes from Ephedra sinica. The Papaver somniferum (opium poppy) yields morphine, codeine, and paregoric, a treatment for diarrhoea. Laudanum, a tincture of the opium poppy, was the favored tranquilizer in Victorian times. The future of higher plants is very promising as they can serve as best sources of medicinal agents in the prevention and treatment of diseases (Lee 2004).

1.2.1. Revival of Traditional Medicine

The history of interest in phytochemicals reveals that crude drugs were the dominant therapy until the time of World War II. During the late 19th century, Western medicine began to supersede the folk and learned medicine that had been gathered and traded between cultures since the time of the Ancient Egyptians and moved away from any interest in folk knowledge of medicinal plants (Duru and Onyedineke 2010). Today, pendulum is swinging back to an interest in the value of traditional medicine. There is currently a rising recognition of the value of experience and historical knowledge gathered by indigenous cultures with medicinal plants. Medicinal plants can save lives, livelihood and cultures. Herbal medicines are an important part of the culture and traditions worldwide. It is therefore no surprise that medicinal plants have raised their importance all over the globe. Recently, the renewed interest in medicinal plants as a re-emergent health aid has been fuelled by the extensive antimicrobial resistance along with rising costs of prescription drugs in the maintenance of personal health and well-being and the bioprospecting of new plant-derived drugs. The revival of interest in herbal medicines is firstly due to increased awareness of the limited horizon of synthetic pharmaceutical products to control major diseases and secondly due to the current widespread belief that 'green medicine' is safe and more accessible and affordable than the costly synthetic drug many of which have adverse side effects. The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products especially from developed countries. According to a WHO estimate, about 80% of the world population relies on traditional systems of
medicines for primary health care, where plants form the dominant component over other natural resources (Zhou 1997)

Today, the renewed interest in traditional pharmacopoeias reveals that researchers are concerned not only with determining the scientific rationale for the plant's usage, but also with the discovery of novel compounds of pharmaceutical value. Instead of relying on trial and error, as in random screening procedures, traditional knowledge helps scientists to target plants that may be medicinally useful (Natarajan et al. 2003). The burgeoning interest in medicinal plants reflect recognition of the validity of many of the traditional claims for the value of natural products in health care. Many medicinal plants exert specific medicinal actions and may be used in response to specific health problems over short or long term intervals. It is estimated that total of 122 drugs from 94 plant species have been discovered through ethnobotanical leads. Now-a-days, many scientists have isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in laboratories for use in pharmaceutical preparations.

1.2.2. Herbal Drug Research Today

The goal of herbal drug research and development program is to discover single entity and multicomponent bioactive natural products that may serve as leads for the development of new pharmaceuticals which address unmet therapeutic needs. Traditional knowledge-driven drug discovery will serve as a powerful search engine and most importantly, will greatly facilitate the focused and safe natural products research to rediscover the drug discovery process (Kelble 2006). There are over 750,000 plants on earth. Relatively speaking, only a very few of the healing herbs have been studied scientifically. Of these, only about 6% have been screened for biologic activity, and a reported 15% have been evaluated phytochemically. Recently, all over the world, there is an upsurge and interest among scientific institutions, biological research institutions in the use of medicinal plants, crude extracts or active ingredients to treat various ailments. Almost all of the current research validating herbal medicine has been done in Germany, Japan, China, India, Taiwan, and Russia.
Most of the research that is done on plants continues to focus on identifying and isolating active ingredients, rather than studying the medicinal properties of whole plants. Herbalists, however, consider that the power of a plant lies in the interaction of all its ingredients. Plants used as medicines offer synergistic interactions between ingredients both known and unknown (Ahmed et al. 1998). Today, rather than using a whole plant, pharmacologists identify, extract, isolate and synthesize individual components to capture the active properties. In addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavonoids, and other substances that are important in supporting a particular herb's medicinal properties. These elements also provide an important natural safeguard. Furthermore, an increasing reliance on the use of herbal products in the industrialized societies has led to the extraction and development of several drugs and chemotherapeutics from plants (Aliero and Afolayan 2005). Some of the wild plants, traditionally used as herbal remedies by ayurvedic practitioners, secured an important place in modern medicine. Nevertheless, many plants, which have curative properties, are yet to be seriously screened for their medicinal value. In an extensive screening programme of plants used in traditional medicine, researchers provide scientific evidence for their rational use in treating infections and diseases (Galanakis 2011).

1.2.3. A Global Trend in Herbal Drug Market

Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs available in markets contain at least one active ingredient derived from plant material. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. WHO also notes that of 122 plant-derived pharmaceutical medicines, about 80% are used in modern medicine that correlated directly with their traditional uses as plant medicines by native cultures. The herbal drug industry is considered to be a high growth industry of the late 90s and seeing the growing demand, it is all set to flourish in the next century. The trend for the increasing popularity of medicinal herbs in developing countries is well supported by statistical data. The world market for plant-derived chemicals alone exceeds several billion dollars per year. Trade in medicinal plants is growing in volume and in
exports. It is estimated that the global trade in medicinal plants is US$ 800 million per year. The botanical market, inclusive of herbs and medicinal plants, in the USA, is estimated, at approximately US$ 1.6 billion p.a. China with exports of over 120,000 tonnes p.a., and India with some 32,000 tonnes p.a. dominate the international markets. The annual export of medicinal plants from India is valued at Rs. 1200 million. It is estimated that Europe, annually, imports about 400,000 tonnes of medicinal plants with an average market value of US$ 1 billion from Africa and Asia. A growing awareness of this new contributor to the foreign-exchange reserves of several national treasuries is beginning to emerge. To satisfy growing market demands, surveys are being conducted to unearth new plant sources of herbal remedies and medicines. Based on current research and financial investments into medicinal plants, it seems that they will continue to play important roles in human health (Slavin et al. 2000).

1.2.4. Approaches to Herbal Drug Discovery

The plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Folk medicine is one of the sources to discover new antimicrobial compounds and systematics screening of them may result in the discovery of novel effective compounds. The quest for plants with medicinal properties continues to receive attention as scientists survey plants for a complete range of biological activities, which range from antibiotics to antitumor. In the light of evidence for the rapid global spread of resistant clinical isolates and the appearance of drug resistant strains among community acquired infections as well as appearance of undesirable side effects of certain antibiotics the need for discovery or development of new antimicrobial agents is of paramount importance. For this reason the search for new antimicrobial drugs became an important alternative. Higher plants have been proved to be a potential source for new antimicrobial agents. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information may be of value for the synthesis of complex chemical substances. There are many approaches to the search for new biologically active principles in
higher plants. Several reviews pertaining to approaches for selecting plants as candidates for drug discovery programs have been published. Different approaches include (i) Random selection followed by chemical screening, (ii) Random selection followed by one or more biologic assays, (iii) Follow-up of biologic activity reports,(iv) Follow-up of ethnomedical uses of plants, (v) Plants used in organized traditional medical systems and (vi) Use of databases. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers. Random collection is to collect readily available plant, prepare extracts, and test each extract for one or more types of pharmacological activity. This, broad screening method is a reasonable approach that eventually should produce useful drugs. Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extraction method and can be followed by various organic extraction methods. Since nearly all of the identified components from plants which are active against microorganisms, are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction (Sharma 1996).

The research on the medicinal plants should be extended with the identification of the active principles in the plants. Scientific examination of the remedies could lead to standardization and quality control of the products to ensure their safety. It is after such evaluations that they can be approved for use in the primary health care. Such research activities could also lead to the development of new drugs. With the development of various analytical methods of high precision, and advances in molecular biology and genetic engineering, it is now possible to isolate compounds in extremely small quantities, study their chemical structure and therapeutic potentialities and then to alter the molecule to be suitable for production of novel and more selective new therapeutic agent 1996 (Balick and Cox 1996). A number of active constituents have been isolated from plants like Azardirachta indica, Senna alata, Terminalia bellerica. Major pharmaceutical companies are currently conducting extensive research on plant materials and encourage large scale pharmacological screening of herbs. Natural products to be used in pharmaceutical preparations are either pure compounds or extracts. The screening of plant extracts
has been of great interest to scientist for the discovery of new drugs effective in the
treatment of several diseases. Extraction of bioactive compounds from medicinal
plants permits the demonstration of their physiological activity and facilitates
pharmacology studies leading to synthesis of a more potent drug with reduced
toxicity. Many reports concerning the antibacterial screening of plant extracts of
medicinal plants have appeared in the literature (Turker and Usta 2008).

1.2.5. Phytomedicinal Actions

The beneficial medicinal effects of phytomedicines typically result from
synergistic actions of secondary products present in the plants. Plant secondary
products have a defensive role against pathogen attack, an attractant role towards
pollinators, protective actions to abiotic stresses such as temperature, water status,
light levels, UV exposure and mineral nutrients and its role at cellular level as plant
growth regulators, modulators of gene expression, and in signal transduction have
also been shown. In contrast to synthetic pharmaceuticals based upon single
chemicals, many phytomedicines exert their beneficial effects through the additive or
synergistic action of several chemical compounds acting at single or multiple target
sites associated with a physiological process (Barbour et al. 2004). This synergistic or
additive pharmacological effect can be beneficial by eliminating the problematic side
effects associated with the predominance of a single xenobiotic compound in the
body. In the role of secondary products as defence chemicals, amixture of chemicals
having additive or synergistic effects at multiple target sites would not only ensure
effectiveness against a wide range of herbivores or pathogens but would also decrease
the chances of these organisms developing resistance or adaptive responses (Vaidya
1982).

1.2.6. Phytomedicine: A Strategy for Future

For future drug development in the 21st century, research should focus not only
on bioactive principles (lead compounds or leads), but also on active fractions and
active formulations from medicinal herbs. In vitro screening programmes, using
Ethnobotanical approach, are important in validating the traditional use of herbal
remedies and for providing leads for newer drugs. The activity identified by an in
vitro test provides a basic understanding of a plant’s efficacy but it does not necessarily confirm that a plant extract is an ineffective medicine or a suitable candidate for drug development (Bradford 2001). Certainly this is a primary concern of ethnopharmacological research in developing new lead compounds. Scientific validation of use of herbal medicine lends support to the continued practice of traditional medicine. Eventually this may lead to more widespread use of traditional medicine in health care system, as in India and China provided thorough toxicological investigations are carried out. In recent years, focus on plant research has increased all over the world and evidence show immense potential of medicinal plants used in various traditional systems. Herbal drugs have got tremendous momentum in global health care system. Many plants have been found to have therapeutic potential and are being used since time immemorial.

1.3. Ethnobotany and Ethnopharmacology

The term ethnobotany lacks a singular, uniformly agreed definition. The term was coined by J. W. Harshberger, who defined it as the use of plants by aboriginal peoples (Clark 1996). Since then, ethnobotany has been redefined and reinterpreted by many scholars in the area. One of the broadest definitions of ethnobotany is that provided by Martin, who described it as the subdiscipline of ethnobotany that is concerned with local people’s interaction with plants. Early ethnobotany was focused on plants of economic significance or potential, while contemporary ethnobotany tends to have a far broader scope and include, for example, traditional agricultural knowledge and traditional vegetation management. Throughout the history of formal ethnobotany, medicinal plants have been an area of keen interest to many ethnobotanists (Chavalittumrong et al. 2004).

Ethnopharmacology is a multidisciplinary field devoted to the study of pharmacologically active agents traditionally used by humans. The term ethnopharmacology was coined as recently as 1967 by Efron, who used the term in the context of hallucinogenic substances. More recently, ethnopharmacology has been defined as the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man. Ethnopharmacology applies
conventional chemical and pharmacological analysis to traditional medicines and in doing so differs from two related disciplines: medical anthropology, which examines health and disease from a cultural perspective, and medical ethnobotany, which is concerned with the use of plants within traditional medical systems. Although ethnopharmacology is not exclusively concerned with plants or plant products, the plant kingdom is the major focus of the discipline, because this kingdom has provided humans with the greatest number of pharmacologically active substances throughout history. The multidisciplinary nature of ethnopharmacology is evidenced by the important roles played by fields such as botany, pharmacognosy, natural product chemistry, pharmacology, toxicology, anthropology and others (Dall Acqua et al. 2008).

Heinrich and Gibbons have noted the differences between ethnopharmacology and bioprospecting, while acknowledging that the two approaches are not mutually exclusive. In brief, ethnopharmacology aims to develop (through increased knowledge and understanding) the use of crude plant preparations in local communities, whereas the goal of bioprospecting is the identification and development of compounds from nature as pharmaceutical drugs in the international market place (Davis 1994). Although the aims of these two approaches to natural products research are vastly different from a socio-economic viewpoint, many of the methodologies will often be the same, and work focussed on one approach might yield results that are relevant to the other. For example, phytochemical and pharmacological investigations of a traditional medicine might lead to the identification of a compound that can be developed into a pharmaceutical drug. Well known examples of this include ephedrine (from Ephedra spp.), atropine (from Atropa bella-donna and other Solanaceae) and more recently the development of the anti-malarial drug artemether from the lead compound artemisinin in the traditional Chinese herb Artemisia annua (Germano et al. 2006).

Different approaches can be employed in the process of bioprospecting. Cotton outlined three main approaches to the collection of plants for screening: the random method, where every species in a given area is included; phylogenetic
targeting, where a particular taxon (such as a family) is targeted, because it is already known to be good source of pharmacologically active metabolites; and the ethno-directed sampling, which is guided by traditional plant use. The latter approach is based on the notion that initial screening and selection has already been conducted effectively by the owners of the traditional knowledge. The ethno-directed approach to identifying plants with biological activity has been shown in a number of studies to be more efficient than the random method at identifying plants with promising pharmacological activity. Such studies include one aimed at identifying plants with anti-HIV activity from Central America and another that showed that plants used ethnomedically to treat viral infections were more than 100 times more likely to yield compounds with anti-viral activity than randomly collected plants. However, it has been argued that the successful development of drugs from traditional medicines is most likely for conditions such as inflammation, gastrointestinal or nervous system disorders, because these pathologies are widely recognised and treated in indigenous systems of medicine (Dudhatra et al. 2010).

1.3.1. Ethnopharmacology and phytotherapy

The term phytotherapy is used to describe the use of plant-based, chemically complex therapeutic agents in contemporary, mostly industrialised societies. Phytotherapy is usually based on a history of traditional use, but it differs from traditional indigenous herbal medicine by employing industrialised extraction and manufacturing methods and by being cosmopolitan in scope. Hence phytomedicines made from plants from around the globe are available in most industrialised countries. Ethnopharmacology has the potential to increase our knowledge and understanding of traditional herbal medicines, how they work, how they are best prepared, and how they can be applied in a safe and efficacious manner (Bhavani and Ballow 2000). Due to the chemical complexity of both traditional herbal medicines and modern phytomedicines, the task of elucidating their pharmacology is a complex one indeed. A full understanding of how complex mixtures of plant compounds interact with the human body and with each other is probably not achievable, and pharmacological investigations of plant extract almost always focus on one or a few 'active' constituents, i.e. compounds with profound biological activity
(Adedayo et al. 2001). It should always be borne in mind that many other compounds present in a plant extract could potentially play a role in the overall activity of that extract, for example by modulating the pharmacokinetic and/or pharmacodynamic properties of the _actives_. Despite this caveat, ethnopharmacological investigations clearly have much to offer modern phytotherapy, and the long-term success of the _herbal renaissance_ currently experienced in most of the industrialised world undoubtedly depends on the scientific underpinning of traditional or anecdotal uses (Akanmu et al. 2005).

### 1.4. Introduction to Bambusa arundinacea

Bamboo is one of the precious plant resources of the earth. It has play a significant role in human civilization since ancient times, and is still contributing to the subsistence of over two billion people living in tropical and subtropical belts in Asia, Latin America and Africa. Bambusa arundinacea(Retz.)Roxb belongs to a family poaceae, a graceful spinous bamboo, distributed throughout the moist parts of India, up to an altitude of 1,250m, particularly near river banks; native to south-east Asia, also cultivated in the plains of North-West India, and on the hills of Andhra Pradesh,Tamil Nadu and Karnataka. Thorny tree, stems many, tufted on a stout rootstock, grows upto 30 meter high; culms 15-18 cm across; nodes prominent, the lower emitting horizontal almost naked shoots armed at the nodes with 2-3 stout recurved spines; internodes upto 45 cm. long. Leaves    linear or linear-lanceolate, tip stiff, glabrous or puberulous beneath, margins scabrous, base ciliate, mid-rib narrow, leaf-sheath ending on a thick callus and shortly briskly auricle. It flowers gregariously once in 30-40 years. Rhizomes short, stout, knotty; culms dense, reaching 24-30m in height and 15-17cm in diameter. Flowers in large panicles, sometimes occupying the whole culm; caryopsis oblong 5-8mm long, grooved on one side. A bamboo culm consists of internodes (which is hollow for most bamboo) and a node, which is solid and provides structural integrity for the plant (Dhar 1988).

The plant is considered as one of the world's most useful trees, as almost every part of the Bamboo tree can be used for medication, food and industrial purposes. Bamboo is used in Chinese medicine for treating infections and healing. It is a low-
calorie source of potassium. It is known for its sweet taste and as a good source of nutrients and protein. When bamboo is heated at very high temperature in an airless vessel, it becomes charcoal, which is used like other charcoal products, as a fuel component, a deodorizer, or an absorbent. The vapor that comes off the heated bamboo can be condensed to produce a liquid known as bamboo vinegar. Bamboo vinegar has been produced in Japan for many years and is used medicinally to treat eczema, atopic dermatitis, and other skin diseases; it is most commonly applied by adding to bath water. Bamboo vinegar is recognized as an anti-inflammatory and anti-fungal. Pickled bamboo, used as a condiment, may also be made from the pith of the young shoot (Devi et al. 2014).
Figure 1.1. Bambusa Tree  
Figure 1.2. Bambusa stem  

Figure 1.3. Bambusa Inflorescence  
Figure 1.4. Mature Inflorescence  

Figure 1.5. Dried Inflorescence  
Figure 1.6. Bambusa Seeds
1.4.1. Classifications

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Liliatae
Order: Cyperales
Family: Poaceae
Bambusa arundinacea (Retz.) Willd.
Scientific Name : Bambusa arundinacea (Retz.) Willd.

Synonym : Bambos arundinacea Retz.
Synonym : Arundo bambos L.
Synonym : Bambusa bambos (L.) Voss
Synonym : Nastus arundinaceus

Common Names:
Assamese   : Mokal Baansh, Jaati Baansh
Bengali    : Baansha
English    : Bamboo
Gujarati   : Baambu, Vaans
Hindi      : Buns, Bambu, Baans
Kannada    : Bidiru, Vamsha
Kashmiri   : Bains, Vanshah, Bons
Konkani    : Vaaso, Vaso, Velu
Malayalam  : Mula, Mula, Illi
Manipuri   : Saneibi
Marathi    : Maanga, Kalaka, Baamboo, Velu, Kalanka
Oriya      : Bans
Sanskrit   : Amupah, Vamsh, Vambhah, Kantakah, Kantakilah, Ardrapatrakah
Tamil      : Peru Varia Mungi, Kulay-munkil, Periya Mungil, Mungil,
Telugu : Vamsamu, Bongu-veduru, Veduru
Others : Giant Thorny Bamboo, Spiny Bamboo, Peria Mungil, Male Baans.

1.4.2. Regional names
   Gujarati (Toncor, Wans, Vanskapur, Vas-nu-mitha); English (Bamboo, Bamboo manna, Giant Thorny Bamboo); Hindi (Bans-lochana, Banskapur, Vanoo, Banz); Bengoli (Bans-Kapur,Baans, Baansh, Baroowa Bans); Sanskrit (Vanshalochana, Venulavanam); Arab (Tabashir); Marthi (Bansa, Baambii, Bansamitha); Tamil(Munga-luppa, Mullumangila, Mulmunkil, Mungil); Telugu (Veduruppu, Mulkas Veduru, Mullu Veduru); Maliyalam (Moleuppa); Kannad (Bidaruppu, Tavakshira); Burma (Vd-chha, Vathega-kiyo, Vasan, Vathe gasu); Unani (Tabashir , Tawashir) (Mahadevan et al. 2003).

1.4.3. Botanical description
   Thorny tree, stems many, tufted on a stout root-stock, grows upto 30 meter high; culms 15-18cm across; nodes prominent, the lower emitting horizontal almost naked shoots armed at the nodes with 2-3 stout recurved spines; internodes upto 45 cm. long. Leaves 17.5 – 20.5 X 2-2.5 cm, linear or linear – lanceolate, tip stiff, glabrous or puberulous beneath, margins scabrous, base ciliolate, mid-rib narrow, leaf-sheath ending on a thick callus and shortly bristly auricle. Inflorescence, enormous panicles often occupying the whole stem. Caryopsis (grain) oblong, 5-8 mm long, grooved on one side. Flowering and Fruiting: Once in life time, often during September – May (Majumder and Banerjee 1985).
Figure 1.7. Descriptive parts of Bambusa
1.4.4. Traditional medicinal uses of Bamboo

The extract of the plant Bambusa aurundinacea Retz. had been used in folk medicines to treat various inflammatory conditions. In Ayurveda, the leaves, the stem and roots are used as astringent, laxative and as diuretic. An ointment from the root is said to be a folk remedy for cirrhosis and hard tumor. Shoot of Bambusa aurundinacea Retz. is used for dislodgelement of worms from ulcer. Leaf bud (decoction) of Bambusa aurundinacea Retz.is used to encourage the free discharge of menses. Leaves are useful in leprosy, hematemesis, fever and haemoptysis. Leaves are also used in cough paralytic complications and in snake bites. An ointment from the Bambusa aurundinacea Retz.root is said to be a folk remedy for cirrhosis and tumors, especially tumor of abdomen, liver, spleen and stomach (Mohan Ram and Gopal 1981).

Tabasheer, a siliceous secretion of Bambusa aurundinacea (up to 97 % SiO2), considered aphrodisiac, cooling, and tonic is used in asthma, cough. Leaves given to horse suffering from coughs and colds. Tribal women around Salem in Tamil nadu chew leaves of Bambusa aurundinacea Retz. in the morning and evening for 1-3 days to induce abortion of an early conception. The extracts of Bambusa aurundinacea have been used in Indian folk medicine to treat various inflammatory conditions (Foster and Cornella 1961). The plant has antiulcer activity also. It is thought that these two properties in the same extract are very useful in the treatment of inflammatory conditions. It is well known fact that the most of the available anti-inflammatory drugs are ulcerogenic. Leaves decoction is used to stimulate menstruation and as an antispasmodic to help relieve menstrual pain, in dysmenorrhoea and amenorrhea. A decoction of bamboo joints is said to increase the flow of lochia after delivery. Bambusa leaf juice is given for strengthening the cartilage in osteoarthritis and osteoporosis. It plays a part in the integrity of the bones, arterial walls, skin, teeth, gums, hair and nails and has been used to alleviate eczema and psoriasis. It is used in diarrhoea, dyspepsia, flatulence and worm problems. Silicates of Bambusa are very useful in creation of the body's structural matrix for forming and repairing connective tissue. An ointment from the root is said to be a folk remedy for cirrhosis and hard tumors, especially tumors of the abdomen, liver, spleen and stomach. Tabasheer, a
siliceous secretion (up to 97% SiO₂), considered aphrodisiac, cooling, and tonic, is used in asthma, cough and debilitating diseases (Sen Gupta 1939).

Seeds of Bambusa arundinacea Willd, by the Kani tribes of Kanyakumari district, southern Western Ghats. Method of seed collection, storage and mode of consumption by indigenous people has been described. The indigenous community not only uses the seeds as a food, but also as commercial commodity to improve the economy. The Kani tribes believe that the seeds of Bambusa arundinacea enhance the fertility, so that there is great demand of seeds of this species in pharmaceutical industry to manufacture drugs to improve fertility (Bhaduri et al. 1968).

![Diagram of Bamboo uses](image)

**Figure 1.8. Uses of Bamboo**

Bamboo leaves have been used in traditional Chinese medicine for treating fever and detoxification for over 1000 years. Recently, some biologically active
components in bamboo leaves and their potential health benefits have been widely studied (Duraipandiyan et al. 2006). An ethanol/water extract of bamboo leaf mainly contains flavone glycosides, phenolic acids, coumarin lactones, anthraquinones and amino acids Many papers have indicated that a flavonoid-rich bamboo leaf extract has multiple biological effects, such as anti-free radical, anti-oxidation, anti-aging, anti-fatigue, anti-bacteria, anti-virus, and prevention of cardiovascular diseases, and can be used as a pharmaceutical intermediate, dietary supplement, cosmetic ingredient, and food additivechemical investigation of this plant, triterpenes and steroidal glycosides were the major phytoconstituents (Jarald et al. 2007).

Bambusa arundinacea has been proven to have great pharmacological potential with a great utility and usage in folklore medicine.Various parts of this plant such as leaf,root,shoot and seed possess anti-inflammatory, anti-ulcer, anti-diabetic, anti oxidant, anthelmintic, astringent, emmenagogue activity. Vamsarocana (bamboo manna) is useful in various disorders/diseases like hyperpiesia, diarrhea, vomiting, heart diseases, cough, asthma, fever, tuberculosis and a general tonic in convalescents. The leaves are cooling, emmenagogue, hence, beneficial in convalescents and dysmenorrheal. The roots are diuretic, tonic, depurative, laxative and cooling and also used in skin diseases, burning sensation, arthralgia, general debility and dysuria. The decoction of the sprouts is beneficial in anorexia, dyspepsia `and worms. Shoot of Bambusa arundinacea is used for dislodgement of worms from ulcer. Leaves given to horse suffering from coughs and colds. Tribal women around Salem in Tamil nadu chew leaves of Bambusa arundinaceain the morning and evening for 1-3 days to induce abortion of an early conception (Kiruba et al. 2007). A decoction of bamboo joints is said to increase the flow of lochia after delivery. Bambusa leaf juice is given for strengthening the cartilage in osteoarthritis and osteoporosis. It plays a part in the integrity of the bones, arterial walls, skin, teeth, gums, hair and nails and has been used to alleviate eczema and psoriasis. It is used in diarrhoea, dyspepsia, flatulence and worm problems (Projan and Shales 2004). Silicates of Bambusaare very useful in creation of the body’s structural matrix for forming and repairing connective tissue. The kani tribes of kanyakumari district used the seeds as food and they believe that the seeds of Bambusa arundinacea

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enhance the fertility. However there is lack of information about the use of bamboo seed. Hence, an attempt has been made to explore the indigenous knowledge about the use of this bamboo seed.

1.5. Selection of the Plant for Present Study

When selecting a plant for pharmacological activities, four basics methods are usually followed:

a) Random choice of plant species
b) Choice based on ethnomedical use
c) Follow up of existing literature on the use of the species
d) Chemotaxonomic approaches

Comparison of the four methods showed that the choice based on folklore has given about 25% more positive leads than other methods. Based on the second and third approach, selection of the plant has been made in the present work. In light of the above context, Bambusa arundinacea seeds were selected for the study. Considering the above, the objectives set forth are: The specific objectives of the investigation are: To exploit and evaluate the physiochemistry, pharmacognosy, qualitative and quantitative analysis of phytochemicals and secondary metabolites, vitamins and minerals of Bambusa arundinacea seeds. Further to exploit various extract of Bambusa arundinacea seeds for their antioxidant, antidiabetic, anticancer, anti-inflammatory, antidiabetic, anti-arhritic, anti-microbial, antihelminthic activity (Invitro).
1. 6. AIMS AND OBJECTIVES

- To study the macroscopic and microscopic characters of Bambusa arundinacea seeds.
- To study the physiochemical characters of Bambusa arundinacea seeds.
- To evaluate the phytochemical substance present in the Bambusa arundinacea seeds.
- To study the quantitative values of secondary metabolites of Bambusa arundinacea seeds.
- To study the qualitative and quantitative values of nutraceuticals present in Bambusa arundinacea seeds.
- To study the Invitro antioxidant effect of Bambusa arundinacea seeds.
- To study the Invitro antidiabetic effect of Bambusa arundinacea seeds.
- To study the Invitro anticancer effect of Bambusa arundinacea seeds.
- To study the Invitro antimicrobial effect of Bambusa arundinacea seeds.
- To study the Invitro antihelminthic effect of Bambusa arundinacea seeds.
- To study the Invitro antiarthritic effect of Bambusa arundinacea seeds.
CHAPTER 2

REVIEW OF LITERATURE

2.1. Bamboo family

Bamboo leaves have been used in traditional Chinese medicine for treating fever and detoxification for over 1000 years. Recently, some biologically active components in bamboo leaves and their potential health benefits have been widely studied. An ethanol/water extract of bamboo leaf mainly contains flavone glycosides, phenolic acids, coumarin lactones, anthraquinones and amino acids. The leaves of Bambusa arundinacea are emmenogogue and used as a folk medicine for the treatment of cough, fever and leprosy. The plant contain flavanoids, phenolics, amino acids, proteins glucosides. The young shoot contain poisonous cynogenic glucosides (0.3%) and taxiphyllain. Bamboo-manna (Vanslochan) contains silica or silicon as hydrates of silicic acid potash, alumina, iron peroxide, and calcium carbonate (Ajay Kumar Rathaur 2013). It was reported that the extract of B. arundinacea showed anti-inflammatory, antiulcer, antifertility, antimicrobial and hypoglycemic activities. The hypoglycemic properties of extracts of bambusa leaves have also been established. Previous research on this plant has resulted in the isolation of flavones glycosides (Rates 2001).

The root (burnt root) is applied to ringworm, bleeding gums, painful joints. Seeds are acrid, laxative, said to be beneficial in strangury and urinary discharge. Bark is used for skin eruptions. Leaf is emmenagogue, antileptic, febrifuge, belchic, used in haemoptysis. The antiinflammatory effect of the methanol extract of the leaves of Bambusa arundinacea against carrageenin-induced as well as immunologically induced paw oedema and also its antiulcer activity in albino rats have been studied and found to be significant when compared to the standard drugs (Macwan et al. 2010).
The antiinflammatory effect of the methanol extract of the leaves of Bambusa arundinacea against carrageenin-induced as well as immunologically induced paw oedema and also its antiulcer activity in albino rats have been studied and found to be significant when compared to the standard drugs. The combination of methanol extract and phenylbutazone (Non-Steroidal Antiinflammatory Agent, NSAIA) has been studied and found to be the most potent antiinflammatory activity experimentally with least toxic (no ulcerogenic) activity. Thus, the combination of herbal product (methanol extract of Bambusa arundinacea) with modern medicine (NSAIAs) will produce the best antiinflammatory drug and will be useful for long-term treatment of chronic inflammatory conditions like rheumatoid arthritis with peptic ulcer, which are common (Muniappan and Sundararaj 2003).

Aqueous ethanolic solvent extracts of Bambusa arundinacea seed were tested for anti-diabetic activity usingalloxan induced diabetic rats and compared with standard. Theresult expressed that aqueous ethanolic extracts had shown significant protection and maximum reduction in blood glucose was observed in alloxan induced diabetic rats. The results of this comprehensive study reveal that Bambusa arundinacea seed shown statistically significant Anti-Diabetic activity in comparison to the standard glibenclamide(Marchala et al.2011).

Water-phase extract of bamboo shavings (WEBS), by supercritical carbon dioxide extraction, was evaluated for its antimicrobial action against the range of food borne and food spoilage pathogens using agar disc diffusion assay in nutrient agar and Czapek Dox Agar media. The WEBS exhibited antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Aspergillus niger, Penicillium citrinum and Saccharomyces cerevisiae with a concentration-dependent relationship (Zhang et al. 2010).

An ethanolic extract of Bambusa arundinacea tender shoots (BASE) caused a reduction in fertility of male rats. After administration of 300 mg/kg per day of BASE for 7 days, the fertility index decreased to 15% for control rats and to 23% after a 7-day recovery period, respectively. The number of cohabited females being
successfully inseminated was reduced especially after 4 days of treatment. Complete recovery of mating behavior was evident 8 days after BASE withdrawal. The number of spermatozoa in the caput and cauda epididymis were decreased concomitant with a decrease in the motility of spermatozoa collected from the cauda epididymis. The weights of testes, epididymides, vas deferens and prostate were also significantly decreased. The serum profile of protein and oxaloacetic/pyruvic transaminase activity show the extract to be relatively non-toxic (Vanithakumari et al. 1989).

Anti-arthritic activity of Bambusa arundinacea in treating Rheumatoid Arthritis (RA) using CFA-induced arthritis animal model was investigated (Vlietinck et al. 1995). The effect of Bambusa arundinacea methanolic extract on the Arthritis was studied by analyzing various markers of Bone erosion like histological, radiological analysis of the joints. For evaluation of anti-arthritic activity other parameters analyzed are Paw volume, Arthritic index, Rheumatoid Factor, Erythrocyte Sedimentation Rate (ESR) and Spleen histopathology. The powdered leaves are used for hot extraction by using methanol as solvent. The anti-arthritic activity of the dry extracts was performed using female rats of about 200 to 250gms. The methanolic extract of Bambusa arundinacea significantly (dose dependent) decreased the bone erosion, spleen enlargement & rheumatoid factor etc. at a dose (100mg/kg, 200mg/kg, 300mg/kg) compared to the control group but less compared to Standard drug (Rathod Jaimik et al. 2011).

Ethanolic extract of the root part of Bambusa arundinacea was investigated for their anthelmintic activity against Pheritima posthuma (Sailaja 2013). The antioxidant activityof flavanoids results from the combination of their iron chelatig activity and their ability to scavenge ageing- induced free radical. Flavanoid can inhibit oxidases such as lipoxygenase, cyclooxygenase and xanthine oxidase, thus preventing the invivo formation of reactive oxygen species and organic hydroperoxidase. Additionally, it has been found that flavanoid inhibit enzymes indirectly involved in oxidative processes. Flavanoids shows antiaging, photoprotection and hair color protection (Nazreen et al. 2011). In Ayurveda, the Indian system of traditional medicine, the silicious concretion found in the culms of the bamboo stem is called
banslochan. It is known as tabashir or tawashir in Unani-Tibb the Indo-Persian system of medicine. In English it is called bamboo manna. This concretion is said to be a tonic for the respiratory diseases (Ghosh et al. 1938).

2.2. Phytochemical constituents

Bamboo leaves have been used in traditional Chinese medicine for treating fever and detoxification for over 1000 years. Recently, some biologically active components in bamboo leaves and their potential health benefits have been widely studied. An ethanol/water extract of bamboo leaf mainly contains flavone glycosides, phenolic acids, coumarin lactones, anthraquinones and amino acids Many papers have indicated that a flavonoid-rich bamboo leaf extract has multiple biological effects, such as anti-free radical, anti-oxidation, anti-aging, anti-fatigue, anti-bacteria, anti-virus, and prevention of cardiovascular diseases, and can be used as a pharmaceutical intermediate, dietary supplement, cosmetic ingredient, and food additive. Chemical investigation of this plant, triterpenes and steroidal glycosides were the major phytoconstituents. In the course of phytochemical investigation of Bambusa, Stigmaster-5, 22-dien-3β-ol, Stigmaster-5-en-3β-ol-β-D glucopyranoside were isolated in good quantities. The synergistic hypoglycaemic effect of these two compounds has been well established. Along with the two new compounds i.e. 17, 20, 20-tri demethyl-20α-isopryl oleanane and a new acid, eicosanyl dicarboxylic acid, another two known compounds α-amyrin acetate and urs-12-en-3β-ol-β-D-glucopyranosid have been isolated for the first time from this plant (Jaral et al. 2007).

The silicious substance found near the joint inside is white camphor like crystalline in appearance, slightly sticky to the tongue and sweet in taste. Shoot has active constituents are Oxalic acid, reducing sugar, resins, waxes, HCN, benzoic aciddiferuloyl arabinofuranhexasaccharide, diferuloyl oligosaccharide (Vilegas et al. 1997), (5, 5'-di--(diferul-9, 9'-diyl)-[α-Larabinofuranosyl-(1→3)-O-β-D-xylopyranosyl-9 (1→4) -D xylopyranose] (taxiphyllin). Seed contain arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylamine, threonine, valine, tyrosine, niacin, riboflavin, thiamine. Leaves mainly contain protein, glutelina, contains lysine, methionine, betain, cholin, proteolytic enzyme, nuclease,
urease. The phytochemical investigation of an ethanolic leaf extract of Bambusa arundinacea Retz. furnished 6 constituents, two of which represented new natural entities. Remaining four compounds which are previously reported from the other plant sources have been isolated for the first time from the leaves of Bambusa arundinacea Retz. Spectroscopic structure elucidation of the new natural products is described (Yokoyama et al. 2004). The new compounds are characterized as 17, 20, 20-tri dimethyl-20α-isoprenyl oleanane and eicosan-1, 20-dioic acid. A number of studies of bamboo have yielded information about the chemical constituents, but no systematic evaluation has been carried out, so it is difficult to determine which of the identified compounds might be among the primary active constituents. The bamboo leaves, obtained from the common tall bamboos (species of Phyllostachys, rather than the small Lophatherum) have recently been utilized as a source of flavonoids (e.g., vitexin and orientin), used as antioxidants (Preethi et al. 2010).

The flavonoids may reduce inflammation, promote circulation, and inhibit allergy reactions. A juice made from the leaves has been made into a bamboo flavored beer. Bambusa Arundinacea extract contains Silicates (60% - 70%) which are responsible for its action in curative disease like diarrhoea, dyspepsia, flatulence and worm problems, inflammations, ulcers and wounds. Nayak S & Rout GR analyzed the isolation and characterization of microsatellites in Bambusa arundinacea and cross species amplification in other bamboos. Six microsatellites, three polymorphic and three monomorphic, were characterized in a bamboo species, Bambusa aruninacea belonging to the family Poaceae(Koshino et al. 1999). Plant acid invertases, which are either associated with the cell wall or present in vacuoles, belong to family 32 of glycoside hydrolases (GH32). Homology modeling of bamboo vacuolar invertase Bobfruct3 using Arabidopsis cell-wall invertase AtcwINV1 as a template showed that its overall structure is similar to GH32 enzymes, and that the three highly conserved motifs, NDPNG, RDP and EC, are located in the active site. This study also used site-directed mutagenesis to examine the roles of the conserved amino acid residues in these three motifs, which include Asp135, Arg259, Asp260, Glu316 and Cys317, and a conserved Trp residue (Trp159) that resides between the NDPNG and RDP motifs (Jang et al. 2004). The
mutants W159F, W159L, E316Q and C317A retained acid invertase activity, but no invertase activity was observed for the mutant E316A or mutants with changes at Asp135, Arg259, or Asp260. The apparent Km values of the four mutants with invertase activity were all higher than that of the wild-type enzyme. The mutants W159L and E316Q exhibited lower kcat values than the wild-type enzyme, but an increase in the kcat value was observed for the mutants W159F and C317A. The results of this study demonstrate that these residues have individual functions in catalyzing sucrose hydrolysis (Sagwan et al. 2010).

2.3. Bamboo and human health

The use of bamboo as traditional medicine by the Chinese dates back to some 2500 years. They used the bamboo leaves, branches, shoots, seeds, roots and juice to treat phlegm, cooling fever, laryngitis, rhinorrhagia (nose bleed) and vomiting (Kosuge et al. 1985). Thus it can safely be asserted that each part of bamboo is not only a treasure but also a medicine. The use of bamboo is endless and because of this it is an indispensable resource for the rural people.

2.4. Pharmacology

All parts of the bambusa arundinacea possess great pharmacological actions. Traditionally leaves, root and stem secretions were used for various ailments.

2.4.1. Bamboo in cardiovascular diseases

With modernization and industrialization the number of death and disability due to chronic heart diseases such a cardiovascular disease, diabetes etc has surpassed the death and disability due to nutritional deficiencies disease. Fu and his co researchers experimentally proved that when the high cholesterol mice were treated with different concentrations of BLE, there was great reduction in the serum cholesterol. Phyllostachys pubescens leaves proved to have protective effect against palmitic acid induced lipo apoptosis (Jantaratnotai et al. 2006). Experiments conducted on rats showed that flavonoids rich bamboo beer could significantly lower the blood triglycerides and cholesterol. Apart
from this the beer could elevate HDL- cholesterol and reduce LDL- cholesterol in a dose dependantmanner. The cardioprotective potential of flavone C-glucoosides i.e. Orientin obtained from the leaves of Phyllostachys nigra has been proved by Fu and his co workers. They also stated that it could also inhibit apoptosis by blocking the mitochondrial apoptotic pathway (AasthaBhardwaj et al. 2014).

2.4.2. Anti-inflammatory and antiulcer activity

The antiinflammatory effect of the methanol extract of the leaves of Bambusa arundinacea against carrageenin-induced as well as immunologically induced paw oedema and also its antiulcer activity in albino rats have been studied and found to be significant when compared to the standard drugs. The combination of methanol extract and phenylbutazone (Non-Steroidal Antiinflammatory Agent, NSAIDS) has been studied and found to be the most potent antiinflammatory activity experimentally with least toxic (no ulcerogenic) activity. Thus, the combination of herbal product (methanol extract of Bambusa arundinacea) with modern medicine (NSAIDs) will produce the best antiinflammatory drug and will be useful for long-term treatment of chronic inflammatory conditions like rheumatoid arthritis with peptic ulcer, which are common. In 2003, Muniappan and Sundararaj validated that the methanol extract of Bambusa arundinacea was effective against both carrageenin induced and immunologically induced paw oedema in albino rats compared to standard drugs. They also proved that the extract in combination with phenybutazone (a non-steroidal antiinflammatory agent) was more effective in comparison to when used individually. They thus concluded that the natural product together with modern medicine can result in the development of the most suited anti-inflammatory drug. Hwang and his co-researchers, provided scientific evidence to the traditional use of Sasa quelpaertensis leaves for the treatment of inflammation related diseases. They documented the hot water extract of S. quelpaertensis could ameliorate inflammation related diseases by suppressing nitric oxide production in pathological event (Habibur et al. 2015).
Methanol extract of the leaves of Bambusa vulgaris have been shown to possess anti-inflammatory activity against the various anti-inflammatory tests performed which includes formaldehyde induced rat paw edema, acetic acid induced vascular permeability test, carrageenan induced peritonitis and cotton pellet granuloma in albino rats.

2.4.3. Anthelmintic Activity

Ethanolic extract of the root part of Bambusa arundinacea was investigated for their anthelmintic activity against Pheritima posthuma. The study involves the determination of paralysis time and death time of the worms in the different doses of the extracts (10, 20 and 50 mg/ml). The extract exhibited significant anthelmintic activity in a dose dependent manner compared to the control. Activity was comparable with the reference standard Pipeazine citrate (15 mg/ml) and Albendazole (10 mg/ml).

2.4.4. Anti diabetic activity

Diabetes Mellitus (DM) is prevalent among almost 200 million people worldwide, which is thought to increase exponentially to 300 million in the next two decades, type 2 being common. In the study conducted by Ding and his coworkers with moso bamboo leaves on 50 diabetic rats, they evaluated that different doses of polysaccharide were found to possess good hypoglycemic effect. Hyun and Hyeon-Skoog in their experiment with Sasa borealis leaf extract found that when substituted for meat in Patty the leaf extract significantly lowered plasma glucose indicating anti diabetic activity of BLE (Vishal Soni et al. 2013).

The anti diabetic activity of Sasa borealis leaf extract was also studied by Choi and his co workers. The inhibitory effect of the leaves of Pseudosasa japonica was evaluated on high fat diet induced obesity and diabetes in C57BL/6J mice. All the mice had access to high fat diet for a week and then switched over to either the bamboo extract diet or control diet. The mice were regularly monitored for their daily intake of food and weight gained. Though the food intake of mice assigned to bamboo extract was found to be slightly higher
than the control, but the weight gain was however restricted in mice on bamboo extract compared to control. Senthilkumar and others made an attempt to scientifically prove the anti-diabetic activity of the petroleum extract of the leaf of Bambusa vulgaris in streptozotocin induced diabetic rats. They found that oral administration of the extract for a period of 15 days was effective in significantly reducing the blood glucose level in a dose dependant manner when compared to the standard drug glibenclamide. Nam and his team, recently in 2013, reestablished the exact mechanism by which the leaves of Sasa borealis exhibit the anti-diabetic activity. In their experiment they found administration of S. borealis extract in STZ-induced diabetic mice increased insulin signaling together with phosphorylation of AMP-activated protein kinase (AMPK) in HepG2 cells. In addition to this the extract also increased glucose uptake and suppressed the expression of gluconeogenic genes. They thus inferred that S. borealis extract exerted the anti-diabetic effect through the activation of AMPK and improvement of insulin signaling (Midya 1994).

Aqueous ethanolic solvent extracts of Bambusa arundinacea seed were tested for anti-diabetic activity using alloxan induced diabetic rats and compared with standard (Zhani and Rigas 2006). The result expressed that aqueous ethanolic extracts had shown significant protection and maximum reduction in blood glucose was observed in alloxan induced diabetic rats. The results of this comprehensive study reveal that Bambusa arundinacea seed showed statistically significant Anti-Diabetic activity in comparison to the standard glibenclamide.

2.4.5. Anti microbial activity

Plants are rich sources of various phytoconstituents which possess different medicinal properties against different microbes and bamboo is no exception. In 2010, Singh et al.45 studied the antimicrobial activity of the aqueous and ethanolic leaf extracts of Bamboosa arundinaceae against Staphylococcus aureus, Escherichia coli, Pseudomonas aureginosa and Bacillus sp. The ethanolic extract was found to be more effective in inhibiting the microbes compared to aqueous extract against the standard penicillin. Later,
Tanaka and his co-researchers 46 studied the antibacterial activity of Phyllostachys pubescens shoot skin (untreated and dichloromethane extract) against Staphylococcus aureus. They found that both the extracts could inhibit the growth of S. aureus and thus possess antibacterial activity. Mulyono evaluated the antibacterial activity of Dendrocalamus asper against E. coli. They found that of the three different extract types (ethanolic, methanolic and methanol-ethanolic) ethanolic and methanol-ethanolic proved to be potential source of antimicrobial drugs. Recently in 2013, Mulyono and his team also studied the antibacterial activity of Gigantochloa apus against the diarrheagenic E. coli. As per their results, they noted that E. coli was sensitive to both the ethanolic and methanolic extracts but in a concentration dependant manner (Malick 1974).

Water-phase extract of bamboo shavings (WEBS), by supercritical carbon dioxide extraction, was evaluated for its antimicrobial action against the range of food borne and food spoilage pathogens using agar disc diffusion assay in nutrient agar and Czapek Dox Agar media. The WEBS exhibited antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Aspergillus niger, Penicillium citrinum and Saccharomyces cerevisiae with a concentration-dependent relationship (Shah 1968).

2.4.6. Protective effects

Two biological activities of bamboo-derived pyrolyzates were investigated; the protective effects against N-methyl-d-aspartate (NMDA)-induced cell death in primary cultured cortical neuron and the anti-plasmin effects determined by using fibrin and fibrinogen degradation products (FDPs) assay. Treatment of neuronal cells with pyrolyzates of Phyllostachys pubescens, Phyllostachys nigra and Phyllostachys bambusoides resulted in restored cell viability when compared to untreated cells in an NMDA-induced neuronal cell death assay. In addition, cortical neurons treated with Phyllostachys pubescens and Phyllostachys nigra showed a reduction of apoptosis following exposure to NMDA, as determined by Hoechst 33342 staining. In addition, Phyllostachys nigra pyrolyzates also exhibited anti-plasmin action in a FDP assay. It is of interest to note that pyrolyzates exhibited activities of NMDA-receptor
antagonist and antifebrin (ogen), since a combination of NMDA receptor antagonists, glucocorticosteroids, GABAergic drugs and heparin are useful for treatment in delayed postischemic injury. Results indicate that the pyrolyzates derived from bamboo may have antiapoptotic effects, and can be useful as a supplement for ischemic injury treatment (Bahadur 1980).

### 2.4.7. Antifertility activity

An ethanolic extract of Bambusa arundinacea tender shoots (BASE) caused a reduction in fertility of male rats. After administration of 300 mg/kg per day of BASE for 7 days, the fertility index decreased to 15% for control rats and to 23% after a 7-day recovery period, respectively. The number of cohabited females being successfully inseminated was reduced especially after 4 days of treatment. Complete recovery of mating behavior was evident 8 days after BASE withdrawal. The number of spermatozoa in the caput and cauda epididymis were decreased concomitant with a decrease in the motility of spermatozoa collected from the cauda epididymis. The weights of testes, epididymides, vas deferens and prostate were also significantly decreased. The serum profile of protein and oxaloacetic/pyruvic transaminase activity show the extract to be relatively non-toxic (Chatterjee 1960).

### 2.4.8. Insecticidal activity

Asian Centers of Diversity, bamboos are reported to tolerate insects, laterites, low pH, slope, and weeds (2n = 72, 70). Eight grams of raw shoots or slightly more improperly cooked shoots can cause death (Banik 2000). Young shoots contain 0.03% HCN (C.S.I.R., 1948–1976). Hairs on various bamboos, and fungi which live thereon, may cause dermatitis. Benzoic acid and traces of cyanogenic glucoside present in shoots have lethal effect on mosquito larvae (has antiseptic and larval properties)

### 2.4.9. Anti arthritic activity:

Anti-arthritic activity of Bambusa arundinacea in treating Rheumatoid Arthritis (RA) using CFA-induced arthritis animal model was investigated. In the present study, the effect of Bambusa arundinacea methanolic extract on the Arthritis was studied by analyzing various markers of Bone erosion like histological, radiological
analysis of the joints. For evaluation of anti-arhritic activity other parameters analyzed are Paw volume, Arthritic index, Rheumatoid Factor, Erythrocyte Sedimentation Rate (ESR) and Spleen histopathology. The powdered leaves are used for hot extraction by using methanol as solvent. The anti-arhritic activity of the dry extracts was performed using female rats of about 200 to 250gms. The methanolic extract of Bambusa arundinacea significantly (dose dependent) decreased the bone erosion, spleen enlargement & rheumatoid factor etc. at a dose (100mg/kg, 200mg/kg, 300mg/kg) compared to the control group but less compared to Standard drug (Dexamethasone 5 mg/kg i.p) (Das 1976).

2.5. Antioxidant potential of bamboo

In the process of economic development, with the increase in income, human society tends to care more about their health. Therefore, demand for healthy herbal organic foods developed from various plants has also increased. Production of more efficient and productive food items by the researchers are on demand. One such plant with multiple qualities is bamboo. Bamboo has been used over centuries by the humans both in daily life and for medicinal purpose in China and other Asian countries. The earliest scientific evidence of use of bamboo in traditional medicine dates back to 1963. This marked the beginning of the use of bamboo as medicine which was followed by series of research carried out by different workers since then 1931 (Banik 1987).

Bamboo has attracted attention world over due to its high antioxidant content and therapeutic effects on inflammation, fatigue, cancer, hyperlipidemia, diabetes, aging and hypertension. Free radicals might occur either by the accidents of chemistry or due to specific metabolic purpose in the body. The free radicals produced by either way have different reactivity with some leading to damage to biomolecules such as DNA, lipids and proteins32. Antioxidants can react with free radicals during the oxidation process by acting as a reactive species scavenger and liberating catalysts, so antioxidants can be used to reduce the oxidative process33 but they are not 100% effective. Mere large doses of diet-derived antibody was thought to be important to stay healthier for long time,
but with the passage of time and development of science and technology the supply of ‘pro-oxidants’ is thought to be a better option. Bioactive compounds like ascorbic acid, carotenoids, tocochromanols and phenols are antioxidants (Adhikari 1928). The bamboo leaf extract (BLE) is thought to be a good source of natural antioxidants and also have great pharmaceutical potential. BLE is mainly composed of flavonoids, lactones and phenolic acid. The flavonoids are represented mainly by the flavones C-glycosides which include homoorientin, isovitexin, orientin and vitexin. Apart from this quercetin, luteolin, rutin, caffeic acid, p-coumaric acid, chlorogenic acid and tricin are also present. The flavonoid content was recorded to be 3.44% in different bamboo leaves species (Baroah 1999).

The antioxidant activity of flavonoids results from the combination of their iron chelating activity and their ability to scavenge ageing-induced free radical. Flavanoid can inhibit oxidases such as lipooxygenase, cyclooxygenase and xanthine oxidase, thus preventing the in vivo formation of reactive oxygen species and organic hydroperoxidase. Additionally, it has been found that flavanoid inhibit enzymes indirectly involved in oxidative processes. Flavanoids show antiaging, photoprotection and hair color protection (Appasamy 1993).

2.5.1. Anticancer activity

The leaves of Sasa senanensis (popularly known as Kumaizasa) have been used in Eastern Asia as a potential source of natural drug since hundreds of years. The alkaline extract prepared from the leaves (in hot water at 100°C) of S. senanensis is popularly known as ‘Sasa health’. Tsunoda et al. from their experiment on mammary tumor strain of SHN virgin mice proved that oral administration of Sasa health for 12 days could significantly inhibit both the development and growth of mammary tumor in experimental models. In 2008, Seki and his team also made an attempt to prove the anti-tumor activity of Sasa health. They used three different temperatures (100°C, 121°C and 196°C) to prepare the Sasa health to evaluate the anti-tumor potential in three mouse tumor models (S-180, C38 and Meth-A). Oral administration of the extract a concentration of 0.05% or
more was found to be effective in suppressing tumor growth in mouse models S-180 and C38. The extract also accelerated immunostimulating activity, which in turn activated the macrophages and human natural killer (NK) cells in tumor models and thus suppress the tumor (Chun-ju et al. 2013).

Panee conducted experiment to test the effect of leaves of Pseudosasa japonica on the development of DMBA (7,12-Dimethylbenz[a]anthracene) induce breast cancer in SD (Sprague-Dawley) rats. He found that oral administration of bamboo extract for 3 weeks prior to DMBA injection could delay the onset of breast cancer by one week as compared to the control. Moreover, the bamboo extract also showed the potential of decreasing the incidence of occurrence of tumor by 44% and restricting the growth rate of the tumor by 67% after 11 weeks of DMBA treatment. Seki and Maeda studied the anti cancer/tumor activity of Kumaizasa bamboo, Sasa senanensis and Caulis bamfusae. In their study, Seki and Maeda tested the cancer preventive effect of aqueous extract of Kumaizasa bamboo both prior to tumor implantation and also after inducing of carcinogenesis using DMBA in experimental mouse (They found that the Kumaizasa bamboo was effective against both the cases and could significantly suppress the incidence of tumor growth and enhanced survival rate on one hand and the extract at the rate of 0.03% fed for two weeks could significantly suppress the cancer. In the same year, the anti tumor activity of Sasa senanensis leaf extract was experimented using three different mouse tumor models viz. S-180, C38 and Meth-A by Seki and his team. Oral administration of the extract at a concentration of 0.05% or more was found to significantly reduce the tumor growth in mouse models S-180 and C38 and also prolonged the survival rate compared to the control (Chun-ju et al. 2010). Lu and his co-researchers were successful in establishing that the savings of Caulis bamfusae had the potential to inhibit the growth of cancer cell lines (P388 and A549) and also notably inhibit the tumor growth on sarcoma-loaded mice model S180 compared to standard cyclophosphamide. Apart from this they also isolated a compound named friedelin and proved its efficacy as anti tumor compound against cancer lines, A375, L929, Hela and THP-1. The ethanolic leaf extract of Sasa
quelpaertensis has also been reported to exert potent cytotoxicity activity against human colon cancer HCT116 cells (Das 1976).

2.5.2. Anti fatigue effect

Leaf extract of Phyllosatchys nigra var henonis have been reported to enhance the anti-fatigue capacity in mice. You and his coworkers64 found that oral administration of 80% ethanol extract of Pseudosasa japonica leaf for 18 days could drastically increase the swimming time in experimental mice up to one and half folds and simultaneously reduce the blood lactate and elevate the removal of lactate suggesting its potential to reduce fatigue compared to the control group. In 2006, an attempt was also made to study the anti-fatigue activity of Bambusa tuloides using BALB/c mice models by Zhang and his team65. They found that the extract at an appropriate concentration not only could prolong the weight-loaded swimming and climbing time but also exerted active effect on the serum urea nitrogen, hepatic glycogen and blood lactic acid level in BALB/c mice thus personified its anti-fatigue activity (Bag and Palani 2013).

2.5.3. Anti obesity effect

Obesity, characterized by the deposition of excessive fats in the adipocytes is considered to be a major obstacle in efforts to improve human health. The effect of Sasa borealis leaf extract (SBE) on inflammatory cytokines and insulin resistance in high fat diet (HFD) induced obese mice. They found that the S. borealis leaf extract was effective at just 5% when administered for 12 weeks. After 12 weeks treatment they recorded that the body weight and the adipose tissue deposition were decreased significantly compared to untreated HFD mice. They also found decrease in glucose, insulin, IAUC, HOMA-IR, TNF-α, IL-6 and leptin levels. These results justified that SBE contains anti-obesity compounds. It was in 2012 that Kang and his researchers made an attempt to explore the anti-obesity effect of Sasa quelpaertensis leaf extract (SQE) in high fat diet (HFD) induced obese mice and mature adipocytes. They noted that the administration of SQE for 70 days to HFD mice not only decreased the body weight, adipose tissue weight, serum cholesterol and triglycerides but also reduced the serum levels of
several enzymes along with deposition of lipid droplets in the liver when compared to untreated mice. They finally concluded that the anti obesity effect of SQE is mediated by the activation of AMPK in adipose tissue (Chun-ju 2009).

2.5.4. Anti hyperlipidemic effect

Alterations in lipid profile are one of the most common complications in diabetes mellitus and affects 40% of all diabetic patients72. The study was carried out by Ding and his team73 to elucidate the anti-hyperlipidemic effect of polysaccharides from Moso bamboo leaves (PMBL). Mice were fed with high fat food to induce hyperlipidemia and then treated with PMBL in a dose dependant manner. The result revealed significant decrease in total cholesterol, tri-glyceride and low- density lipoprotein in serum together with decrease in crude fat in liver concluding PMBL exhibited could improve hepatic function in mice and possesses antihyperlipidemic effect (Banik 1994).

2.5.5. Vessles protection

Flavanoids are vein active and vessel-protective agents because they reduce the permeability and increase the resistance of blood capillaries. Flavanoids are used in the treatment of blood vessel disorders such as varices, chronic venous insufficiency, low capillary resistance etc. Oral administration of flavanoid s has been observed to effectively improve the capillary resistance in animal models (Ayana et al. 2012).

2.5.6. Acute and subchronic toxicity studies

The anti-oxidant of bamboo leaves (AOB) has recently been certificated as a novel kind of natural anti-oxidant by the Ministry of Health of the Peoples Republic of China, and has been used in various food systems. Here, AOB was subjected to a series of acute and subchronic toxicological tests to evaluate its safety. The results showed that the maximum tolerated dose (MTD) of AOB was >10 g/kg body weight in both rats and in mice, which can be regarded as virtually non-toxic. No mutagenicity evidence was detected in any of the three mutagenic tests. Administration at levels of 1.43, 2.87 and 4.30 g/kg per day to the rats for 90 days did not induce significant hematological, clinic, chemical and histopathological changes,
and suggested a no-observed adverse- effect level (NOAEL) of 4.30 g/kg per day. These results indicate that AOB can be generally regarded as safe for use as a food additive (Sharma et al. 2014).

2.5.7. Effects of bamboo buds

An ethanolic extract of the tender shoots of Bambusa arundinaceae was administered at 300 mg/kg per rat per day for 7 days to adult male rats to assess epididymal structural and functional activity. Sperm motility decreased markedly in the cauda epididymal fluid and sperm count decreased significantly in both caput and caudal segments of the epididymis. Histologically, a reduction in epithelial and stereocilia height and lumen diameter was noted. An increase in intertubular stroma was also evident. Epididymal weights, activities of acid phosphatase and total LDH were reduced in both epididymal segments. Protein concentration was appreciably increased only in the caudal segment. Extract therapy impaired the structural and functional integrity of the epididymis (Lakshmi et al. 2014).

Bamboo is the main food of the Giant Panda; it makes up 99% of the Panda's diet. Soft bamboo shoots, stems, and leaves are the major food source of the Giant Panda of China. Bamboo is used in Chinese medicine for treating infections and healing (Akanmu et al. 2004). It is a low-calorie source of potassium. It is known for its sweet taste and as a good source of nutrients and protein. When bamboo is heated at very high temperature in an airless vessel, it becomes charcoal, which is used like other charcoal products, as a fuel component, a deodorizer, or an absorbent. The vapor that comes off the heated bamboo can be condensed to produce a liquid known as bamboo vinegar. Bamboo vinegar has been produced in Japan (where it is called chikusaku-eki) for many years and is used medicinally to treat eczema, atopic dermatitis, and other skin diseases; it is most commonly applied by adding to bath water. Bamboo vinegar is recognized as an anti-inflammatory and anti-fungal. Pickled bamboo, used as a condiment, may also be made from the pith of the young shoots. In addition, bamboo is frequently used for cooking utensils within many cultures (Sharma and Bala 2006). In modern times, some see bamboo tools as an eco-friendly alternative to other manufactured utensils. In Ayurveda, the Indian system of
traditional medicine, the silicious concretion found in the culms of the bamboo stem is called banslochan. It is known as tabashir or tawashir in Unani-Tibb the Indo-Persian system of medicine. In English it is called "bamboo manna". This concretion is said to be a tonic for the respiratory diseases. In addition, the fiber of bamboo has been used to make paper in China since early times (De 1940).

A high quality hand-made paper is still produced in small quantities. Coarse bamboo paper is still used to make spirit money in many Chinese commun Bamboo's natural hollow form makes it an obvious choice for many instruments, particularly wind and percussion. There are numerous types of bamboo flute made all over the world, such as the dizi, xiao, shakuhachi, palendag, jinghu, angklung. Bamboo was in widespread use in early China as a medium for written documents. The earliest surviving examples of such documents, written in ink on string-bound bundles of bamboo strips (or "slips"), date from the 5th c. BC during the Warring States period.

2.5.8. Important formulations

- Sitopaladi churna
- Talisadi churna

Genuine Bamboo-manna is difficult to procure. Curcuma angustifolia is in use as a substitute. Its original name, Tabashiri, was equated in due course the Tabasheer of Unani medicines.

Possible Combinations: Bambusa arundinacea + Piper longum + Cinnamomum camphora (curing lung disease)

Recommended dose: The recommended dose is between 0.5% and 5.0%.

2.6. Pharmacognosy

Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be safe. Another factor which emphasizes this attention is the incidences of harmful nature of synthetic drugs which are regarded as harmful to human beings and environment. The advantage of natural drugs is their easy availability, economic
and less or no side effects but the disadvantage is that they are the victims of adulteration. The more effective the natural drug more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material (Verpoorte 2000). Adulteration or substitution is nothing but replacement of original plant with another plant material or intentionally adding any foreign substance to increase the weight or potency of the product or to decrease its cost. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The most common error is one common vernacular name is given to two or more entirely different species (Yoon and Baek 2005). All these problems can be solved by pharmacognostic studies of medicinal plants. It is very important and in fact essential to lay down pharmacognostic specifications of medicinal plants which are used in various drugs. Pharmacognosy is the study of medicines derived from natural sources, mainly from plants. It basically deals with standardization, authentication and study of natural drugs. Most of the research in pharmacognosy has been done in identifying controversial species of plants, authentication of commonly used traditional medicinal plants through morphological, phytochemical and physicochemical analysis. The importance of pharmacognosy has been widely felt in recent times (Zhang et al. 2006). Unlike taxonomic identification, pharmacognostic study includes parameters which help in identifying adulteration in dry powder form also. This is again necessary because once the plant is dried and made into powder form, it loses its morphological identity and easily prone to adulteration. Pharmacognostic studies ensures plant identity, lays down standardization parameters which will help and prevents adulterations. Such studies will help in authentication of the plants and ensures reproducible quality of herbal products which will lead to safety and efficacy of natural products (Patwardhan et al. 2004).

2.7. Antioxidants

Oxidative stress: Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under
normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation in vivo and in vitro and create oxidative stress which results in numerous diseases and disorders (Moore et al. 2006).

Causes for oxidative stress: Free radicals formed in the body are due to many environmental and biological factors. Environmental factors include exposure to ultraviolet sunlight, X-rays and gamma rays, radiation, smoking, pollution, ozone, and certain drugs, chemicals or pesticides. Biologically produced ROS are a result of metabolic reactions where oxygen species are intermediate electron donors/acceptors. The production of free radicals is a natural process that can occur with or without the aid of enzymes and only becomes a health concern when defense mechanisms are not able to neutralize them.

Antioxidant enzymes: Exposure to free radicals from a variety of sources has led to the evolution of a series of defense mechanisms in organisms. Defense mechanisms against free radical-induced oxidative stress include: (i) preventive mechanisms, (ii) repair mechanisms, (iii) physical defenses, and (iv) antioxidant defenses. Enzymatic antioxidant defenses include three primary enzymes, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (Gpx), which are involved in direct elimination of ROS, and secondary enzymes, namely glutathione reductase (GR), glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase (G6PDH), and ascorbate peroxidase (Apx), which help in the detoxification of ROS by decreasing peroxide levels or by maintaining a steady supply of metabolic intermediates (glutathione, NADPH) that are necessary for optimum functioning of the primary antioxidant enzymes (Alberti and Zimmet 1998). Non-enzymatic antioxidants are represented by ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids,
and other antioxidants which as a whole, play a homoeostatic or protective role against ROS (Adom et al. 2005).

Natural sources of antioxidants: The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth’s inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components. Medicinal plants have great antioxidant potential which is due to their contents of variable phyto constituents. A large number of experiments have been carried out concerning the antioxidant activity of several plant extracts and powders. The results of these experiments reveal that, the activity is due to several secondary metabolites especially, e.g., phenolic compounds (tannins, flavonoids, anthocyanins, chalcones, xanthones, liganans, depsides, and depsidones), terpenes (sesquiterpenes and diterpines), alkaloids, and organic sulfur compounds (Zajicek 1996).

2.8. Cancer

Cancer is a complex disease that is normally associated with a wide range of escalating effects both at the molecular and cellular levels. It therefore seems unlikely that chemoprevention follows simplistic rules and formulations. The old saying "Prevention is always better than cure" is particularly true in the case of cancer where a cure, if at all possible, is associated with high cytotoxic loads and/or invasive procedures. Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer diseases are characterized by abnormal proliferation of cells. They constitute the second cause of mortality behind cardiovascular diseases in developed countries and the third after infectious and cardiovascular diseases in developing countries. Cancer is a major public health problem worldwide with millions of new cancer patients diagnosed each year and many deaths resulting from this disease. Cancer is fundamentally a disease of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, genes which regulate cell growth and differentiation must be altered. Genetic changes can occur at many levels,
from gain or loss of entire chromosomes to a mutation affecting a single DNA nucleotide. There are two broad categories of genes which are affected by these changes. Oncogenes may be normal genes which are expressed at inappropriately high levels, or altered genes which have novel properties. In either case, expression of these genes promote the malignant phenotype of cancer cells. Tumor suppressor genes are genes which inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes. Typically, changes in many genes are required to transform a normal cell into a cancer cell (Jenab and Thompson 1998).

Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism). The first FDA approved chemopreventive agent was tamoxifen, for reducing the risk of breast cancer. This agent was found to reduce the breast cancer incidence by 50% in women at high risk. With tamoxifen, there is an increased risk of serious side effects such as uterine cancer, blood clots, ocular disturbances, hypercalcemia, and stroke. The serious side effects of the FDA approved chemopreventive drugs is an issue of particular concern when considering long-term administration of a drug to healthy people who may or may not develop cancer. This clearly indicates the need for agents, which are safe and efficacious in preventing cancer. Diet derived natural products will be potential candidates for this purpose. Several classes of anticancer drugs have been developed and many of them are of natural origin. Natural products have been the mainstay of cancer chemotherapy for the past 30 years. However, most of the currently used anticancer drugs cause undesirable side effects due to lack of tumor specificity and multidrug resistance. Therefore the search for potent, safe and selective anticancer compounds is crucial for new drug development in cancer research. Natural products, due to their structural diversity, provide excellent templates for the construction of novel compounds (Thompson 2000).
It is well established that plants have been a useful source of clinically relevant antitumor compounds. Plants have long history of use in the treatment of cancer. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. For example, Hartwell has collected data on about 3000 plants, those of which possess anticancer properties are subsequently used as potent anticancer drugs. The use of plant extracts and derived products in the treatment of cancers is of exceptional value in the control of malignancies, due to the fact that most of the anticancer drugs severely affect the normal cells. It has been recommended that ethnopharmacological usages, such as immune and skin disorders, inflammatory, infectious, parasitic and viral diseases be taken into account when selecting plants used to treat cancer, since these reflect disease states bearing relevance to cancer or cancer symptoms. Plant secondary metabolites and their semi-synthetic derivatives continue to play an important role in anticancer drug therapy. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin and paclitaxel (taxol). Sixty percent of currently used anticancer agents are derived in one way or another from natural sources (Qu et al. 2005). In light of the continuing need for effective anticancer agents, and the association of fruit and vegetable consumption with reduced cancer risk, edible plants are increasingly considered as sources of anticancer drugs.

Plant derived anticancer agents in clinical use: The isolation of the vinca alkaloids, vinblastine and vincristine from the Madagascar periwinkle, Catharanthus roseus G. Don. introduced a new era of the use of plant material as anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer. Vinblastine and vincristine are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers, including leukemia, lymphomas, advanced testicular cancer, breast and lung cancers and Kaposi’s sarcoma (Dimayuga and Garcia 1991). The discovery of paclitaxel (Taxol) from the bark of the Pacific Yew, Taxus brevifolia Nutt. is another evidence of the success in natural product drug discovery. Taxus baccata was reported to be used in the Indian Ayurvedic medicine for the treatment of cancer. The structure of
paclitaxel was elucidated in 1971 and was clinically introduced to the US market in the early 1990s. Paclitaxel is significantly active against ovarian cancer, advanced breast cancer, small and non-small cell lung cancer. Camptothecin, isolated from the Chinese ornamental tree Camptotheca acuminata Decne, was advanced to clinical trials by NCI in the 1970s but was dropped because of severe bladder toxicity. Topotecan and irinotecan are semi-synthetic derivatives of camptothecin and are used for the treatment of ovarian and small cell lung cancer, and colorectal cancer, respectively (Zhueang et al. 1994). Epipodophyllotoxin is an isomer of podophyllotoxin, which was isolated as the active anti-tumor agent from the roots of Podophyllum species, Podophyllum peltatum Linnaeus and Podophyllum emodi Wallich. Etoposide and teniposide are two semi-synthetic derivatives of epipodophyllotoxin and are used in the treatment of lymphomas and bronchial and testicular cancers. Homoharringtonine, isolated from the Chinese tree Cephalotaxus harringtonia var. drupacea is another plant-derived agent in clinical use. A racemic mixture of harringtonine and homoharringtonine has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia. Elliptinium, a derivative of ellipticine, isolated from a Fijian medicinal plant Blekeria vitensis A.C. Sm., is marketed in France for the treatment of breast cancer (Sang et al. 2006).

2.9. Inflammation

Inflammation is being implicated in the pathophysiology of an increasing number of diseases. In addition to conditions traditionally considered to be inflammatory in nature, inflammation is now considered to have a role in a wide range of pathologies, including cardiovascular disease, cancer, diabetes age-related macular degeneration, Parkinson's disease, Alzheimer's disease, and possibly depression (Bonnet et al. 1997).

Overview over the inflammatory process: Inflammation is a rapid and non-specific response to cellular injury in vascularised tissues. The inflammatory response is produced and controlled by complex interactions between cellular and plasma protein components. The cellular component involves intercellular
communication effected by a range of cytokines. The inflammatory response commences with a brief constriction of arterioles followed by vasodilation and exudation of protein-containing plasma and blood cells into the injured tissue. This causes swelling and oedema (Zhang et al. 2007). Meanwhile leukocytes adhere to vessel walls and cause the endothelial cells to contract, creating enough space between these cells for the leukocytes to enter the extravascular tissue. Increased vascular permeability is maintained until the inflammatory state is resolved, and it is the interplay between blood cells and plasma proteins in the affected tissue that controls the inflammatory response and interacts with part of the immune response. The principal cell types involved in inflammation are mast cells, endothelial cells, phagocytic leukocytes (polymorphonuclear neutrophils, macrophages, and eosinophils), and platelets (Brand Williams et al. 1995).

Mast cells play a key role in the initiation of the inflammatory response. Degranulation leads to the release of stored chemicals such as histamine, which causes increased vascular permeability, and mast cells also synthesise pro-inflammatory mediators such as prostaglandins, leukotrienes and platelet-activating factor (PAF). Endothelial cells express adhesion molecules (selectins) for leukocytes and platelets, and also produce nitric oxide (NO), which causes vasodilation but also may play a regulatory role by suppressing mast cell and platelet function. Endothelial cells also produce two prostaglandin derivatives with opposite action: the vasoconstrictor thromboxane A2 (TxA2) and the vasodilator prostacyclin (PGI2), and it is the interplay between these two regulatory compounds that allows for platelet aggregation to occur only at the site of injury. Cellular products as inflammatory mediators: The various cells involved in the inflammatory response produce a range of compounds that act as inflammatory mediators, including cytokines and products of arachidonic acid metabolism, i.e. prostaglandins and leukotrienes. Cytokines are proteins produced by a range of different cell types. The major types of cytokines are the interleukins and the interferons, but the class also includes tumour necrosis factors, colony-stimulating factors, transforming growth factor, and others (Cao et al. 1993).
Arachidonic acid metabolism: Products of arachidonic acid metabolism such as prostaglandins and leukotrienes play key roles in inflammation, and their syntheses are well established targets in the pharmacological treatment of inflammation. Arachidonic acid (5Z,8Z,11Z,14Z-eicosatetraenoic acid) is an unsaturated, 20-carbon, omega-6 fatty acid found in cell membranes. It can be obtained from the diet or be derived from linoleic acid. In the cell membrane, arachidonic acid is esterified to phospholipid, and arachidonate must be liberated from phospholipid before it can act as a substrate for enzymatic modification. These modifications, catalysed by various enzymes, are known as arachidonic acid metabolism and can lead to the formation of inflammatory mediators collectively known as eicosanoids, i.e. prostaglandins (PGs), thromboxanes (TXs), hydroxyeicosatetraenoic acids (HETEs) and leukotrienes (LTs).

Arachidonic acid is released from the cell membrane by phospholipase enzymes, in particular phospholipase A2, and the free acid can be metabolised to eicosanoids by cyclo-oxygenase (COX) and lipoxygenase (LOX) enzymes. Metabolism catalysed by COX enzymes gives rise to prostaglandins of the 2-series as well as thromboxanes, while LOX metabolism leads to the formation of leukotrienes. COX is also known as prostaglandin endoperoxide synthase. This protein possesses two discrete activities: the cyclo-oxygenase activity first inserts two oxygen molecules into arachidonic acid resulting in PGG2; this is followed by the reduction of PGG2 to PGH2, a result of the protein's peroxidase activity. Since prostaglandins of the 2-series, including PGE2, are involved in many inflammatory processes, the inhibition of the COX pathway of arachidonic acid metabolism is a prime pharmacological target and one that has been exploited long before the pathway was known. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the cyclo-oxygenase activity (but not the peroxidase activity) of prostaglandin endoperoxide synthase. Acetylsalicylic acid (aspirin) does so in an irreversible fashion, by acetylating the enzyme. Two isoforms of the COX enzyme are known; these are known as COX-1 and COX-2 and their respective protein products show differential distribution. COX-1 is constitutively expressed in most tissues, where it catalyses the biosynthesis of eicosanoids (prostaglandins and thromboxanes) that
regulate numerous cellular processes. In contrast, COX-2 activity is generally undetectable in most tissues, but the expression of COX-2 can be rapidly induced in inflammatory cells in response to stimulation by pro-inflammatory cytokines or by growth factors. A new class of anti-inflammatory and analgesic agents that are selective COX-2 inhibitors were developed in the 1990s (e.g. celecoxib, refecoxib). The rationale for this drug development was that selective COX-2 inhibitors were not expected to interfere with homoeostatic physiological processes and should therefore be less likely than non-selective COX inhibitors to cause the unwanted side-effects typical of traditional NSAIDs (Ou et al. 2001).

Already in the late 1990s there was growing evidence, however, that viewing COX-2 as solely pro-inflammatory and COX-1 as essentially benign was too simplistic. COX-2 has now been shown to be involved in normal physiology such as the regulation of vascular and renal blood flow, and Sautebin reviewed work suggesting that COX-2 could have anti-inflammatory action, while COX-1 could contribute to inflammation under certain circumstances. Nevertheless, COX-2 inhibitory drugs (coxibs) were aggressively marketed and became very widely prescribed for inflammatory conditions in industrialised countries in the early 2000s, only for some of them to be withdrawn from the market a few years later due to the increased cardiovascular risks associated with their use (rofecoxib [Vioxx®] was withdrawn; celecoxib [Celebrex®] is still used, but in a far more discriminate manner). A so-called splice variant of COX-1, known as COX-3, COX-1b or COX-1v, is also known, but after initial speculation that it might be a target for paracetamol, medical interest in it has waned. The alternative metabolic pathway of arachidonic acid is controlled by the lipoxygenase (LOX) enzymes (Obied et al. 2005). They catalyse the insertion of molecular oxygen into polyunsaturated fatty acids with a 1Z,4Z-pentadiene system. Depending on the location of the oxygen insertion into arachidonic acid, mammalian lipoxygenase enzymes are classified as 5-, 12- or 15-lipoxygenases. The primary products are 5-, 12- and 15-hydroperoxyeicosatetraenoic acids (HPETEs), which are subsequently reduced to the corresponding hydroxyeicosatetraenoic acids (HETEs) or, in case of the 5-LOX pathway, converted to the eicosanoids known as leukotrienes (Prior et al. 2005).
Prostaglandins: The prostaglandins are a group of cyclic, 20-C unsaturated fatty acids, which all share a double-bond at C13-C14. Based on structural differences, the prostaglandins (PGs) are divided into 9 groups, named A-I. A subscript numeral indicates the number of unsaturated carbon bonds in the compound, and the subscripts α- and β- denotes the orientation of a hydroxyl-group on C9 below or above the molecular plane (e.g. PGF2α). Originally discovered in seminal fluid from the prostate (giving rise to their name), prostaglandins are known to be synthesised and released in virtually all body tissues. Notissue (except seminal fluid) appears to have the capacity to store prostaglandins, so rate of release reflects the rate of biosynthesis. Prostaglandins are produced in response to a variety of stimuli, including inflammation, allergic responses and trauma, and they usually exert their action locally, close to their site of release. Several prostaglandins are known to be metabolised rapidly in the liver, kidneys and lungs (Foster, 1996). Five prostanoid receptors named DP, EP, FP, IP and TP have been identified that show some degree of selectivity for PGD2, PGE2, PGF2α, PGI2 and thromboxane A2 (TXA2), respectively. The existence of several subtypes of the EP receptor has been proposed and it is believed that PGE2 exerts different actions at these. The formation of different PGs from the unstable metabolite PGH2 is cell-specific. For example, PGI2 (prostacyclin) is the predominant prostaglandin in the vascular endothelium, where it inhibits platelet aggregation and causes vasodilation. In mast cells, PGD2 is the major COX product, while PGE2 is the prevailing prostaglandin in the kidney. PGE2 has several pro-inflammatory activities, including vasodilation and increasing vascular permeability, inducing fever, and enhancing pain and oedema caused by other mediators such as bradykinin and histamine. Large amounts of PGE2 and PGF2 can be produced by stimulated monocytes and macrophages, whereas stimulated neutrophils produce moderate amounts of PGE2 (Balogh et al. 2004).

Thromboxanes: The thromboxanes are eicosanoids arising from the COX metabolic pathway like the prostaglandins. Thromboxane A2 (TXA2) is the major metabolite of PGH2 in platelets. Unlike its metabolite thromboxane B2
(TxB2), which is inactive, TxA2 contracts vascular smooth muscle, induces platelet aggregation and causes serotonin release.

Leukotrienes: Leukotrienes (LTs) are potent biologically active compounds produced by the 5-LOX pathway via 5-HPETE. LTC4, LTD4, and LTE4 are produced mainly by mast cells, basophils, eosinophils and endothelial cells from the precursor LTA4, while LTB4 is produced in neutrophils, monocytes and macrophages (Kulmatycki and Jamali 2006). LTB4 is a potent chemotactic agent for leucocytes, it increases vascular permeability, causes the release of lysosomal enzymes, and promotes the generation of reactive oxygen species and the production of inflammatory cytokines including tumour necrosis factor α (TNFα), interleukin-1 (IL-1) and interleukin-6 (IL-6). LTC4, LTD4, and LTE4 increase vascular permeability, but also cause bronchoconstriction and promote hypersensitivity reactions. Leukotrienes have been associated with a range of inflammatory conditions including asthma, colitis, dermatitis, rheumatoid arthritis and septic peritonitis, and recent work has suggested an important role for leukotrienes in the development and progression of atherosclerosis. The modification of leukotrienes as a pharmacological target has only been achieved relatively recently, but several drugs (inhibitors of leukotrienes biosynthesis and receptor antagonists) are now approved for the treatment of asthma and allergic rhinitis (Chang et al. 2002).

Plants as anti-inflammatory agents: When you examine a man with an irregular wound and that wound is inflamed [there is] a concentration of heat; the lips of that wound are reddened and that man is hot in consequence. Then you must make cooling substances for him to draw the heat out leaves of willow. The bark and leaves of willow (Salix spp.) are among the most famous plants with anti-inflammatory properties. A source of a range of salicylates, mostly in glycoside form, willow has been used medicinally for millennia. The parent compound of these salicylates, salicylic acid, was shown to be highly effective in the treatment of rheumatic fever in one of the earliest clinical trials in history (Akanji et al.1993). Its corrosive effects in the gastrointestinal tract lead to the development of a derivative,
acetylsalicylic acid, which was marketed by the German pharmaceutical company Bayer in 1899 under the name Aspirin. The most successful pharmaceutical drug of all time, acetylsalicylic acid became the prototype for the class of pharmaceuticals known as non-steroidal anti-inflammatory drugs (NSAIDs). However, it was not until 1971 that the main mechanism of action for Aspirin was elucidated. In groundbreaking work led by John Vane, who was later rewarded with a Nobel Prize, the key to Aspirin’s anti-inflammatory action was shown to be its potent ability to inhibit cyclooxygenase (Gaitan et al. 1989).

Over the past two decades, numerous plant extracts and plant compounds have been investigated for their ability to modulate inflammation. Most of these investigations have been conducted in vitro or in animal models, while only a relatively small number of human trials have been conducted in this area. Plant compounds with anti-inflammatory activity have been reviewed and arachidonic acid metabolism, nitric oxide and nuclear factor kappa B (NFkB) identified as major targets. In many cases such anti-inflammatory activity appears to be the result of the ability of a compound to inhibit the action and/or biosynthesis of pro-inflammatory cytokines, chemokines or adhesion molecules involved in the inflammatory process, for example by activating transcription factors (incl. NFkB) and protein kinases. In terms of arachidonic acid metabolism, it is noteworthy that a number of plant compounds and extracts have been shown to be dual inhibitors of cyclo-oxygenase and 5-lipoxygenase enzymes in vitro (Ajitha and Rajnarayana 2001).

Medicinal plants for antimicrobial property: The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency (Adebayo et al. 2009). Isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world. In addition to this problem antibiotics are sometimes associated with adverse side effects on the host, which include hypersensitivity, depletion of beneficial gut
and mucosal microorganisms, immunosuppression and allergic reactions. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrumantibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection. Examples include methicillin-resistant staphylococci, pneumococci resistant to penicillin and macrolides, vancomycin-resistant Enterococci as well as multi-drug resistant gram-negative organisms (Mohanasundri et al. 2007).

There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross-infection however, the development of new antibiotics should be continued as they are of primary importance to maintain the effectiveness of antimicrobial treatment. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles (Jahn 1998). Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today (Zhang et al. 2010).

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Over the years, the World Health Organization advocated that countries should encourage traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (Eilrt et al. 1981). In recent years, pharmaceutical
companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century. It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Dash et al. 2005).

The medicinal plants around the world contain many compounds with antibacterial activity. Many efforts have been made to discover new antimicrobial compounds from various sources such as micro-organisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds. The use of botanical medicines is generally on the rise in many parts of the world (Mangale et al. 2012). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents. Numerous experiments have been carried out to screen natural products for antimicrobial property. Considering the above, it can be stated that plants are valuable sources for new compounds and should receive special attention in research strategies to develop new antimicrobials urgently required in the near future.

Toxicity study: Plants, vegetables and herbs used as food and in the folk treatment have been accepted currently as one of the main source of drug discovery and development, but only a few of them have been scientifically investigated, especially about their toxic aspects. Phytotherapy has never stopped gaining popularity. In low and middle income countries, it often represents the main, if not, only therapeutic system to which majority of people are referred to for their primary health care. Its widespread use is further substantiated by the
affordability, knowledge of medicinal plants and the belief that they are harmless. The increase in number of users as opposed to the scarcity of scientific evidences on the safety of the medicinal plants, have raised concerns regarding toxicity and detrimental effects of these remedies (Dapar et al. 2007).

To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a safe dose in humans. The highest overall concordance of toxicity in animals with humans is with hematological, gastrointestinal, and cardiovascular adverse effects. Acute toxicity test gives clues on the range of doses that could be toxic to the animal; it could also be used to estimate the therapeutic index (LD50/ED50) of drugs and xenobiotics. Safety of herbal medicine: Systemic toxicity from the herbal extracts depends on the route, and site of exposure. Direct tissue damage is usually the result of cellular destruction and this may have a biochemical or immunological basis but many pathological lesions are of unknown mechanism, particularly as regards the intermediate stages between the interaction of the toxin or its metabolite with cellular constituents, and the start of the final degenerative changes that lead to cell death (Barger 2003).

Toxic effects may be detected by gross pathological examination in the post mortem or histopathological examination after toxicity studies have been carried out. Some may also be detected using clinico-chemical analysis of body fluids. Efficacy and safety of these medicinal plants have been studied by use of model animals such as BALB/c mice, albino mice, Wistar mice, rats and rabbits.

2.10. Diabetes Mellitus

In 2007, the global diabetes mellitus burden estimate was 246 million, and the International Diabetes Federation (IDF) estimates that this figure is likely to rise to 380 million by the year 2025. The World Health Organization estimates that close to 347 million people are diabetic and this number is likely to rise to 438 million by the year 2030, if proper control and management strategies are
not put in place. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000, and is expected to rise to 4.4% in 2030. WHO estimated that globally 7.1 million deaths could be attributed to high blood pressure, 4.4 million to high cholesterol, and 2.6 million to excessive body weight. In developing countries, those most frequently affected by type II diabetes are in the middle, productive years of their lives, aged between 35 and 64 years, and 55% deaths occur in women. The major factors identified for developing diabetes are inheritance (genetic predisposition) and environmental factors such as nutrition and chemical toxins (Abreu et al. 2006).

Africa and Asia are identified as regions with greatest potential where diabetics could raise two to three folds above the present level. In sub-Saharan Africa, diabetes mellitus prevalence was estimated to be 0.01% in 2000 and was projected to rise to 0.12% by the year 2025. In Kenya, the prevalence of diabetes mellitus in 2010 was estimated to be 3.3% based on regional population, and is likely to be an underestimation because over 60% of the people diagnosed with diabetes mellitus usually present to the health care facility with seemingly unrelated complaints. Similarly, two thirds of Kenyans with diabetes mellitus do not know they have the disease (Hallfrisch and Facnbehall 2000).

Pathogenesis of diabetes mellitus: The chronic hyperglycemic nature of diabetes is associated with prolonged damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathophysiological processes are involved in the development of diabetes mellitus. These range from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficiency and insufficient action of insulin on target tissues leads to carbohydrates, fats and proteins metabolism abnormalities. The presenting symptoms of hyperglycemia include; polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute life threatening consequences of uncontrolled diabetes are hyperglycemia with
ketoacidosis or the non-ketotic hyperosmolar syndrome. Long term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, charcot joints and autonomic neuropathy causing gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction. Oxidative stress has been suggested as a contributory factor in the pathogenesis of diabetes mellitus. Diabetes increases the production of tissue damaging reactive oxygen species (ROS) by glucose autoxidation and / or non-enzymatic protein glycosylation (Venn and Mann 2004).

One of the major sites at which oxidative complications to diabetes take place is the vascular endothelium. Hyperglycemia has been found to increase production of ROS such as superoxide anion (O2–), and hydrogenperoxide (H2O2) which reduce nitrogen oxide (NO) bioavailability in cultured endothelial cells, and in vascular tissue. Endothelial dysfunction is a well-documented characteristic phenomenon in diabetes mellitus, and is attributed to decreased vasorelaxant, and increased contractile responses to physiological, and pharmacological stimuli. The number of people with diabetes mellitus is on constant rise globally due to: population growth, low birth weight, aging, urbanization, sedentary lifestyles with physical inactivity, over-processed diets, smoking, psychological stress and increase in prevalence of obesity (Anderson et al. 2000).

The worldwide survey reported that DM is affecting nearly 10% of the population. This pandemic is characterized by excessive sugar in the blood due to deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced to control blood glucose. This disorder affects carbohydrate, protein and fat metabolism and chronic hyperglycemia causes glycation of body proteins that in turn leads to secondary complications that affects eyes, kidneys and nerves.

Classification of diabetes mellitus: World Health Organization classifies diabetes mellitus into insulin-dependent diabetes mellitus (IDDM) or type I, and
non-insulin-dependent diabetes mellitus (NIDDM) or type II. Insulin-dependent diabetes mellitus, also referred to as juvenile onset diabetes, is usually first diagnosed in children, teenagers and young adults. In these patients the β-cells of the pancreas no longer make insulin because the body’s immune system has destroyed them. Treatment for IDDM usually involves taking insulin shots or use of an insulin pump, wise food choices, regular exercise, controlling blood pressure and cholesterol. Type I diabetes mellitus, has multiple genetic predispositions and is related to environmental factors that are still poorly defined. It accounts for 5-10% globally of individuals with diabetes. The rate of β-cell destruction is quite variable, being rapid mainly in infants and children and slow in adults. Children and adolescents may present with keto-acidosis as the first manifestation of the disease, while others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or keto-acidosis in the presence of infection or other stress (Cohen et al. 1980).

The other category is type II diabetes whose onset is usually after 40 years of age and accounts for approximately 90-95% of the diabetes mellitus cases worldwide. It usually develops later in life though there is an increase in numbers of young patients. It is also called adults onset diabetes which affects individuals who have insulin resistance, and usually have relative insulin deficiency. Insulin resistance is defined as an inadequate response to circulating insulin by insulin target tissues like adipose, skeletal muscle and liver; and this usually precede the characteristic hyperglycemia in type II diabetes. Most patients with type II diabetes are obese which causes some degree of insulin resistance. This form of diabetes frequently goes undiagnosed for many years because hyperglycemia develops gradually and earlier stages are often not severe enough for the patients to notice any of the classic symptoms of diabetes. Although patients with this form of diabetes may have insulin levels that appear normal or elevated, higher blood glucose levels would be expected to result in even higher insulin values had their β-cells function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. The risk of developing this form of diabetes increases with age, obesity and lack of physical
activity. Other forms of diabetes mellitus include; gestational diabetes and brittle diabetes. These forms are associated with monogenic defects in β-cell function inherited in an autosomal dominant pattern, and are frequently characterized by onset of hyperglycemia at an early age, generally before age of 22 years. They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action (Beattie et al. 1988)

Major organs affected by diabetes mellitus: The physiologic effects of insulin in the body are far reaching. These also directly correlate to the effects seen in the body of either too much or too little insulin in circulation. The net effect of the hormone involves the storage, and utilization of carbohydrates, proteins and fats. Most of the effects of insulin are seen in the adipose tissue, skeletal, cardiac and smooth muscle, liver and the pancreas. Adipose tissue is involved in the maintenance of normal glucose levels in the body. Its primary role is the storage of energy in form of triglycerides with glucose disposal being the primary role for the skeletal muscle. Adipose tissue has a number of glucose transporters such as GLUT 4, GLUT 8 and GLUT 12 that are responsible for shuttling glucose into the cells. GLUT 4 is the main hexose transporter and is highly expressed in the adipose tissue. Visceral fat depots found in adipose tissue have very high lipolytic rates resulting in the release of large amounts of fatty acids into the system. Insulin normally suppresses the lipase and adipocytes triglyceride lipase enzyme that hydrolyses intracellular triglyceride but in the insulin resistant state, the activity of this enzyme is enhanced resulting in a free fatty acid flux. Adipose tissue releases large amounts of a protein known as tumour necrosis factor (TNF-α) that plays a major role in the repression of many genes in the body which are responsible for the uptake and storage of glucose as well as fatty acids. TNF-α also mediates the inflammatory process which is associated with obesity and type II diabetes (Jenkins et al. 2002).

Skeletal muscle stores glucose as glycogen which it oxidizes when needed to produce energy. It accounts for about 75% of the whole body insulin
stimulated glucose uptake. About 500-600 g of glycogen is stored in the muscle tissue of a 70 kg man, but because of the lack of glucose-6-phosphatase in this tissue, it cannot be used as a source of blood glucose except by indirectly supplying the liver with lactate for conversion to glucose. Glucose is transported into the cells through a specialized transmembrane sugar transporter known as GLUT 4, which catalyses transport of glucose through the plasma membrane. This transporter works in tandem with others like GLUT 1, 5 and 12 to enhance glucose transport via facilitative diffusion. Insulin has many effects on the muscle, with the most important ones being the increased entry of glucose. When insulin binds to its receptors, tyrosine phosphorylation of protein substrates occurs and this activates the P13 kinase pathway (Holm and Bjorck 1992).

Subsequent signaling pathways are activated with GLUT 4 eventually moving from its intracellular stores to the plasma membrane. This transporter has a unique characteristic such that its N and COOH terminals direct both endocytic and exocytic processes. Abel demonstrated that sensitivity to insulin was markedly higher as was the response of uptake of glucose in transgenic mice which had higher expression levels of GLUT 4. In type II diabetic patients there is as much as a 90% reduction in levels of GLUT 4 that are responsive to insulin. Translocation of transporters and subsequent signaling pathways are interfered with. This is one way that results in the characteristic insulin resistant (IR) state found in type II diabetics. There is also an increased GLUT 4 expression in muscle in response to exercise (Zhou et al. 2004).

The liver is the first major organ reached by insulin via the bloodstream. Insulin exerts effects on the liver by either promoting anabolism or inhibiting catabolism. The liver helps the body to maintain normal blood glucose concentrations in fasting and postprandial states. When insulin levels are low, then glycogenolysis and increased hepatic glucose production results. The liver has a maximum storage capacity of 100-110 g of glycogen or approximately 440 kilocalories of energy. Insulin promotes glycogen synthesis and storage as well as inhibits breakdown of glycogen into glucose. These effects are mediated by changes in the
activity of enzymes in the glycogen synthesis pathway. Insulin inhibits the expression of key gluconeogenic enzymes such as G-6-phosphatase leading to elevated levels of glucose production in the liver. Insulin increases both protein and triglyceride synthesis and very low density lipoproteins (VLDL) formation by the liver. Individuals with type II diabetes have a higher incidence of liver function transferases (LFTs) abnormalities than individuals without diabetes. The most common abnormality is elevated alanine aminotransferase (ALT). Antidiabetic agents have generally been shown to decrease ALT levels as tighter blood glucose levels are achieved (Drankhan et al. 2003).

The human pancreas is made up of two types of tissues, namely exocrine and endocrine. The exocrine tissue (acini) secretes digestive enzymes that help to breakdown proteins, carbohydrates, fats and acids in the duodenum while the endocrine pancreas (islets of langerhans) has a hormonal function. It produces insulin, somatostatin, gastrin and glucagon. These hormones have important roles to play in maintaining glucose and salt homeostasis in the body (Colborne and Laidman 1975).

Contributing factors for diabetes mellitus: The continued increase in prevalence of diabetes in the developing nations can be largely attributed to urbanization, westernization and economic development. The major contributing risk factors related to these are population ageing, obesity, sedentary lifestyles, over-processed diets, smoking, psychological stress and low birth weight. In obesity associated type II diabetes mellitus, there is an increased accumulation of visceral fat which contains pro-inflammatory molecules such as α-tumour necrosis factor (TNF-α), which is involved in the regulation of insulin sensitivity in the body. Other molecules such as adiponectin whose levels are low in obesity improves insulin sensitivity, reduce glucose output and fatty acid oxidation in the liver (Baravalia et al. 2009).

Poor dietary choice is a major contributing factor to obesity and associated disorders like type II diabetes mellitus. Epidemiological evidence has
demonstrated that saturated fatty acid intake is associated with increased risk of insulin resistance, diabetes and impaired glucose tolerance. The inclusion of foods rich in trans-fatty acids and high ratios of saturated to unsaturated fats results in weight gain and predisposition to diabetes. Foods such as red meats, refined grains, sweets and high fat dairy products have been linked to risks of type II diabetes. In contrast, weight loss is characterized by reduction in fat cell mass especially visceral fat which contain inflammatory markers associated with insulin resistance and decreased insulin sensitivity. Reduced visceral fat due to weight loss is accompanied by decreased adipose TNF-α release resulting to improved insulin sensitivity.

Diagnosis of diabetes mellitus: The accepted WHO criteria for diagnosis of diabetes mellitus are based on a venous plasma glucose concentration of > 11.1 mM, 2-hour after a 75 g glucose tolerance test. The criteria for abnormal glucose tolerance in pregnancy are those of Carpenter and Coustan. Recommendations from the American Diabetes Association’s Fourth International Conference on Gestational Diabetes Mellitus held in March, 1997 support the use of Carpenter and Coustan diagnostic criteria as well as the alternative use of a diagnostic 75-g, 2-hour oral glucose tolerance test (OGTT). The new diagnostic criteria for diabetes mellitus have been greatly simplified. The OGTT which was previously recommended in 1979 by the National Diabetes Data Group, has been replaced with the recommendation that diagnosis be based on two fasting plasma glucose levels of 2500 mg/L (13.8 mM) or higher.

Measurement of the fasting plasma glucose level is the preferred diagnostic test, but any combination of two abnormal test results can be used. Fasting plasma glucose is considered as the primary diagnostic test because it predicts adverse outcomes like retinopathy. Conventional management of diabetes mellitus: The mainstay of non-pharmacological treatment of diabetes is diet and physical activity. Other methods of treatment include; acupuncture, hydrotherapy, mineral supplementation and conventional drugs which include; exogenous insulin, oral hypoglycemic agents and transplantation (Hallikainen et al.2000).
Oral glucose lowering drugs belong to five classes of oral agents approved for the management of diabetes mellitus. Oral therapy is indicated in any patient in whom diet and exercise fail to achieve acceptable glycemic control. Although initial response may be good, oral hypoglycemic drugs may lose their effectiveness in a significant percentage of patients. The drug category includes; sulfonylurea, biguanide, α-glucosidase inhibitor, thiazolidinedione and meglitinide. These drugs have various side effects. For instance; sulfonylurea causes weight gain due to hyperinsulinemia, biguanide cause body weakness, fatigue, lactic acidosis and alpha glucosidase inhibitor may cause diarrhea while thiazolidinediones may increase LDL-cholesterol level. Insulin is commonly included in an oral agent when glucose control is sub-optimal at maximal dose of oral medication. Weight gain and hypoglycemia are common side effects of insulin. Vigorous insulin treatment may also carry an increase in atherogenesis (Gezr et al. 2006).

Oral glucose-lowering agent sulfonylurea, tolbutamide and glyburide act by enhancing insulin secretion from the pancreatic β cells. These act on liver cells stimulating breakdown of glucose in glycolytic pathway and inhibiting glucose generation. Sulphonylureas acts by inhibiting KATP channels in plasma membranes of pancreatic β cells. The inhibition works to stimulate the secretion of insulin which is similar to that produced by glucose in the body but is of a distinct mechanism. They may be used as first-line drugs in a case where oral hypoglycemic medication is required particularly in patients who cannot tolerate metformin. Newer drugs in this category such as glipizide and glimipramide appear to afford similar efficacy than older drugs such as gliclazide. All sulphonylureas drugs have a sulphonic acid-urea nucleus, and different chemical moieties are added at various positions on the nucleus to make different drugs. The action of the resultant drugs may have the desired effect, however, the potency and efficacy may differ significantly (Gao et al. 1999).

Sulphonylureas drugs are typically not indicated for type 1 diabetic patients since they require the functioning of the β cells to produce the desired effect
on blood glucose. They have been found to be most effective in non-obese patients with mild maturity onset diabetes and whose high glucose levels have not responded appropriately to diet alterations. Biguanides such as metformin act by increasing glucose transport across cell membrane of the skeletal muscle. They act in the presence of endogenous insulin, and are effective only where there are residual functioning pancreatic islet cells. Metformin is widely used in treatment of patients with insulin resistance because it can be used safely as an adjunct to diet therapy in obese patients to control their high glucose levels especially those who are not responsive to other therapies. The exact mode of action of metformin is disputable. It indicated that it activates adenosine monophosphate protein kinase (AMPK) in liver cells leading to increased fatty acid oxidation and glucose uptake by cells. An overall reduction in lipogenesis and hepatic glucose production is normally observed. Metformin has antioxidant properties which are useful in its use in treatment of diabetes and cardiovascular disease. It has been demonstrated to inhibit xanthine oxidase and phosphodiesterase, advanced glycation end product formation and decreased production of tumour necrosis factor (Chanda et al. 2010).

The main problem with metformin is the risk of lactic acidosis which is particularly common in patients with renal insufficiency, cardiovascular disease, peripheral vascular disease, liver disease, pulmonary disease and in individuals over 65 years. Weakness, fatigue, shortness of breath, nausea, dizziness and kidney toxicity are the side effects.

Thiazolidinediones are known to act by increasing the sensitivity of peripheral tissues to insulin by affecting the expression of specific genes. They achieve this by binding and activating \( \gamma \) peroxisome proliferator-activated receptor (PPAR-\( \gamma \)), a nuclear receptor. Some of the effects of this gene expression include the increase in the expression of the glucose transporters, decreased hepatic glucose output as well as the increased differentiation of pre-adipocytes into adipocytes. The high affinity of this drug to PPAR-\( \gamma \) is important in the management of insulin resistance since large adipocytes that differentiate from smaller ones produce TNF-\( \alpha \) which increase insulin resistance.
Thiazolidinediones therefore suppresses the expression of these adipokines involved in insulin resistance (Truswell 2002).

Alpha-glucosidase acts by inhibiting alpha glucosidase enzyme in the brush border of the small intestine. This delays the absorption of glucose by decreasing the breakdown of complex carbohydrates by enteric digestive enzymes. Some of the most commonly used α-glucosidase inhibitors like acarbose have severe gastrointestinal side effects such as diarrhoea, flatulence and abdominal pains. This raises the need for other sources of these inhibitors that have fewer side effects. The most obvious choice for these alternatives would be plants with ethnomedical uses in management of diabetes (Slavin 2003).

Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind especially in the line of medicine and pharmacology. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action in human body. The World Health Organization (WHO) has estimated that for some 3.4 billion people in the developing world, plants represent the primary source of medicines. This means that 88% of the world’s population mainly relies on traditional herbal medicines for their primary health care. Between 1983 and 1994, 41% of all globally prescribed drugs were derived from natural products. The percentage of natural products-derived drugs was 40% in 2000 which has remained approximately constant at 26% after 2001.

The phytomedicines have a long history and they are the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences of different cultures. Amerindians, black slaves from Africa and Caucasians whether explicable or not used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. Most of the plant species in the world have not yet been subjected to phytochemical studies for possible biological active constituents. On the other hand, the majority of
secondary metabolites that are identified in medicinal plants show a pleiotropic ability to interact with several target (Vanithakumari et al. 1989).

Therefore, traditional medicine offers promising solutions to face the global increasing demands for new therapeutic agents. Insufficient data exist for most plants to guarantee their quality, efficacy and safety. Plants contain hundreds of constituents and some of them are very toxic such as the most cytotoxic anti-cancer plant-derived drugs, digitalis and the pyrrolizidine alkaloids. However, the adverse effects of phytotherapeutic agents are less frequent compared with synthetic drugs, but well-controlled clinical trials have now confirmed that such effects really exist. World Health Organization Alma-Ata Declaration in 1978 opened the door for a dialogue between traditional and modern health care on the understanding that unsafe practices should be eliminated and that only what is both safe and effective should be promoted. Safety should be the overriding criterion in the selection of phytomedicines. Screening, chemical analysis, clinical trials and regulatory measures should be undertaken in respect to phytomedicines (Ferguson and Harris 1999).

Herbal management of diabetes mellitus: The systematic study of herbal medicines and the investigation of the biologically active principles of phytomedicines including their clinical applications, standardization, quality control, mode of action and potential drug interactions have emerged as one of the most exciting developments in modern therapeutics and medicine. Healthcare practitioners and medical scientists have come to accept that herbal medicines are different from the pharmacologically active molecules that they may contain. Several comparative clinical studies have been published to show that herbal medicines could have full therapeutic equivalence with chemotherapeutic agents while retaining the simultaneous advantage of being devoid of serious adverse effects. Developments in molecular biology and information technology have enhanced the understanding of the mechanism of action of many herbal drugs and the associated phytomedicines which differ in many respects from that of
synthetic drugs or single chemical entities. Herbal medicinal products are now generally available in both developed and developing countries (Dhriti et al. 2014).

Phytochemicals from roots of ginseng have been used for over 2000 years in Far East because of their health promoting effects in diabetic cases. The ginseng species most commonly used include Panax ginseng (Asian ginseng) and Panax quinquefolius (American ginseng). Their pharmacological activity is mostly attributed to ginsenosides, a family of steroids named steroidal saponins. The most commonly reported side effects of ginseng are nervousness and excitation but these diminish with continued use or dosage reduction. The hypoglycaemic activity of some medicinal plants have been identified and experimentally demonstrated in in-vivo and in-vitro diabetic models and documented in several studies. These plants include; Azadirachta indica, Cassia occidentalis linnaeus, Colocynthis citrullus, Ocimum gratissimum, Momordica charantia and Zingeber officinale among many others. The chemical composition and potency of herbal products depends on the plant extract derivative, the age of the plant part used season when harvested and the method of processing (Gazda et al. 2006).

Spices commonly used as diet adjuncts that contribute to the taste and flavour of foods have been demonstrated to have hypoglycemic activity. Among the spices, fenugreek seeds (Trigonella foenumgraecum), garlic (Allium sativum), onion (Allium cepa) and turmeric (Curcuma longa) have been experimentally documented to possess hypoglycemic potential. Herbal medicines are usually perceived by the public as being natural, safe and free from side effects and the rationale for their continued use has largely rested on long-term clinical experience. Plants contain hundreds of constituents and some of them may have toxic side effects which make it necessary to carry out toxicity studies. The continued use of herbal medicines will necessitate a thorough scientific investigation, and should go a long way in validating their folkloric usage (Okwu and Josiah 2006).
2.11. Essential micronutrients from herbs

Essential mineral elements from herbs: Medicinal plants are good and balanced sources of essential micronutrients minerals that are valuable in the management of diabetes mellitus. These micronutrients function as essential coenzymes and cofactors for metabolic reactions and thus help support basiccellular reactions such as glycolysis, the citric acid cycle, lipid and amino acid metabolism required to maintain energy production and life (O’Dell et al. 1972).

Micronutrients have been investigated as potential preventive and treatment agents for both type I and type II diabetes and for common complications of diabetes. For instance, magnesium is a cofactor in the glucose-transporting mechanism of the cell membrane and various enzymes in carbohydrate oxidation, and is thought to play a role in the release of insulin. A deficiency of magnesium is significantly more common in type II diabetes than the general population. Magnesium deficiency has been associated with complications of diabetes particularly retinopathy. Studies have shown patients with most severe retinopathy also presents with the lowest magnesium levels. On the other hand, manganese is a cofactor of various enzymes critical in cellular biochemical reactions such as activation of manganese superoxide dismutase, an antioxidant enzyme involved in the protection of cell membranes and tissues from degeneration and disruption helping the body to catabolize carbohydrates, lipids, proteins and in energy production (Dost and Tokul 2006).

Zinc is involved in the regulation of insulin receptor-initiated signal transduction mechanisms and insulin receptor synthesis. Zinc plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells. The abilities to synthesize and secrete insulin and use glucose are impaired in the zinc deficient state. Intestinal zinc absorption rates and plasma zinc levels are reduced in diabetic patients. The trace element trivalent chromium (Cr3+) is required for the maintenance of normal glucose metabolism. Experimental chromium deficiency leads to impaired glucose tolerance which improves upon the addition of chromium to the diet. Chromium plays
an important role in glucose and lipid metabolism, and dietary deficiency can cause impaired glucose tolerance which is of great importance to diabetes. Oral supplementation with chromium corrects these problems in-patients with type II diabetes and in children with protein-energy malnutrition. Such supplements have no effect in people with normal chromium intakes.

Chromium is an essential micronutrient which functions as a cofactor in insulin-regulating activities. It facilitates insulin binding and subsequent uptake of glucose into the cell and therefore decreases fasting glucose levels, improves glucose tolerance and lowers insulin levels. It also lowers total cholesterol in normal, elderly and type II diabetic subjects. Without chromium, insulin's action is blocked and glucose levels are elevated. Chromium picolinate is a form of chromium that exhibits biological activity (Maga 1982).

Trivalent chromium has long been considered to be a safe nutritional supplement. Although the hexavalent form of chromium is a known human respiratory tract carcinogen when inhaled in high-exposure industrial settings, there is no evidence of any carcinogenic effects in humans from the trivalent form of chromium found in chromium supplements. A reasonable amount of supplemental chromium is 200 μg/day. The current adequate intake (AI) for chromium is 25 μg for women and 35 μg for men. No tolerable upper intake level (UL) which has been established. Previous recommendations placed a daily intake at ≤ 200 μg/day within a safe and adequate range (Gerhardt and Galo 1998). Another important mineral element is vanadium which is known to play critical role in regulation of intracellular signaling and as a cofactor of enzymes essential in energy metabolism. It reduces the rate of gluconeogenesis and increases glycogen deposition. A reasonable amount of supplemental vanadium is 20 μg/day. Vanadyl sulfate at a dose of 100 mg/day is effective in improving insulin sensitivity (Abukakar et al. 2008).

Molybdate is an effective anti-hyperglycemic agent in diabetics with severe insulin resistance. It is associated with substantial reduction of hyper-
Insulinaemia and an increase in pancreatic insulin stores. The glucose-lowering effect of molybdenum may be partly related to reduction of hepatic glucose production, and possibly to increased glucose usage. Medicinal plants toxicities could be attributed to the high levels of mineral elements. For instance, trivalent chromium sources are not toxic. However, hexavalent chromium toxicity from industrial exposure through inhalation has been associated with increased incidence of lung cancer. In experimental animals, ingestion of chromate resulted in liver and kidney damage. Epigastric pain, diarrhea and vomiting have been observed from high zinc intake from food stored in galvanized containers. Supplements of as little as 25 mg of zinc have resulted in diminished absorption of copper, presumably because of competition. Lead toxicity produces neurological, gastrointestinal, renal, immunological, endocrinological and hematopoietic changes in humans. Supplementation of human volunteers with vanadyl compounds at oral doses of 50-125 mg/day caused cramps, loosened stools, green tongue in all patients, fatigue and lethargy in some individuals (Mecham 1971).

Essential vitamins with hypoglycemic activity from herbs: The efficacy and safety of medicinal plants in the management of diabetes mellitus is further potentiated by presence of vitamins in appropriate amounts. These vitamins includes; α-tocopherol, retinol, ascorbic acids and carotenoids such as lycopene, β-carotene and β-cryptoxanthin. Retinol also termed as vitamin A refers to a sub-class of retinoic acids, long understood to help regulate immune functions and to reduce morbidity of infectious diseases. Vitamin A is required for normal functioning of the visual system, maintenance of cell function for growth, epithelial integrity, production of red blood cells, immunity, and reproduction. Different forms of vitamin A include β carotene, which is found in plants, and preformed vitamin A, which is found in animals. Vitamin A is an essential nutrient that cannot be synthesized so it must be obtained through diet (Wieringa 1967).

Vitamin A deficiency increases vulnerability to a range of illnesses including diarrhoea, measles, and respiratory infections. These are leading causes of mortality among children in low and middle income countries, where risk of
infection and risk of mortality can be compounded by coexisting under-nutrition. The bioavailability of provitamin A carotenoids in fruit and vegetables is lower than once believed and it is difficult for children to fulfill their daily requirements through plant foods alone. Consequently, vitamin A deficiency is common among children whose families cannot afford eggs and dairy products. Preformed vitamin A (retinol, retinal, retinoic acid, and retinyl esters) is the most active in humans; it is usually used in supplements in the form of retinyl esters. High intake of synthetic vitamin A over a prolonged period can lead to toxicity, but toxicity from food sources is rare. Periodic supplementation should not cause serious adverse effects (Evans et al. 1973).

Alpha-tocopherol also called vitamin E is a collective name for a group of fat-soluble compounds with distinctive antioxidant activities that includes tocopherols and tocotrienols. It is found naturally in some foods, added to others, and available as a dietary supplement. Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma- and delta-tocopherol and alpha-, beta-, gamma- and delta-tocotrienol) that have varying levels of biological activity. The only form of tocopherol that is nutritionally important to humans is α-tocopherol (Miller et al. 2000).

Serum concentrations of α-tocopherol depend on the liver, which takes up the nutrient after the various forms are absorbed from the small intestine. The liver preferentially re-secretes only α-tocopherol via the hepatic α-tocopherol transfer protein, the liver metabolizes and excretes the other vitamin E forms. As a result, blood and cellular concentrations of other forms of vitamin E are lower than those of α-tocopherol and has been the subject of less research. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species (ROS) formed when fat undergoes oxidation. Scientists are investigating whether, by limiting free-radical production and possibly through other mechanisms, vitamin E might help prevent or delay the chronic diseases associated with free radicals. In addition to its activities as an antioxidant, vitamin E is involved in cell signaling, regulation of gene expression and other metabolic processes. Usual dietary
intakes are estimated at 7–11 mg/day. The recommended daily/dietary allowance (RDA) for alpha-tocopherol is 15 mg/day for people of 15 years of age and older. The UL for alpha-tocopherol is 1,000 mg/day from supplemental sources. Natural vitamin E (d-alpha tocopherol) has approximately twice the bioactivity of synthetic forms of the vitamin (Slavin et al. 2001).

Vitamin B1 is also termed as thiamine and has two primary functions; alpha-keto acid decarboxylation and transketolation. Decarboxylation reactions are an integral part of carbohydrate metabolism. Thiamine is involved in the alpha-keto acid decarboxylation of pyruvate, alpha-ketoglutarate and the branched-chain alpha-keto acids such as leucine, isoleucine and valine metabolites. Transketolation is involved in the pentose phosphate pathways (Nawar 1985).

Thiamine is converted to its active form, thiamine pyrophosphate. The thiamine-dependent enzymes are important for the biosynthesis of neurotransmitters and for the production of reducing substances used in oxidative stress defenses, as well as for the biosynthesis of pentoses used as nucleic acid precursors. Thiamine plays a central role in cerebral metabolism. Its deficiency results in dry and wet beriberi, a peripheral neuropathy, acardiomyopathy with edema and lactic acidosis and Wernicke–Korsakoff syndrome, whose manifestations consist of nystagmus, ophthalmoplegia and ataxia evolving into confusion, retrograde amnesia, cognitive impairment and confabulation. Vitamin B3 occurs in two forms: nicotinic acid and nicotinamide. The active coenzyme forms, nicotinamide adenine dinucleotide (NAD) and NAD phosphate are essential for the function of hundreds of enzymes and normal carbohydrate, lipid and protein metabolism. As a vitamin, the two compounds function similarly, but in pharmacological doses they have distinct effects (Yamaguchi et al. 2000).

Nicotinic acid at a dose range of 1-3 g/day is an effective treatment for dyslipidemia, although its use in people with diabetes has been limited because of its negative effect on glycemic control. The daily requirements intakes (DRIs) for niacin are reported in niacin equivalents (NE) because niacin can be
synthesized by the body from tryptophan. The recommended daily/dietary allowance (RDA) is 14 mg NE for women and 16 mg NE for men. The UL is 35 mg NE/day for adults (Miller et al. 1993).

Animal studies suggests that nicotinamide acts by protecting pancreatic β-cells from autoimmune destruction by maintaining intracellular NAD levels and inhibiting the enzyme poly (ADP-ribose) polymerase (PARP), an enzyme involved in DNA repair. Nicotinamide also acts as a weak antioxidant of nitric oxide radicals. Natural ascorbic acid is vital for the body performance. Vitamin C is an antioxidant which acts as an electron donor for 8 human enzymes; three of which participate in collagen hydroxylation and two in carnitine biosynthesis; of the three enzymes which participate in collagen hydroxylation, one is necessary for biosynthesis of the catecholamine and norepinephrine, the other is involved in amidation of peptide hormones, and the last one in tyrosine metabolism (Yu et al. 2002).

Vitamin C protects low-density lipoproteins ex vivo against oxidation and may function similarly in the blood. A common feature of vitamin C deficiency is anemia. The antioxidant property of vitamin C stabilizes folate in food and in plasma. Vitamin C promotes absorption of soluble non-haem iron by chelation or by maintaining the iron in the reduced (ferrous, Fe2+) form. However, the amount of dietary vitamin C required to increase iron absorption ranges from 25 mg upwards and depends on the amount of inhibitors, such as phytates and polyphenols, present in the meal. Carotenoids from herbs: Among all the pigments present in living organisms, there is no doubt that the carotenoids are the most widely distributed in nature. They are found throughout the plant kingdom in both photosynthetic and non-photosynthetic tissues in bacteria, in fungi and in animals. The latter are unable to synthesize carotenoids and therefore they incorporate them from dietary plants (Acqua and Innocenti 2004).

Carotenoids are natural pigments synthesized by plants and microorganisms, but not by animals. These include; lycopenes which have two
acyclic end groups and β-carotene which has two cyclohexene type end groups; oxygenated carotenoids also called xanthophylls. Examples of these compounds are; a) zeaxanthin and lutein (hydroxy), b) spirilloxanthin (methoxy), c) echinenone (oxo) and d) antheraxanthin (epoxy).

Many epidemiologic studies have associated high carotenoid intake with a decrease in the incidence of chronic disease. However, their biological mechanism remains elusive. Multiple possibilities exist. Certain carotenoids i) can be converted to retinoids to have provitamin A activity, ii) can modulate the enzymatic activities of lipoxygenases such as proinflammatory and immunomodulatory molecules, iii) can have antioxidants properties which are well above what is seen with vitamin A, iv) can activate the expression of genes which encode the message for production of a protein, connexin 43, which is an integral component of the gap junctions required for cell to cell communication. Such gene activation is not associated with antioxidant capacity and is independent of provitamin A activity (Baki et al. 2007).

Beta-carotene is the main compound with provitamin A activity. When incorporated into the diet, it is broken down into two molecules of retinol (vitamin A) by action of the enzyme β-carotene-15 15'-dioxygenase in the intestine. However, β-carotene is not the only carotenoid with provitamin A activity, and, any carotenoid with at least one unsubstituted β-ring can undergo similar cleavages and give rise to a vitamin A molecule. However, carotenoids such as α-carotene and β-cryptoxanthin can thus contribute substantially to the nutritional value of fruits and vegetables. Current attention is centered on the action of β-carotene as an antioxidant, as it may interfere with free radical oxidation such as the peroxidation of lipids, typical of many degenerative diseases. Although it has been clearly demonstrated that β-carotene has a significant antioxidant effect in vitro, there is still no real proof that it's in vivo function at the low concentrations in which it is found and under physiological conditions (Ajitha and Rajnarayana 2001).
There are 650 known naturally occurring carotenoids. Most of the carotenoids found naturally in fruits and vegetables present skeleton of 40 carbon atoms (C40) and are biosynthesized from molecules of an intermediary C20 (geranylgeranyldiphosphate), giving rise to a phytoene as generic precursor of the whole wide range of carotenoids present in the plant kingdom. The phytoene molecule undergoes a series of successive desaturations (up to four), introducing new double bonds into the carbon chain, resulting in spreading of the double bond conjugation and thus of the chromophore that is typical of these natural pigments and responsible for their chromatic properties (Akah et al. 2009).

Lycopene (carotene), is an acyclic carotenoid containing 11 conjugated double bonds, is naturally present in trans-form in raw tomatoes and imparts red color to the tomatoes. It is one of the most abundant non-vitamin analogues present in human blood from food consumption. Among the common dietary carotenoids, lycopene has the highest single oxygen quenching capacity in vitro and its antioxidant properties are probably related to risk reduction of certain types of cancers. It has been found that after air-drying at 80°C, the number of hydroxyl phenol groups increases owing to the hydrolysis of flavonoid glycosides and or the release of cell wall phenolics. Processing promotes different side reactions that could affect the antioxidant activity of the plant products.
CHAPTER 3

MATERIALS AND METHODS

3.1. Plant Collection & Identification

Dried seeds of Bambusa arundinacea (Retz) Roxb were collected from amirthi forest & wild life conservation centre, javathu hills, vellore district. The seeds and plants were authenticated by Prof. Dr. Jayaraman. Director, Plant Anatomy Research Institute, Tambaram, Chennai.

3.2. Pharmacognostic analysis

It is very important and in fact essential to lay down pharmacognostic specifications of medicinal plants which are used in various drugs.

3.2.1. Macroscopic characteristics

The seed was macroscopically examined for shape, size, surface characteristics, texture, color, consistency, odour, taste, etc. (Khandelwal, 2007).

3.2.2. Microscopic characteristics

The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μm. Dewaxing of the sections was by customary procedure. The sections were stained with Toluidine blue as per the method. Same
procedure was followed for microscopic characteristics of powdered material of seed of Bambusa arundinacea (Khandelwal 2007).

3.3. Extraction of Plant material

The plant materials were air-dried at room temperature (26°C) for two weeks, after which it was ground to a uniform powder. The powdered (100gm) was extracted three times by cold percolation method with 300 ml of Hexane, acetone and hydro ethanol at room temperature for 72hrs the filtrates were concentrated under reduced pressure at 40°C and stored in refrigerator at 2-8°C for use in subsequent experiments (Bele and Khale2011).

3.4. Physicochemical analysis

The following physicochemical parameters were carried out in dried seed powder and methanolic extract of Bambusa arundinacea (Holecheck et al. 1982).

- Loss on drying
- Total ash
- Acid insoluble ash
- Water soluble ash
- Petroleum ether soluble extractive
- Alcohol soluble extractive
- Methanol soluble extractive
- Water soluble extractive
- Solubility test
- pH
- Melting and Boiling point
- Heavy metal analysis

3.4.1. Loss on drying:

Two grams of crude powder of B. arundinacea (seed) was taken in an evaporating dish and then dried in an oven at 105 °C till constant weight was
obtained. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially.

3.4.2. Total ash

Two grams of dried powder of B.arundinacea (seed) was taken in a silica crucible and ignited it by gradually increasing the heat to 500 °C in a muffle furnace until it was white, indicating the absence of carbon. Ash was cooled in a desiccator and weighed without delay. The percentage was calculated on the basis of sample taken initially.

3.4.3. Acid insoluble ash

To the crucible containing total ash, 25 ml of hydrochloric acid (HCl, ~70g/l) was added; it was covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and it was washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible; it was dried on a hot plate and ignited to constant weight. The residue was allowed to cool and then weighed without delay. The percentage was calculated on the basis of sample taken initially.

3.4.4. Water soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper. It was washed with hot water and ignited in a crucible for 15 minutes. The residue was allowed to cool and then weighed without delay. Weight of insoluble matter was subtracted from the weight of total ash. The percentage was calculated on the basis of sample taken initially.

3.4.5. Determination of petroleum ether soluble extractive

Five grams of dried seed powder of B. arundinacea was taken in 100 ml of petroleum ether in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated
to dryness at 105 °C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.6. Determination of alcohol soluble extractive

Five grams of dried seed powder of B. arundinacea was taken in 100 ml of alcohol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.7. Determination of methanol soluble extractive

Five grams of dried seed powder of B. arundinacea was taken in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.8. Determination of water soluble extractive

Five grams of dried seed powder of B. arundinacea was taken in 100 ml of water in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, it was filtered and the filtrate was evaporated to dryness at 105°C till constant weight was obtained. The percentage of extractable matter was calculated with reference to the sample taken initially.

3.4.9. Solubility

The quantitative solubility test of hydro ethanolic extract of B. arundinacea was determined in different solvents. 5 mg of extract was weighed for solubility test for different solvents. The extract was added in each solvent until saturated solution developed. Solubility was calculated in mg/ml.
3.4.10. **Determination of pH**

The hydro ethanolic extract of B. arundinacea was dissolved in distilled water and was kept in a water bath for 20 min. It was then filtered and the pH of the filtrate was noted with the help of a Systronic pH meter (pH system 361).

3.4.11. **Determination of melting and boiling point**

The melting and boiling point of the hydroethanolic extract of B. arundinacea were done at Department of Biotechnology, Bharat University, chennai, India by open capillary method.

3.4.12. **Determination of heavy metals**

The analysis for heavy metals like arsenic, chromium, cadmium, lead, and mercury for hydro ethanolic extract of B. arundinacea were done at Biozone private Limited,Chennai.

3.5. **Phytochemical Analysis**

Phytochemical examinations were carried out for the extract as per the standard methods (Bele and Khale2011).

3.5.1. **Qualitative phytochemical analysis**

Various extracts of Bambusa arundinaca seeds were tested for the presence of phytochemicals.

3.5.1.1. **Test for Carbohydrates**

To 2ml of plant extract, 1ml of Molisch’s reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.
3.5.1.2. Test for Flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

3.5.1.3. Test for Alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

3.5.1.4. Test for Quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

3.5.1.5. Test for Glycosides

To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

3.5.1.6. Test for Cardiac Glycosides

To 0.5ml of extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at the interface indicates presence of cardiac glycosides.

3.5.1.7. Test for Tannins

To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

3.5.1.8. Test for Saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.
3.5.1.9. Test for Terpenoids
To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

3.5.1.10. Test for Phenols
To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

3.5.1.11. Test for Coumarins
To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

3.5.1.12. Test for Steroids and Phytosteroids
To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

3.5.1.13. Test for Phlobatannins
To 1ml of plant extract few drops of 2% HCL was added appearance of red color precipitate indicates the presence of phlobatannins.

3.5.1.14. Test for Anthraquinones
To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

3.5.2. Quantitative Phytochemical Analysis
The amount of phytochemicals were quantified by using standard methods of HPLC.
3.5.2.1. Determination of Total Phenol

The amount of total phenol content was determined by Folin-Ciocalteu reagent method (Sagwan et al. 2010). The samples were shaken on a reciprocating shaker for 24 h at room temperature. The contents were centrifuged at 10,000 x g for 5 min and the supernatant was used for further analysis. The extracted free phenolics were estimated following the method. One-ml aliquots of the above extract were pipetted into different test tubes to which 1-ml of folin-phenol reagent and 2 ml of 20 % (w/v) Na2CO3 solution were added. The tubes were shaken and placed in a boiling water bath for exactly 1 min and then were cooled under running tap water. The resulting blue solution was diluted to 25 ml with distilled water and the absorbance was measured at 650 nm with a Spectronic 20D spectrophotometer. If precipitation occurred, it was removed by centrifugation at 5000 x g for 10 min before measuring the absorbance. The amount of phenolics present in the sample was determined from a standard curve prepared with catechol. A blank containing all the reagents minus plant extract was used to adjust the absorbance to zero. Average values of triplicate estimations were expressed as g 100 g-1 of the sample on a dry weight basis.

3.5.2.2. Determination of Flavonoids

Chromatographic system: The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph Standard solution 1, and record the peak responses (Harshal and Priscilla 2011). Procedure: Separately inject equal volumes (about 20 μL) of each of the Standard solutions and the Test solution into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each flavonoids in sample.

Extraction solvent- Prepare a mixture of alcohol, water, and hydrochloric acid (50:20:8).

Mobile phase - Prepare a mixture of methanol, water, and phosphoric acid (100:100:1).

Make adjustments if necessary (System Suitability under Chromatography à 621 ų).
Standard solutions-Transfer accurately weighed quantities of USP Quercetin RS, kaempferol, and isorhamnetin to separate volumetric flasks, dissolve each in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain Standard solutions 1 mg per mL, respectively.

Test solution-Transfer about 10.0 g of sample given finely powdered and accurately weighed, to a 250-mL flask fitted with a reflux condenser. Add 78 mL of Extraction solvent, and reflux on a hot water bath for 135 minutes. [NOTE: The solution will turn deep red. The color of the solution is not a definitive indication of reaction completeness.] Allow to cool at room temperature. Decant to a 100-mL volumetric flask. Add 20 mL of methanol to the 250-mL flask, and sonicate for 30 minutes. Filter, collect the filtrate in the 100-mL volumetric flask, wash the residue on the filter with methanol, collect the washing in the same 100-mL volumetric flask, dilute to volume and mix.

Method:2: The amount of total flavonoid content was determined by Aluminium chloride method (Aastha Bhardwaj et al.2014). The reaction mixture consisted of 1.0 ml extract, 1 ml methanol, 0.5 ml aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 415 nm. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

3.5.2.3. Determination of Alkaloids

Chromatographic system: The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm × 150-mm column that contains packing L1. The flow rate is about 1.8 mL per minute. Procedure: Separately inject equal volumes (about 10 μL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentages in the portion of 100(CV/W) (r U / r S), in which C is the concentration, in mg per mL, Reference Standard in the Standard solution using the correction factors as noted above; V is the final volume, in mL, of the Test solution; W is the weight, in mg, of taken; and r U and r S are the peak areas from the Test solution and the Standard solution. Mobile phase- Dissolve 9.93 g of monobasic potassium phosphate in 730
mL of distilled water. Add 270 mL of acetonitrile, mix, filter, and degas. Make other adjustments if necessary. Standard solution- Dissolve accurately weighed quantities of in a mixture of water and methanol (1:1) to obtain a solution containing about 0.2 mg of each USP Reference Standard per mL. Test solution- Finely powder an amount of sample and transfer 1.0 g, accurately weighed, to a continuous-extraction apparatus with a 500-mL round-bottom flask. Treat the Golden seal with 150 mL of methanol, and extract for 6 hours, or until the solvent is clear. The volume of the thimble should be at least one-half that of the volume of methanol. Cool to room temperature, and transfer the methanol extract to a 200-mL volumetric flask. Rinse the extraction unit with methanol, quantitatively transfer the contents to the volumetric flask, and dilute with methanol to volume.

3.5.2.4. Determination of Glycosides

Transfer the organic layer (sample in methanol) to a suitable flask and remove the solvent by distillation, evaporating almost to dryness. Dissolve the residue in 0.3 ml to 0.5 ml of methanol R and transfer to a volumetric flask, rinsing the first flask with warm water R and adding the rinsings to the methanolic solution. Allow to cool and dilute to 50.0 ml with water R. Transfer 20.0 ml of the solution to a 100 ml round-bottomed flask with a ground-glass neck and containing 2 g of ferric chloride R and 12 ml of hydrochloric acid R. Attach a reflux condenser and place the flask in a water-bath so that the level of the water is above that of the liquid in the flask and heat for 4 h. Allow to cool, transfer the solution to a separating funnel and rinse the flask successively with 3 ml to 4 ml of 1 M sodium hydroxide and 3 ml to 4 ml of water R, adding the rinsings to the separating funnel. Shake the contents of the separating funnel with three quantities, each of 30 ml, of a mixture of 1 volume of ether R and 3 volumes of hexane R. Wash the combined organic layers with two quantities, each of 10 ml, of water R and discard the rinsings. Dilute the organic phase to 100.0 ml with the mixture of ether and hexane. Take 20.0 ml, evaporate carefully to dryness on a water-bath and dissolve the residue in 10.0 ml of a 5 g/l solution of magnesium acetate R in methanol R. Measure the absorbance at 515 nm using methanol R as the compensation liquid (Adebajo et al. 2006).
Calculate the percentage content of hydroxyanthracene glycosides, expressed as cascaroside A, from the expression:

\[ A \times 6.95 \]

\[ m \]

i.e. taking the specific absorbance to be 180.

\[ A = \text{absorbance at } 515 \text{ nm}, \]

\[ m = \text{mass of the sample in grams}. \]

Measure the absorbance of the test solution at 440 nm

### 3.5.2.5. Determination of steroids

Standard solution: Weigh accurately a suitable quantity of the reference substance specified in the individual monograph, previously dried under the conditions specified in the monograph, and dissolve in a suitable volume of aldehyde - free ethanol. dilute quantitatively and stepwise with aldehyde - free ethanol to obtain a solution containing about 10 \( \mu \text{g} \) of the steroid per ml.

Test Solution: Prepare as directed in the individual monograph.

Method: Into a glass - stoppered, 50 ml, conical flask pipette 20.0 ml of the test solution. Into two similar flasks pipette 20.0 ml of the standard solution and 20.0 ml of aldehyde - free ethanol (Blank) respectively. To each flask add 2.0 ml of blue tetrazolium solution and mix; to each flask add 2.0 ml of the mixture of 10 volumes of tetraneethylammonium hydroxide solution (10%) and 90 volumes of aldehyde - free ethanol, mix and allow to stand in the dark a temperature between 25° c and 35 °c . At the end of exactly 90 minutes add to each flask 1.0 ml of glacial acetic acid and mix. Measure the absorbences of the solutions obtained from the test solution and the standard solution at about 525 nm against the blank.

The quantity in mg, of the steroid in the 20-ml aliquot of the test solution is given by the expression

\[ \text{At} / \text{As} \times \text{Cs} \]
Where At = absorbance of the test solution;

As = absorbance of the standard solution;

Cs = quantity, in mg, of the reference substance in the 20-ml aliquot of the standard solution.

Calculate the quantity of the steroid in the substance being examined on the basis of the aliquot the test solution taken for the determination and from the declared content of the steroid in the appropriate reference substance.

3.5.2.6. Determination of Quinones

Mobile phase-Prepare a filtered and degassed mixture of methanol an dehydrated alcohol (13:7). Make adjustments if necessary.

System suitability preparation- Dissolve accurately weighed quantities of USP quinines RS in dehydrated alcohol, heating at about 50° for 2 minutes if necessary, to obtain a solution having known concentrations of about 0.5 mg of each per mL.

Standard preparation- Dissolve an accurately weighed quantity of USP dehydrated alcohol, heating at about 50° for 2 minutes if necessary, to obtain a solution having a known concentration of about 1.0 mg per mL. Assay preparation- Transfer about 5.0 gm itter gourd accurately weighed, to a 50-mL volumetric flask, dissolve in dehydrated alcohol, heating at about 50° for 2 minutes if necessary, cool, dilute with dehydrated alcohol to volume, and mix.

Chromatographic system: The liquid chromatograph is equipped with a 275-nm detector and a 5-mm × 15-cm column that contains packing L1, and is maintained at a temperature of 35°. The flow rate is adjusted to obtain a retention time of about 11 minutes. Chromatograph the System suitability preparation, and record the peak responses the Standard preparation, respectively.

3.5.2.7. Determination of saponins

Solvent 1- Prepare a mixture of methanol and water (65:35).

Solvent 2- Use the lower phase of a mixture of 30 mL of 0.1 N hydrochloric acid, 20 mL of 1-propanol, and 50 mL of chloroform.
Reagent-Dissolve 75 mg of ferric chloride in 50 mL of glacial acetic acid. Add 50 mL of sulfuric acid, while shaking and cooling. Prepare immediately before use.

Escin standard solutions-Dissolve an accurately weighed quantity of USP Escin RS in glacial acetic acid, shaking for 1 minute. Dilute quantitatively, and stepwise if necessary, to obtain solutions having known concentrations of about 0.6, 0.4, and 0.2 mg per mL.

Test solution- Accurately weigh 1.00 g of ground seeds, and place in a 250-mL round-bottom flask. Add exactly 100 mL of Solvent 1, and weigh the filled flask with a precision of ±0.1 g. Attach a condenser to the flask, reflux for 30 minutes, and allow to cool. Adjust to the initial weight by adding Solvent 1 as needed, mix, and filter. Transfer 30.0 mL of the filtrate to a round-bottom flask, and evaporate the solvents under vacuum. Dissolve the residue with 20 mL of 0.1 N hydrochloric acid, and quantitatively transfer with the aid of two additional 5-mL portions of 0.1 N hydrochloric acid to a 250-mL separation funnel. Add 20 mL of 1-propanol and 50 mL of chloroform, and shake vigorously for 2 minutes. Separate the chloroform layer, and add Solvent 2 to the upper phase remaining in the separation funnel. Shake vigorously for 2 minutes, and separate the chloroform layer. Combine the chloroform layers in a round-bottom flask, and evaporate to dryness under vacuum. Evaporate the remaining solvents with the aid of a current of air. Wash the residue with two 10-mL portions of ether, filter, wash the filter with 10 mL of ether, and discard the ether filtrates. After evaporation of the residual ether, add to the residue a 10-mL portion of glacial acetic acid, and pass through the previously used dried filter into a 50-mL volumetric flask. Repeat the addition of glacial acetic acid followed by filtration two additional times, combining the filtrates in the volumetric flask. Wash the round-bottom flask with small quantities of glacial acetic acid, and filter into the volumetric flask. Dilute with glacial acetic acid to volume.

Procedure: Transfer 1 mL each of the Escin standard solutions, the Test solution, and glacial acetic acid to separate test tubes with stoppers. Add 4.0 mL of Reagent to each tube, cap the tubes, and place them in a water bath at 60° for 25 minutes, shaking occasionally. Measure the absorbances at 540 nm of the reacted Test solution and the reacted Escin standard solutions, using glacial acetic acid as the blank. Plot the absorbances obtained from the reacted Escin standard solutions versus
concentrations, in mg per mL, of USP Escin RS in the corresponding Escin standard solution. From the graphs so obtained, determine the concentration, C, in mg per mL, of triterpene glycosides as escin (C 55H 86O 24) in the Test solution. Calculate the percentage of triterpene glycosides in the portion of Horse Chestnut taken by the formula: (50/3)(C/W), in which C is the concentration, in mg per mL, of triterpene glycosides in the Test solution as obtained above; and W is the weight, in g of bambusa seed taken to prepare the Test solution.

3.5.2.8. Determination of Tannins

Carry out all the extraction and dilution operations protected from light. In the case of a herbal drug or a dry extract, to the stated amount of the powdered drug (180) or the extract in a 250 ml round-bottomed flask add 150 ml of water R. Heat on a water-bath for 30 min. Cool under running water and transfer quantitatively to a 250 ml volumetric flask. Rinse the round-bottomed flask and collect the washings in the volumetric flask, then dilute to 250.0 ml with water R. Allow the solids to settle and filter the liquid through a filter paper 125 mm in diameter. Discard the first 50 ml of the filtrate (Wainer 1995).

In the case of a liquid extract or a tincture, dilute the stated amount of the liquid extract or tincture to 250.0 ml with water R. Filter the mixture through a filter paper 125 mm in diameter. Discard the first 50 ml of the filtrate.

Total Polyphenols: Dilute 5.0 ml of the filtrate to 25.0 ml with water R. Mix 2.0 ml of this solution with 1.0 ml of phosphomolybdate tungstic reagent R and 10.0 ml of water R and dilute to 25.0 ml with a 290 g/l solution of sodium carbonate R. After 30 min measure the absorbance (2.2.25) at 760 nm (A_1), using water R as the compensation liquid.

Polyphenols Not Adsorbed by Hide Powder: To 10.0 ml of the filtrate, add 0.10 g of hide powder CRS and shake vigorously for 60 min. Filter and dilute 5.0 ml of the filtrate to 25.0 ml with water R. Mix 2.0 ml of this solution with 1.0 ml of phosphomolybdate tungstic reagent R and 10.0 ml of water R and dilute to 25.0 ml with a 290 g/l solution of sodium carbonate R. After 30 min measure the absorbance at 760 nm (A_2), using water R as the compensation liquid.
Standard: Dissolve immediately before use 50.0 mg of pyrogallol R in water Rand dilute to 100.0 ml with the same solvent. Dilute 5.0 ml of the solution to 100.0 ml with water R. Mix 2.0 ml of this solution with 1.0 ml of phosphomolybdotungstic reagent R and 10.0 ml of water R and dilute to 25.0 ml with a 290 g/l solution of sodium carbonate R. After 30 min measure the absorbance at 760 nm (A₃), using water R as the compensation liquid.

Calculate the percentage content of tannins expressed as pyrogallol from the expression:

\[ \frac{62.5(A₁ - A₂)m₂}{A₃Xm₁} \]

\( m₁ \) = mass of the sample to be examined, in grams,
\( m₂ \) = mass of pyrogallol, in grams.

### 3.5.2.9. Determination of Terpenoids

Test solution: Dilute the mixture of sample extract and xylene R to 10 ml Standard: Anethole is mixed with xylene to 10 ml. The chromatographic procedure may be carried out using:

A capillary column 30 m to 60 m long and 0.3 mm in internal diameter coated with macrogol 20 000 R. Nitrogen for chromatography R as the carrier gas at a flow rate of 0.40 ml/min and split at a ratio of 1 to 200, a flame-ionisation detector, maintaining the temperature of the column at 60 °C for 4 min, then raising the temperature linearly at a rate of 5 °C per minute to 170 °C and maintaining at 170 °C for 15 min and maintaining the temperature of the injection port at 220 °C and that of the detector at 270 °C. Inject 1 µl of each solution. Determine the content of anethole by normalisation.

### 3.5.2.10. Estimation of Carbohydrate by Gravimetry

Procedure: Pipet 50 mL of alkaline cupric tartrate TS into a 400-mL beaker, add 48 mL of water, mix, and pipet into the mixture 2 mL of Injection that has been diluted quantitatively with water, if necessary, to a 5.0% concentration. Cover the beaker with
a watch glass, heat the solution, regulating the heat so that boiling begins in 4 minutes, and continue boiling for 2.0 minutes. Filter the hot solution at once through a tared porcelain filtering crucible, wash the precipitate with water maintained at 60°, then with 10 mL of alcohol. Dry at 105° to constant weight. Perform a blank determination, and make any necessary correction (Giddings and Ra 1984). The corrected weight of the precipitate is compared with dextrose of known concentration.

3.5.2.11. Estimation of Protein Content

Place a quantity of the substance to be examined (m g) containing about 2 mg of nitrogen in a combustion flask, add 4 g of a powdered mixture of 100 g of dipotassium sulphateR, 5 g of copper sulphate R and 2.5 g of selenium R, and three glass beads. Wash any adhering particles from the neck into the flask with 5 ml of sulphuric acid R, allowing it to run down the sides of the flask, and mix the contents by rotation (Lowry et al. 1951). Close the mouth of the flask loosely, for example by means of a glass bulb with a short stem, to avoid excessive loss of sulphuric acid. Heat gradually at first, then increase the temperature until there is vigorous boiling with condensation of sulphuric acid in the neck of the flask; precautions should be taken to prevent the upper part of the flask from becoming overheated. Continue the heating for 30 min, unless otherwise prescribed. Cool, dissolve the solid material by cautiously adding to the mixture 25 ml of waterR, cool again and place in a steam-distillation apparatus. Add 30 ml of strong sodium hydroxide solution R and distil immediately by passing steam through the mixture. Collect about 40 ml of distillate in 20.0 ml of 0.01M hydrochloric acid and enough waterR to cover the tip of the condenser. Towards the end of the distillation, lower the receiver so that the tip of the condenser is above the surface of the acid. Take precautions to prevent any water on the outer surface of the condenser from reaching the contents of the receiver. Titrate the distillate with 0.01M sodium hydroxide , using methyl red mixed solution R as indicator (n₁ ml of 0.01M sodium hydroxide ).

Repeat the test using about 50 mg of glucose R in place of the substance to be examined (n₂ ml of 0.01M sodium hydroxide ).
Content of nitrogen = 0.01401(n2 – n1) percent

m

Nitrogen content x 6.25 = protein.

3.2.5.12. Aminoacid Quantification by HPLC

Test solution: Dissolve the substance to be examined in the mobile phase to obtain a concentration of 1.0 mg/ml.

Reference solution (a) Dissolve mixed amino acids CRS in the mobile phase to obtain a concentration of 1.0 mg/ml.

Column: size: l = 0.10 m, Ø = 4.6 mm;

stationary phase: octadecysilyl silica gel for chromatography R (3 µm).

Mobile phase Dissolve about 15.2 g of triethylamine R in 800 ml of water R, adjust to pH 3.0 with phosphoric acid R and dilute to 1000 ml with water R. Add 850 ml of this solution to 150 ml of a mixture of 2 volumes of propanol R and 3 volumes of acetonitrile R.

Flow rate 1.0-1.5 ml/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µl of test solution /standardsolution.

Run time 90 min.

3.5.2.13. Estimation of Total Lipids

Dissolve 30 g in 100 ml of water, transfer to a separating funnel, acidify with 1M sulphuric acid and extract with successive quantities of 50, 40 and 30 ml of ether. Mix the ether solutions in a separating funnel and wash with water until the washings are free from mineral acid. Transfer the ether solution to a tared flask, remove the ether and dry the residue of fatty acids to constant weight at 80° (Bartnik and Jakubczyk 1989).
3.2.5.14. Estimation of Fatty acid

Test solution: Introduce about 0.45 g of the substance to be examined into a 10 ml volumetric flask, dissolve in hexane R containing 50 mg of butylhydroxytoluene R per litre and dilute to 10.0 ml with the same solvent. Transfer 2.0 ml of the solution into a quartz tube and evaporate the solvent with a gentle current of nitrogen R. Add 1.5 ml of a 20 g/l solution of sodium hydroxide R in methanol R, cover with nitrogen R, cap tightly with a polytetrafluoroethylene lined cap, mix and heat in a water-bath for 7 min. Cool, add 2 ml of boron trichloride-methanol solution R, cover with nitrogen R, cap tightly, mix and heat in a water-bath for 30 min. Cool to 40-50 °C, add 1 ml of trimethylpentane R, cap and vortex or shake vigorously for at least 30 s. Immediately add 5 ml of saturated sodium chloride solution R, cover with nitrogen R, cap and vortex or shake thoroughly for at least 15 s. Allow the upper layer to become clear and transfer to a separate tube. Shake the methanol layer once more with 1 ml of trimethylpentane R and combine the trimethylpentane extracts. Wash the combined extracts with 2 quantities, each of 1 ml, of water R and dry over anhydrous sodium sulphate R. Prepare 2 solutions for each sample.

Columnmaterial: fused silica,

Size: 1 = 30 m, Ø = 0.25 mm,
Stationary phase: macrogol 20 000 R (film thickness 0.25 μm).
Carrier gas hydrogen for chromatography R or helium for chromatography R, where oxygen scrubber is applied.
Split ratio1:200.
Temperature: 170°C – 225°C
Detection Flame ionisation.
Injection1 μl, twice.
System suitability: The 15 fatty acids to be tested are satisfactorily identified from the chromatogram

3.5.2.15. Quantification of Vitamins

Vitamin A, Ascorbic Acid, Pyridoxine Hydrochloride, Riboflavin, and Thiamine Hydrochloride or Thiamine Mononitrate; and one or more minerals derived
from substances generally recognized as safe, furnishing one or more of the following elements in ionizable form: calcium, chromium, copper, fluorine, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, and zinc (Yu et al. 2003). Mobile phase: Use n-hexane.

Standard preparation: Dissolve an accurately weighed quantity of USP Vitamin A RS in n-hexane, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 15 μg of retinyl acetate per mL. System suitability preparation: Dissolve an accurately weighed quantity of retinyl palmitate in n-hexane to obtain a solution having a concentration of about 15 μg of retinyl palmitate per mL. Mix equal volumes of this solution and the Standard preparation to obtain a solution having concentrations of 7.5 μg each of retinyl acetate and retinyl palmitate per mL.

Assay preparation: Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 5 Tablets, to a container having a polytetrafluoroethylene screw cap, add 10 mL of dimethyl sulfoxide and 15 mL of n-hexane, and shake for 45 minutes on a wrist-action shaker in a water bath maintained at 60°. [NOTE: Set up the wrist-action shaker to ensure that the contents of the container are mixed vigorously and thoroughly.] Centrifuge at 3000 rpm for 10 minutes, and transfer the hexane layer by means of a pipet to a 100-mL volumetric flask. Add 15 mL of n-hexane to the dimethyl sulfoxide layer, shake thoroughly for 5 minutes, and transfer the hexane layer by means of a pipet to the 100-mL volumetric flask. Repeat this extraction with three additional 15-mL portions of n-hexane. Dilute the extracts in the volumetric flask with n-hexane to volume, and mix. Quantitatively dilute a 10-mL volume of this solution with n-hexane to obtain a solution having a final concentration of about 15 μg of vitamin A per mL. Retain the remaining solution for use in the assays for vitamin D, vitamin E, and phytonadione.

Chromatographic system (Chromatography á 621 ñ): The liquid chromatograph is equipped with a 325-nm detector and a 4.6-mm × 15-cm column that contains 3-μm packing L8. The flow rate is about 1 mL per minute. Chromatograph the System
suitability preparation, and measure the peak responses as directed for Procedure: the resolution, \( R \), between retinylacetate and retinyl palmitate is not less than 10; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure: Separately inject equal volumes (about 40 \( \mu \)L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak area for retinyl acetate obtained from the Standard preparation and the peak area for retinylacetate or retinyl palmitate in the chromatogram of the Assay preparation. For products containing vitamin A acetate or vitamin A palmitate, calculate the quantity, in mg, of vitamin A as the retinol equivalent (C 20H 300) in the portion of Tablets taken by the formula: 0.872CD(r U / r S), in which 0.872 is the factor used to convert retinyl acetate, obtained from USP Vitamin A RS, to its retinol equivalent; \( C \) is the concentration, in mg per mL, of USP Vitamin A RS in the Standard preparation; \( D \) is the dilution factor, in mL, for the Assay preparation; and \( r U \) and \( r S \) are the peak areas of the all-trans retinyl ester obtained from the Assay preparation and the Standard preparation, respectively. [NOTE: The molar responses of retinyl acetate and retinyl palmitate are equivalent].

2,2,4-trimethylpentane, mix on a vortex mixer to form a suspension, and shake for 10 minutes. Centrifuge for about 10 minutes to break up the emulsion and to clarify the supernatant layer. [NOTE: The supernatant layer is used for the determination of vitamin A, and also vitamin D and vitamin E, if present in the formulation. ] If necessary, quantitatively dilute a volume of the supernatant layer with 2,2,4-trimethylpentane to obtain a solution having a concentration close to that of the Standard preparation.

Chromatographic system (Chromatography á 621 ñ ): The liquid chromatograph is equipped with a 325-nm detector and a 4.6-mm \( \times \) 25-cm column that contains 5-\( \mu \)m packingL24. The flow rate is about 1.5 mL per minute. Chromatograph the System suitability preparation, and measure the peak areas as directed for Procedure: the resolution, \( R \), between retinyl acetate and retinyl palmitate is not less than 8.0; and the relative standard deviation for replicate injections is not more than 3.0%.
Procedure: Separately inject equal volumes (about 40 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak area of retinyl acetate obtained from the Standard preparation and the peak area of retinylacetate or retinyl palmitate obtained from the Assay preparation. Calculate the quantity, in mg, of vitamin A, as the retinol (C 20H 30O) equivalent, in the portion of Tablets taken by the formula: 26.5ECD(r U / r S), in which E is the factor used to convert the retinyl acetate, obtained from USP Vitamin A RS, to its retinol equivalent, the factor being 0.872; C is the concentration, in mg per mL, of USPVitamin A RS in the Standard preparation; D is the dilution factor, in mL, used to prepare the Assay preparation from the supernatant layer; and r U and r S are the peak responses of the all-trans retinyl ester obtained from the Assay preparation and the Standard preparation, respectively. [NOTE: The initial extraction into 26.5 mL of 2,2,4-trimethylpentane is already accounted for in this equation. The molar responses of retinyl acetate and retinyl palmitate are equivalent].

**Assay for vitamin A:** Method 3: [NOTE -Where a vitamin A ester (retinyl acetate or retinyl palmitate) is indicated in the following procedure, use the chemical form present in the formulation. USP Vitamin A RS is all-trans retinyl acetate. It is to be used where USP Vitamin A RS is specified. Use low-actinic glassware throughout this procedure].

Extraction solvent-Prepare a mixture of n-hexane and methylene chloride (3:1).

Potassium hydroxide solution-Cautiously add 80 g of potassium hydroxide to 100 mL of water, mix, and cool.

Diluting solution- Transfer 1.0 g of pyrogallol to a 100-mL volumetric flask, and add alcohol to dissolve. Dilute with alcohol to volume, and mix.

Mobile phase-Prepare a mixture of n-hexane and isopropyl alcohol (92:8). Make adjustments if necessary (System Suitability under Chromatography á 621 #).

Standard stock solution-Dissolve an accurately weighed quantity of USP Vitamin A RS in Diluting solution, and dilute quantitatively, and stepwise if necessary, with
Diluting solution to obtain a solution having a known concentration of about 30 \( \mu \text{g} \) per mL. This solution may be stored in a refrigerator for one week.

Standard preparation—Quantitatively dilute an accurately measured volume of Standard stock solution with Diluting solution to obtain a solution having a known concentration of about 1 \( \mu \text{g} \) of USP Vitamin A RS per mL. Transfer 10.0 mL of this solution to a stoppered 125-mL flask, and add 5 mL of water, 5 mL of Diluting solution, and 3 mL of Potassium hydroxide solution. Insert the stopper tightly, shake for 15 minutes over a water bath maintained at 60 ± 5°, and cool to room temperature. Add 7 mL of water and 25.0 mL of Extraction solvent. Insert the stopper tightly, and shake vigorously for 60 seconds. Rinse the sides of the flask with about 60 mL of water, and allow to stand for about 10 minutes until the layers separate. Withdraw a portion of the organic layer for injection into the chromatograph. This Standard preparation contains about 0.34 \( \mu \text{g} \) of retinol per mL.

Assay preparation—Weigh and finely powder a counted number of Tablets. Transfer an inaccurately weighed portion of the powder, equivalent to about 1.5 mg of retinyl acetate, to a stoppered 125-mL flask. Add 5 mL of water, 15 mL of Diluting solution, and 3 mL of Potassium hydroxide solution. Insert the stopper tightly, shake for 15 minutes over a water bath maintained at 60 ± 5°, and cool to room temperature. Add 7 mL of water and 25.0 mL of Extraction solvent. Insert the stopper tightly, and shake vigorously for 60 seconds or longer, if necessary, for complete extraction. Rinse the sides of the flask with about 60 mL of water, and allow to stand for about 10 minutes until the layers separate. [NOTE—Do not shake, as an emulsion may form]. Withdraw a portion of the organic layer, and dilute quantitatively, and stepwise if necessary, with Extraction solvent to obtain a solution having a concentration of about 0.34 \( \mu \text{g} \) of retinol per mL.

Chromatographic system (Chromatography á 621 ã): The liquid chromatograph is equipped with a 335-nm detector and a 6.2-mm \( \times \) 8-cm column that contains packing L3. The column temperature is maintained at 40°, and the flow rate is about 4 mL per minute. Chromatograph the Standard preparation, and measure the peak areas.
as directed for Procedure: the relative retention times are about 0.92 for 13-cis retinol and 1.0 for all-trans retinol; and the relative standard deviation for replicate injections is not more than 5.0%.

Procedure: Separately inject equal volumes (about 50 μL) of the Standard preparation and the assay preparation into the chromatograph, record the chromatograms, and measure the peak areas for all-trans retinol and 13-cis retinol. Calculate the quantity, in mg, of vitamin A, as theretinol (C 20H 30O) equivalent, in the portion of Tablets taken by the formula: 0.872CD(r U / r S), in which 0.872 is the factor used to convert retinyl acetate, obtained from USP Vitamin A RS, to its retinol equivalent; C is the concentration, in mg per mL, of USP Vitamin A RS in the Standard preparation; D is the dilution factor, in mL, used to prepare the Assay preparation; r U is the sum of the areas of the all-trans retinol and 13-cis retinol peaks obtained from the assay preparation; and r S is the peak areas of all-trans retinyl acetate obtained from the Standard preparation.

Assay for cholecalciferol: or ergocalciferol (vitamin D), Method 1: [NOTE Where vitamin D (cholecalciferol or ergocalciferol) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard. Use low-actinic glassware throughout this procedure]. Mobile phase: Prepare a filtered and degassed mixture of n-hexane and isopropyl alcohol(99:1). Make adjustments if necessary (System Suitability under Chromatography á 621 ū).

Standard preparation: Dissolve an accurately weighed quantity of USP Cholecalciferol RS or USP Ergocalciferol RS in n-hexane, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 2 μg per mL. System suitability preparation: Heat a volume of the Standard preparation at 60° for 1 hour to partially isomerize vitamin D (cholecalciferol or ergocalciferol) to its corresponding precursor.

Assay preparation: Transfer not less than 20 mL, accurately measured, of the solution retained as specified in the directions for Assay preparation in the Assay for vitamin A, Method 1, to a suitable container, and evaporate, if necessary, in vacuum at
room temperature to obtain a solution having a concentration of about 2 µg of cholecalciferol or ergocalciferol per mL.

Chromatographic system (Chromatography á 621 ñ): The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm × 15-cm column that contains 3-µm packing L8. The flow rate is about 1 mL per minute. Chromatograph the System suitability preparation, and record the peak heights as directed for Procedure: the resolution, R, between the vitamin D3 form present and its corresponding precursor is not less than 10. Chromatograph the Standard preparation, and record the peak heights as directed for Procedure: the relative standard deviation for replicate injections is not more than 3.0%.

Procedure: Separately inject equal volumes (about 100 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the assay for vitamin E, Method I: [NOTE -Where vitamin E (alpha tocopherol, alphatocopheryl acetate, or alpha tocopheryl acid succinate) is specified in the following procedure, use the chemical form present in the formulation and the relevant USP Reference Standard. Use low-actinic glassware throughout this procedure].

Mobile phase - Dilute 10 mL of phosphoric acid with water to 1000 mL to obtain Solution A. Prepare a filtered and degassed mixture of methanol and Solution A (95:5). Make adjustments if necessary (System Suitability under Chromatography á 621 ñ).

Standard preparation: Dissolve an accurately weighed quantity of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, or USP Alpha Tocopheryl Acid Succinate RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 2 mg per mL.

System suitability preparation: Dissolve an accurately weighed quantity of USP Ergocalciferol RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a concentration of 0.65 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask containing about 100 mg of USP Alpha Tocopheryl Acetate RS, accurately weighed. Dissolve in 30 mL of methanol,
with the aid of sonication if necessary, dilute with methanol to volume, and mix. Store this solution in a refrigerator. Volume to obtain a solution having a concentration of about 2 mg of alpha tocopherol, alphatocopheryl acetate, or alpha tocopheryl acid succinate per mL.

Chromatographic system (Chromatography á 621 ŋ ) : The liquid chromatograph is equipped with a 254-nm detector and an 8-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the System suitability preparation, and record the peak areas as directed for Procedure: the relative retention times are about 0.5 for ergocalciferol and 1.0 for alpha tocopheryl acetate; the resolution, R, between ergocalciferol and alpha tocopheryl acetate is not less than 12; and the tailing factor is between 0.8 and 1.2. Chromatograph the Standard preparation, and record the peak areas as directed for Procedure: the relative standard deviation for replicate injections is not more than 3.0%. Procedure - Separately inject equal volumes (about 100 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the quantity, in mg, of alpha tocopherol (C 29H 50O 2), alphatocopherylacetate (C 31H 52O 3), or alpha tocopheryl acid succinate (C 33H 54O 5) in the portion of Tablets taken by the formula:

$$CD(r U / r S)$$

in which C is the concentration, in mg per mL, of the corresponding USP Reference Standard in the Standard preparation; D is the dilution factor, in mL, for the Assay preparation; and r U and r S are the peak responses for the relevant vitamin E form obtained from the Assay preparation and the Standard preparation, respectively. Calculate the alpha tocopherol equivalent of alphatocopheryl acetate or alpha tocopheryl acid succinate by multiplying the content, in mg, by the factor 0.91 or 0.81, respectively. Assay preparation - Transfer not less than 20 mL, accurately measured, of the solution retained as specified in the directions for assay preparation in the Assay for vitamin A, Method 1 to a suitable container, and evaporate in vacuum at room temperature to dryness. Transfer the residue with the aid of methanol to a suitable volumetric flask, and dilute with methanol to Mobile phase - Transfer 240 mL of methanol to a 1000-mL volumetric flask, add 10 mL of water followed by 0.5 mL of
50 percent phosphoric acid, dilute with acetonitrile to volume, mix, filter, and degas. Make adjustments if necessary System Suitability under Chromatography á621 ſ).

System suitability preparation: Dissolve accurately weighed quantities of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, and USP Alpha Tocopheryl Acid Succinate RS in methanol, and dilute quantitatively with methanol to obtain a solution having known concentrations of about 2 mg of each USP Reference Standard per mL. Standard preparation: Dissolve an accurately weighed quantity of USP Alpha Tocopherol RS, USP Alpha Tocopheryl Acetate RS, or USP Alpha Tocopheryl Acid Succinate RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 2 mg per mL (Beniwal et al. 1996).

Assay preparation: Proceed as directed for Assay preparation in the Assay for vitamin A, Method 2. Transfer an accurately measured volume of the supernatant 2,2,4-trimethylpentane layer to a suitable volumetric flask, the volume of the specimen withdrawn from the 2,2,4-trimethylpentane layer and the size of the volumetric flask being such that the final concentration of the Assay preparation is equivalent to that of the Standard preparation. Evaporate nearly to dryness, add several mL of methanol, and evaporate the remaining 2,2,4-trimethylpentane. Dilute with methanol to volume, and mix.

Chromatographic system (Chromatography á621 ſ): The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the System suitability preparation, and record the peak areas as directed for Procedure: the relative retention times for alphatocopheryl acid succinate, alpha tocopherol, and alpha tocopheryl acetate are about 0.6, 0.8, and 1.0, respectively; the resolution between alpha tocopheryl acid succinate and alphatocopherol is not less than 4.0; and the resolution between alpha tocopherol and alphatocopheryl acetate is not less than 3.0. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 3.0%.
Procedure: Separately inject equal volumes (about 25 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the quantity, in mg, of alpha tocopherol (C 29H 50O 2), alpha tocopheryl acetate(C 31H 52O 3), or alpha tocopheryl acid succinate (C 33H 54O 5) in the portion of Tablets taken by the formula:

\[ 26.5CD(r \ U / r \ S), \]

in which C is the concentration, in mg per mL, of the corresponding USP Reference Standard in the Standard preparation; D is the dilution factor used in exchanging the solvent from 2,2,4-trimethylpentane to methanol; and r U and r S are the peak areas of the relevant vitamin E form obtained from the Assay preparation and the Standard preparation, respectively. [NOTE- The initial extraction to 26.5 mL of 2,2,4-trimethylpentane has been accounted for in the calculation formula]. Calculate the alpha tocopherol equivalent of alpha tocopheryl acetate or alpha tocopheryl acid succinate by multiplying the content, in mg, by the factor 0.91 or 0.81.

**Assay for beta carotene:** [NOTE-Use low-actinic glassware throughout this procedure]. Potassium hydroxide solution: Dissolve 58.8 g of potassium hydroxide in 50 mL of water. Iodine solution. Transfer about 10 mg of iodine to a 100-mL volumetric flask. Dissolve incyclohexane, dilute with cyclohexane to volume, and mix. Dilute 10 mL of this solution with cyclohexane to 100 mL, and mix. [NOTE- Prepare this solution fresh daily].

Assay preparation: Weigh accurately not fewer than 20 Tablets. Grind the Tablets to a fine powder, and transfer an accurately weighed quantity of the powder, equivalent to about 2 mg of beta carotene, to a 500-mL saponification flask. Add 100 mL of alcohol, 6 mL of Potassium hydroxide solution, and a magnetic stirring bar. Attach an air condenser to the flask, and heat under reflux for 45 minutes with constant stirring. Cool to room temperature, add 170 mL of solvent hexane, and stir for 30 minutes. Quantitatively transfer the contents of the flask to a 500-mL separatory funnel with portions of solvent hexane. Allow the layers to separate for 5 to 10 minutes, and transfer the upper organic layer to a 500-mL volumetric flask. Transfer the lower aqueous layer into the saponification flask, add 170 mL of solvent
hexane, and stir for an additional 20 minutes. Quantitatively transfer the contents of the saponification flask to the separatory funnel with the aid of portions of solvent hexane. Allow the layers to separate for 10 minutes. Drain the lower aqueous layer, and discard. Transfer the organic layer to the volumetric flask containing the previously collected organic layer. Rinse the separatory funnel with small portions of solvent hexane, and transfer the washings to the volumetric flask. Dilute the hexane extracts with solvent hexane to volume, add 3 g of anhydrous sodium sulfate, shake, and allow to settle. Quantitatively transfer a volume of this solution, equivalent to about 100 µg of betacarotene, to a 50-mL volumetric flask. Evaporate under a stream of nitrogen to dryness, and immediately add cyclohexane. Add 2 mL of iodine solution, and heat for 15 minutes in a water bath maintained at 65°C. Cool rapidly, dilute with cyclohexane to volume, and mix.

Procedure: Determine the absorbance of the Assay preparation at the wavelength of maximum absorbance at about 452 nm, using cyclohexane as the blank. Calculate the quantity, in mg, of beta carotene (C40H56) in the Tablets taken by the formula:

\[(L/D)(A_{U}/223),\]

in which L is the labeled amount, in mg, of beta carotene in each Tablet; D is the concentration, in mg per mL, of beta carotene in the Assay preparation, based on the labeled quantity per Tablet and the extent of dilution; A U is the absorbance of the Assay preparation; and 223 is the absorptivity of beta carotene at 452 nm.

**Assay for ascorbic acid**: Method 1: Weigh and powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of ascorbic acid, to a 200-mL volumetric flask, and add about 75 mL of metaphosphoric-acetic acids TS. Insert a stopper into the flask, and shake by mechanical means for about 30 minutes. Dilute with water to volume, and mix. Transfer a portion of the solution to a centrifuge tube, and centrifuge until an clear supernatant is obtained. Pipet 4.0 mL of this solution into a 50-mL conical flask, add 5 mL of metaphosphoric-acetic acids TS, and titrate with standard dichlorophenol-indophenol solution.
**Assay for niacin:** or niacinamide, pyridoxine hydrochloride, riboflavin, and thiamine.

Method 1: [NOTE -Use low-actinic glassware throughout this procedure].

Diluting solution- Prepare a mixture of water, acetonitrile, and glacial acetic acid (94:5:1).

Mobile phase- Prepare a mixture of water, methanol, and glacial acetic acid (73:27:1) containing 140 mg of sodium 1-hexanesulfonate per 100 mL. Make adjustments if necessary (System Suitability under Chromatography á 621 á).

Standard preparation: [NOTE -Use USP Niacin RS in place of USP Niacinamide RS forformulations containing Niacin] . Transfer about 80 mg of USP Niacinamide RS, 20 mg ofUSP Pyridoxine Hydrochloride RS, 20 mg of USP Riboflavin RS, and 20 mg of USP ThiamineHydrochloride RS, each accurately weighed, to a 200-mL volumetric flask, and add about 180 mL of Diluting solution. Immerse the flask in a hot water bath maintained at 65° to 70° for about10 minutes with regular shaking or using a vortex mixer, until all the solid materials aredissolved. Chill rapidly in a cold water bath for about 10 minutes to room temperature, dilute withDiluting solution to volume, and mix.

Assay preparation: Weigh and finely powder not fewer than 30 Tablets, and transfer anaccurately weighed portion of the powder, equivalent to about 10 mg of niacinamide and 2.5 mg each of pyridoxine hydrochloride, riboflavin, and thiamine hydrochloride, to a 50-mL centrifuge tube. Add 25.0 mL of Diluting solution, and mix, using a vortex mixer, for 30 seconds tocompletely suspend the powder. Immerse the centrifuge tube in a hot water bath maintained at65° to 70°, heat for 5 minutes, and mix on a vortex mixer for 30 seconds. Return the tube to thehot water bath, heat for another 5 minutes, and mix on a vortex mixer for 30 seconds. Filter portion of the solution, cool to room temperature, and use the clear filtrate. [NOTE- Use thefiltrate within 3 hours of filtration].
Chromatographic system (Chromatography á 621 n): The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak areas as directed for Procedure: the relative retention times of niacinamide, pyridoxine, riboflavin, and thiamine are about 0.3, 0.5, 0.8, and 1.0, respectively; and the relative standard deviation for replicate injections is not more than 3.0%.

Procedure: Separately inject equal volumes (about 10 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas for niacin or niacinamide, pyridoxine, riboflavin, and thiamine. Calculate the quantity, in mg, of niacinamide (C 6H 6N 2O) in the portion of Tablets taken by the formula:

\[ 25C(r \ U / r \ S), \]

in which C is the concentration, in mg per mL, of USP Niacinamide RS in the Standard preparation; and r U and r S are the peak responses of niacinamide obtained from the Assay preparation and the Standard preparation, respectively. For formulations containing niacin, C is the concentration, in mg per mL, of USP Niacin RS in the Standard preparation; and r U and r S are the peak areas for niacin obtained from the assay preparation and the Standard preparation, respectively. Separately calculate the quantities, in mg, of pyridoxine hydrochloride (C 8H 11NO 3-HCl), riboflavin (C 17H 20N 4O 6), and thiamine hydrochloride (C 12H 17CIN 4OS-HCl) in the portion of Tablets taken by the same formula, in which C is the concentration, in mg per mL, of the relevant USP Reference Standard in the Standard preparation; and r U and r S are the peak areas for the corresponding vitamin obtained from the Assay preparation and the Standard preparation, respectively. For products containing thiamine mononitrate, calculate the quantity, in mg, of thiamine mononitrate (C 12H 17N 5O 4S), in the portion of Tablets taken by the formula:

\[ (327.36/337.27)(25C(r \ U / r \ S)), \]

in which 327.36 and 337.27 are the molecular weights of thiamine mononitrate and thiamine hydrochloride, respectively; and the other terms are as previously defined.
Assay for niacin, Method 2: [NOTE: Use low-actinic glassware throughout this procedure].

Extraction solvent: Transfer 1 mL of glacial acetic acid and 2.5 g of edetate disodium to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Prepare a mixture of this solution and methanol (75:25). Mobile phase: Prepare a 0.1 M sodium acetate solution by dissolving 13.6 g of sodium acetate in 1000 mL of water. Adjust with acetic acid to a pH of 5.4, and mix. Make adjustments if necessary (System Suitability under Chromatography á 621 ř). [NOTE: A small amount of methanol (up to 1%) may be added to the Mobile phase to improve resolution].

Standard stock solution: Dissolve an accurately weighed quantity of USP Niacin RS in Extraction solvent, and dilute quantitatively, and stepwise if necessary, with Extraction solvent to obtain a solution having a known concentration of about 1 mg per mL.

Standard preparation: Transfer 5.0 mL of Standard stock solution to a 25-mL volumetric flask, dilute with Extraction solvent to volume, and mix.

Assay preparation: [NOTE: This preparation is suitable for the determination of niacin or niacinamide, pyridoxine, and riboflavin, when present in the formulation]. Weigh and finely powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 2 mg of riboflavin, to a 200-mL volumetric flask. If riboflavin is not present in the formulation, transfer a portion equivalent to about 2 mg of pyridoxine. If pyridoxine is not present in the formulation, transfer a portion equivalent to about 20 mg of niacin or niacinamide. Add 100.0 mL of Extraction solvent, and mix for 20 minutes, using a wrist-action shaker. Immerse the flask in a water bath maintained at 70° to 75°, and heat for 20 minutes. Mix on a vortex mixer for 30 seconds, cool to room temperature, and filter. Use the clear filtrate.

Chromatographic system (Chromatography á 621 ř): The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak areas as directed for Procedure: the relative standard
deviation for replicate injections is not more than 3.0%. If necessary, flush the column with methanol between injections.

Procedure: Separately inject equal volumes (about 20 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas for niacin. Calculate the quantity, in mg, of niacin (C6H5NO2) in the portion of Tablets taken by the formula:

\[ 100C(\text{r U} / \text{r S}) , \]

in which C is the concentration, in mg per mL, of USP Niacin RS in the Standard preparation; and \text{r U} and \text{r S} are the peak responses of niacin obtained from the Assay preparation and the Standard preparation, respectively.

**Assay for niacinamide:** Method 2: [NOTE: Use low-actinic glassware throughout this procedure].

Extraction solvent, Mobile phase, Standard stock solution, Standard preparation, Assay preparation, and Chromatographic system—Using USP Niacinamide RS in place of USP Niacin RS, proceed, as directed in the Assay for niacin.

Method 2: Procedure—Separately inject equal volumes (about 20 μL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas for niacinamide. Calculate the quantity, in mg, of niacinamide (C6H6N2O) in the portion of Tablets taken by the formula:

\[ 100C(\text{r U} / \text{r S}) , \]

in which C is the concentration, in mg per mL, of USP Niacinamide RS in the Standard preparation; and \text{r U} and \text{r S} are the peak responses of niacinamide obtained from the Assay preparation and the Standard preparation, respectively.

**Assay for pyridoxine hydrochloride**, Method 2: [NOTE-Use low-actinic glassware throughout this procedure].

Extraction solvent and Mobile phase—Prepare as directed in the assay for niacin.
Method 2: Standard stock solution- Dissolve an accurately weighed quantity of USP Pyridoxine Hydrochloride RS in Extraction solvent, and dilute quantitatively, and stepwise if necessary, with Extraction solvent to obtain a solution having a known concentration of about 0.1 mg per mL.

Standard preparation-Transfer 5.0 mL of Standard stock solution to a 25-mL volumetric flask, dilute with Extraction solvent to volume, and mix.

Assay preparation- Prepare as directed for Assay preparation in the Assay for niacin.

Method 2: Chromatographic system (Chromatography â 621 n ) : The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak areas as directed for Procedure: the relative standard deviation for replicate injections is not more than 3.0%.

Procedure: Separately inject equal volumes (about 20 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas for pyridoxine. Calculate the quantity, in mg, of pyridoxine hydrochloride (C8H11NO3·HCl) in the portion of Tablets taken by the formula:

\[ 100C(rU/\sigma S), \]

in which C is the concentration, in mg per mL, of USP Pyridoxine Hydrochloride RS in the Standard preparation; and \( rU \) and \( rS \) are the peak areas obtained from the Assay preparation and the Standard preparation, respectively.

Assay for riboflavin: Method 2 : [NOTE - Use low-actinic glassware throughout this procedure].

Extraction solvent-Prepare as directed in the Assay for niacin.

Mobile phase- Dissolve 6.8 g of sodium acetate in 1000 mL of water, and mix. Prepare a mixture of this solution and methanol (65:35). Add 2 mL of triethylamine for every 1000 mL of the mixture, adjust with glacial acetic acid to a pH of 5.2, and mix. Make adjustments if necessary (System Suitability under Chromatography â 621 n).
Standard stock solution: Transfer about 20 mg of USP Riboflavin RS, accurately weighed, to a 200-mL volumetric flask. Add about 180 mL of Extraction solvent, and immerse the flask for about 5 minutes in a water bath maintained at 65° to 75°. Mix well, and repeat if necessary until dissolved. Chill rapidly in a cold water bath to room temperature, dilute with Extraction solvent to volume, and mix.

Standard preparation: Quantitatively dilute 5.0 mL of Standard stock solution with Extraction solvent to 25.0 mL, and mix.

Assay preparation: Prepare as directed for Assay preparation in the Assay for niacin.

Method 2: Chromatographic system (see Chromatography \( \lambda \) 621 \( \bar{n} \)). The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm x 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the Standard preparation, and record the peak areas as directed for Procedure: the relative standard deviation for replicate injections is not more than 3.0%.

Procedure: Separately inject equal volumes (about 20 \( \mu \)L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas for riboflavin. Calculate the quantity, in mg, of riboflavin (C17H20N4O6) in the portion of Tablets taken by the formula:

\[
100C(rU / rS),
\]

in which C is the concentration, in mg per mL, of USP Riboflavin RS in the Standard preparation; and \( rU \) and \( rS \) are the peak areas obtained from the Assay preparation and the Standard preparation, respectively.

**Assay for thiamine**: Method 2: [NOTE - Use low-actinic glassware throughout this procedure]. Mobile phase—Dissolve 1.88 g of sodium 1-hexanesulfonate in 1 liter of 0.1% phosphoric acid. Prepare a mixture of this solution and acetonitrile (92:18). Make adjustments if necessary (see System Suitability under Chromatography \( \lambda \) 621 \( \bar{n} \)).

Standard stock solution—Dissolve an accurately weighed quantity of USP Thiamine Hydrochloride RS in 0.2 N hydrochloric acid, and dilute quantitatively, and
stepwise if necessary, with 0.2 N hydrochloric acid to obtain a solution having a
known concentration of about 0.1 mg per mL.

Standard preparation- Transfer 5.0 mL of Standard stock solution to a 25-mL
volumetric flask, dilute with 0.2 N hydrochloric acid to volume, and mix.

Assay preparation: Weigh and finely powder not fewer than 20 Tablets. Transfer an
accurately weighed portion of the powder, equivalent to about 2 mg of thiamine, to a
200-mL volumetric flask. Add 100.0 mL of 0.2 N hydrochloric acid, shake for 15
minutes with a wrist-action shaker, and heat to boiling for 30 minutes. Cool to room
temperature, and filter. Use the clear filtrate.

Chromatographic system (Chromatography á 621 ñ) The liquid chromatograph
is equipped with a 254-mm detector and a 4.6-mm × 25-cm column that contains
packing L1. The flow rate is about 2 mL per minute. Chromatograph the Standard
preparation, and record the peak areas as directed for Procedure: the relative standard
deviation for replicate injections is not more than 3.0%.

Procedure: Separately inject equal volumes (about 20 µL) of the Standard preparation
and the Assay preparation into the chromatograph, record the chromatograms, and
measure the areas for the major peaks. For products containing thiamine
hydrochloride, calculate the quantity, in mg, of thiamine hydrochloride (C12H17NOS·HCl) in the portion of Tablets taken by the formula:

\[100C \times \left(\frac{r \text{ U}}{r \text{ S}}\right)\]

in which C is the concentration, in mg per mL, of USP Thiamine Hydrochloride RS
in the Standard preparation; and \( r \text{ U} \) and \( r \text{ S} \) are the peak areas for thiamine obtained
from the Assay preparation and the Standard preparation, respectively. For products
containing thiamine mononitrate, calculate the quantity, in mg, of thiamine
mononitrate (C12H17N5O4S) in the portion of Tablets taken by the formula:

\[(327.36/337.27) \times (100C) \times \left(\frac{r \text{ U}}{r \text{ S}}\right)\]

in which 327.36 and 337.27 are the molecular weights of thiamine mononitrate and
thiamine hydrochloride, respectively; and the other terms are as previously defined.
Assay for niacin or niacinamide, pyridoxine hydrochloride, riboflavin, and thiamine.

Method 3 [NOTE -Use low-actinic glassware throughout this procedure].

Reagent: Prepare a solution containing 25 g of edetate disodium in 1000 mL of water.

Mobile phase: Transfer 0.4 mL of triethylamine, 15.0 mL of glacial acetic acid, and 350 mL of methanol to a 2000-mL volumetric flask, dilute with 0.008 M sodium 1-hexanesulfonate to volume, and mix. Filter, and degas. Make adjustments if necessary (see System Suitability under Chromatography § 621 ñ).

Standard stock solution: Dissolve accurately weighed quantities of USP Niacin RS or USPNiacinamide RS, USP Pyridoxine Hydrochloride RS, USP Riboflavin RS, and USP Thiamine Hydrochloride RS in Reagent, with heating if necessary, to obtain a solution having known concentrations of about 1.5 mg of USP Niacin RS or USP Niacinamide RS per mL, 0.24 mg of USP Pyridoxine Hydrochloride RS per mL, 0.08 mg of USP Riboflavin RS per mL, and 0.24 mg of USP Thiamine Hydrochloride RS per mL.

Standard preparation: Transfer 5.0 mL of Standard stock solution to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 minutes in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Assay preparation: Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 7.5 mg of niacin or niacinamide, 1.2 mg of pyridoxine hydrochloride, 0.4 mg of riboflavin, and 1.2 mg of thiamine hydrochloride, to a stoppered 125-mL flask. Add 10.0 mL of a mixture of methanol and glacial acetic acid (9:1) and 30.0 mL of a mixture of methanol and ethylene glycol (1:1). Insert the stopper, shake for 15 minutes in a water bath maintained at 60°, and cool. Filter, discarding the first few mL of the filtrate.

Chromatographic system (Chromatography § 621 ñ ) The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The column is maintained at 50°, and the flow rate is about 2.0 mL per
minute. Chromatograph the Standard preparation, and record the peak areas as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure: Separately inject equal volumes (about 5 µL) of the Assay preparation and the Standard preparation into the chromatograph, record the chromatograms, and measure the areas of the peak responses. Calculate the quantity, in mg, of niacin (C6H5NO2) or niacinamide (C6H6N2O) in the portion of Tablets taken by the formula:

\[ 40C(r U / r S), \]

in which C is the concentration, in mg per mL, of USP Niacin RS or USP Niacinamide RS in the Standard preparation; and r U and r S are the peak areas for niacin or niacinamide obtained from the Assay preparation and the Standard preparation, respectively. Separately calculate the quantities, in mg, of pyridoxine hydrochloride (C8H11NO3·HCl) and riboflavin (C17H20N4O6) in the portion of Tablets taken by the same formula, in which the terms are as defined therein. For products containing thiamine hydrochloride, calculate the quantity, in mg, of thiamine (C12H17CIN4O4S) in the portion of Tablets taken by the same formula. For products containing thiaminemononitrate, calculate the quantity, in mg, of thiamine mononitrate (C12H17N5O4S) in the portion of Tablets taken by the formula:

\[ (327.36/337.27)(40C)(r U / r S), \]

in which 327.36 and 337.27 are the molecular weights of thiamine mononitrate and thiaminehydrochloride, respectively; and the other terms are as previously defined.

NOTE: Commercially available atomic absorption standard solutions for the minerals, where applicable, may be used where preparation of a standard stock solution is described in the following Assays. Use deionized water where water is specified. Where atomic absorptionspectrophotometry is specified in the Assay, the Standard preparations and the Assay preparation may be diluted quantitatively with the solvent specified, if necessary, to yield solutions of suitable concentrations adaptable to the linear or working range of the instrument.
3.5.2.16. Quantification of Minerals

Lanthanum chloride solution: Dissolve 26.7 g of lanthanum chloride heptahydrate in 0.125 N hydrochloric acid to make 100.0 mL (Ajaiyeobu 2002). Calcium standard stock solution: Weigh accurately about 1.001 g of calcium carbonate, previously dried at 300° for 3 hours and cooled in a desiccator for 2 hours, and dissolve in 25 mL of 1 N hydrochloric acid. Boil to expel carbon dioxide, and dilute with water to 1000 mL to obtain a solution having a known concentration of about 400 µg of calcium per mL.

Standard preparations: Quantitatively dilute a volume of Calcium standard stock solution with 0.125 N hydrochloric acid to obtain a standard solution having a known concentration of about 100 µg of calcium per mL. Into separate 100-mL volumetric flasks, pipet 3.0 mL of the standard solution, each flask add 1.0 mL of Lanthanum chloride solution, dilute with water to volume, and mix to obtain solutions having known concentrations of about 3.0 µg of calcium per mL.

Assay preparation: Transfer 1 gram of sample in a crucible in a muffle furnace maintained at about 550° for 6 to 12 hours, and cool. Add about 60 mL of hydrochloric acid, and boil gently on a hot plate or steam bath for 30 minutes, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask. Rinse the crucible with small portions of 6 N hydrochloric acid, and add the rinsings to the flask. Dilute with water to volume, mix, and filter, discarding the first 5 mL of the filtrate. Dilute this solution quantitatively, and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a solution having a concentration of about 21 µg of calcium per mL, adding 1 mL of Lanthanum chloride solution per 100 mL of the final volume.

Procedure: Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the calcium emission line at 422.7 nm with an atomic absorption spectrophotometer (see Spectrophotometry and Light-Scattering á 851 ņ) equipped with a calcium hollow-cathode lamp and a nitrous oxide-acetylene flame, using 0.125 N hydrochloric acid containing 0.1% Lanthanum chloride solution as the
blankin μg per mL, of calcium in the Assay preparation. Calculate the weight, in mg, of calcium (Ca) in the portion of sample y.

**Assay for Chromium:** Chromium standard stock solution: Transfer about 2.829 g of potassium dichromate, previously dried at 120° for 4 hours and accurately weighed, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix to obtain a solution having a known concentration of about 1000 μg of chromium per mL. Store in a polyethylene bottle (Kubota et al. 1998).

Standard preparations: Transfer 10.0 mL of the Chromium standard stock solution to a 1000-mL volumetric flask, add 50.0 mL of 6 N hydrochloric acid, dilute with water to volume, and mix to obtain a standard solution having a known concentration of about 10 μg of chromium per mL. Transfer 10.0 mL and 20.0 mL of the standard solution to separate 100-mL volumetric flasks, and transfer 15.0 mL and 20.0 mL of the standard solution to separate 50-mL volumetric flasks. Dilute the contents of each of the four flasks with 0.125 N hydrochloric acid to volume, and mix to obtain solutions having known concentrations of about 1.0, 2.0, 3.0, and 4.0 μg of chromium per mL.

Assay preparation: Proceed as directed for Assay preparation in the Assay for calcium, except to prepare the Assay preparation to contain about 1 μg of chromium per mL and to omit the use of the Lanthanum chloride solution.

Procedure: Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the chromium emission line at 357.9 nm with an atomic absorption spectrophotometer (Spectrophotometry and Light-Scattering á 851 ŋ) equipped with a chromium hollow-cathode lamp and an air-acetylene flame, using 0.125 N hydrochloric acid as the blank. Plot the absorbances of the Standard preparations versus concentration, in μg per mL, of chromium, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, C, in μg per mL, of chromium in the Assay preparation. Calculate the quantity, in μg, of chromium (Cr) in the portion of Tablets taken by the formula: CD, in which D is the dilution factor used to prepare the Assay preparation.
**Assay for copper:** Copper standard stock solution: Dissolve about 1.00 g of copper foil in a minimum volume of a 50% (v/v) solution of nitric acid, and dilute with a 1% (v/v) solution of nitric acid to 1000 mL. This solution contains 1000 μg of copper per mL.

Standard preparations: Transfer 10.0 mL of Copper standard stock solution to a 100-mL volumetric flask, and dilute quantitatively with 0.125 N hydrochloric acid to volume to obtain a standard solution having a concentration of 100 μg of copper per mL. To separate 200-mL volumetric flasks, transfer 1.0, 2.0, 4.0, 6.0, and 8.0 mL of the standard solution, dilute with water to volume, and mix to obtain solutions having known concentrations of about 0.5, 1.0, 2.0, 3.0, and 4.0 μg of copper per mL.

Assay preparation: Proceed as directed for Assay preparation in the Assay for calcium, except to prepare the Assay preparation to contain about 2 μg of copper per mL and to omit the use of Lanthanum chloride solution.

Procedure: Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the copper emission line at 324.7 nm with an atomic absorption spectrophotometer (Spectrophotometry and Light-Scattering â 851 ñ) equipped with a copper hollow-cathode lamp and an air-acetylene flame, using 0.125 N hydrochloric acid as the blank. Plot the absorbances of the Standard preparations versus concentration, in μg per mL, of copper, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, C, in μg per mL, of copper in the Assay preparation. Calculate the quantity, in mg, of copper (Cu) in the portion of Tablets taken by the formula:

\[0.001CD,\]

in which D is the dilution factor used to prepare the Assay preparation.

**Assay for fluoride:** Method 1: [NOTE-Store all solutions in plastic containers]
Sodium acetate solution: Dissolve 408 g of sodium acetate in about 600 mL of water contained in a 1000-mL volumetric flask. Allow the solution to equilibrate to room temperature, dilute with water to volume, and mix. Adjust with a few drops of acetic acid to a pH of 7.0. Sodium citrate solution: Dissolve 222 g of sodium citrate in
250 mL of water in a 1000-mL volumetric flask. Add 28 mL of perchloric acid, dilute with water to volume, and mix.

Fluoride standard stock solution: Transfer an accurately weighed quantity of about 1.105 g of sodium fluoride, previously dried at 100° for 4 hours and cooled in a desiccator, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume, and mix to obtain a solution having a known concentration of about 500 µg of fluoride per mL. Intermediate stock solution 1: Pipet 20.0 mL of Fluoride standard stock solution into a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 100 µg of fluoride per mL. Intermediate stock solution 2: Transfer 2.0 mL of Fluoride standard stock solution to a 100-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 10 µg of fluoride per mL. Standard preparations: To five separate 100-mL volumetric flasks, transfer 3.0, 5.0, and 10.0 mL of Intermediate stock solution 2 and 5.0 and 10.0 mL of Intermediate stock solution 1. To each flask, add 10.0 mL of 1 N hydrochloric acid, 25 mL of 3 M sodium acetate solution, and 25.0 mL of sodium citrate solution. Dilute the contents of each flask with water to volume, and mix to obtain solutions having known concentrations of about 0.3, 0.5, 1.0, 5.0, and 10.0 µg of fluoride per mL.

Assay preparation: Weigh and finely powder a counted number of Tablets. Transfer an inaccurately weighed quantity of the powder, equivalent to about 200 µg of fluoride, to a 100-mL volumetric flask. Dissolve in 10.0 mL of 1 N hydrochloric acid, 25.0 mL of 3 M sodium acetate solution and 25.0 mL of sodium citrate solution, dilute with water to volume, and mix. Procedure: To separate plastic beakers, each containing a plastic-coated stirring bar, transfer 50.0 mL each of the Standard preparations and the Assay preparation. Concomitantly measure the potentials (see pH à 791 ñ), in mV, of the Standard preparations and the Assay preparation, with a pH meter capable of a minimum reproducibility of ±0.2 mV and equipped with a fluoride-specific ion-indicating electrode and a calomel reference electrode. [NOTE: When taking measurements, immerse the electrodes in the solution, stir on a magnetic stirrer having an insulated top until equilibrium is attained (1 to 2 minutes), and
record the potential. Rinse and dry the electrodes between measurements, taking care to avoid damaging the crystal of the specific-ion electrode. Plot the logarithms of fluoride concentrations, in µg per mL, of the Standard preparations versus potential, in mV. From the standard response curve so obtained and the measured potential of the Assay preparation, determine the concentration, C, in µg permL, of fluoride in the Assay preparation. Calculate the quantity, in mg, of fluoride in the portion of Tablets taken by the formula: 0.1C.

Method 2: [NOTE -Use plastic containers and deionized water throughout this procedure] pH 10.0 Buffer- Add 214 mL of 0.1 N sodium hydroxide to 1000 mL of 0.05 M sodium bicarbonate.

Mobile phase- Prepare a filtered and degassed mixture of water, alcohol, and 0.1 N sulfuric acid (175:20:5). Make adjustments if necessary (System Suitability under Chromatography á 621 ų).

Standard stock solution: Dissolve an accurately weighed quantity of USP Sodium Fluoride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 220 µg per mL. This solution contains about 100 µg of fluoride per mL.

Standard preparation: [NOTE -Condition the solid-phase extraction column specified for use in the Standard preparation and the Assay preparation in the following manner. Using vacuum, at a pressure not exceeding 5 mm of mercury, wash the column with one column volume of methanol followed by one column volume of pH 10.0 Buffer. Do not allow the column top to dry. If the top of the column becomes dry, recondition the column]. Transfer 10.0 mL of Standard stock solution to a 100-mL volumetric flask. Add 75 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 10.4 ± 0.1. Dilute with water to volume, and mix. Filter, discarding the first 15 mL of the filtrate. Transfer 25.0 mL of the filtrate to a 50-mL volumetric flask, add 15.0 mL of water, and adjust with 0.1 N sodium hydroxide to a pH of 10.0. Dilute with pH 10.0 Buffer to volume, and mix. Elute a portion of this solution through a 3-mL solid-phase extraction column containing L1 packing that is connected through an adaptor to a second solid-phase extraction column containing sulfonylpropyl strong
cation-exchange packing. Discard the first 3 mL of the eluate, and collect the rest of the eluate in a suitable flask for injection into the chromatograph.

Assay preparation: Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 mg of fluoride, to a stoppered 100-mL volumetric flask, add 15 mL of water, and shake vigorously. Rinse the sides of the flask with 15 mL of water, and allow to stand for 10 minutes. Dilute with water to about 85 mL, adjust with 1N sodium hydroxide to a pH of 10.4 ± 0.1, dilute with water to volume, and mix. Proceed as directed for Standard preparation, beginning with filter, discarding the first 15 mL of the filtrate.

Chromatographic system (Chromatography á 621 ñ ) The liquid chromatograph is equipped with a conductivity detector, a 4.6-mm x 30-cm guard column that contains packing L17, and a 7.8-mm x 30-cm analytical column that contains packing L17. The flow rate is about 0.5 mL per minute. Chromatograph the Standard preparation, and measure the peak areas as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

Procedure: Separately inject equal volumes (about 100 µL) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the peak areas for fluoride. Calculate the quantity, in mg, of fluoride in the portion of Tablets taken by the formula:

0.2C(r U / r S),

in which C is the concentration, in µg per mL, of fluoride in the Standard preparation; and r U and r S are the peak areas obtained from the Assay preparation and the Standard preparation, respectively.

**Assay for Iodide:** Method 1: Bromine water: To about 20 mL of bromine in a glass-stoppered bottle, add 100 mL of water. Insert the stopper into the bottle, and shake. Allow to stand for 30 minutes, and use the supernatant layer (Jimenez et al. 2002).

Procedure: Weigh a counted number of Tablets, and grind them to a fine powder. Transfer an accurately weighed quantity of the powder, equivalent to about 3 mg of iodide, to a nickel crucible. Add 5 g of sodium carbonate, 5 mL of 50% (w/v) sodium
hydroxide solution, and 10 Mlof alcohol, taking care that the entire specimen is moistened. Heat the crucible on a steam bath to evaporate the alcohol, then dry the crucible at about 100° for about 30 minutes to prevent spattering upon subsequent heating. Transfer the crucible with its contents to a furnace heated to about 500°, and heat the crucible for about 15 minutes. [NOTE: Heating at about 500° is unnecessary to carbonize any organic matter present; higher temperature may be used, if necessary, to ensure complete carbonization of all organic matter] Cool the crucible, add 25mL of water, cover the crucible with a water glass, and boil gently for about 10 minutes. Filter the solution, and wash the crucible with boiling water, collecting the filtrate and washings in a beaker. Add phosphoric acid until the solution is neutral to methyl orange, then add 1 mL excess of phosphoric acid. Add excess of Bromine water, and boil the solution gently until colorless and then for 5 minutes longer. Add a few crystals of salicylic acid, and cool the solution to about 20°. Add 1 mL of phosphoric acid and about 0.5 g of potassium iodide, and titrate the liberated iodine with 0.005 N sodium thiosulfate VS, adding starch solution when the liberated iodine color has nearly disappeared. Calculate the quantity, in μg, of iodide in the portion of Tablets taken by the formula:

\[ 105.8V \times N \]

in which V is the volume, in mL, of sodium thiosulfate consumed; and N is the normality of the sodium thiosulfate solution used.

**Estimation of iron by atomic spectrometry:** Iron standard stock solution: Transfer about 100 mg of iron powder, accurately weighed, to a 1000-mL volumetric flask, dissolve in 25 mL of 6 N hydrochloric acid, dilute with water to volume, and mix. Standard preparations: To separate 100-mL volumetric flasks, transfer 2.0, of Iron standard stock solution. Dilute the contents of each flask with water to volume, and mix to obtain solutions having known concentrations of about 2.0, of iron per mL.

Sample preparation: Transfer 1 gram of sample in a crucible in a muffle furnace maintained at about 550° for 6 to 12 hours, and cool. Add about 60mL of hydrochloric acid, and boil gently on a hot plate or steam bath for 30 minutes, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric
acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask. Rinse the crucible with small portions of 6 N hydrochloric acid, and add the rinsings to the flask. Dilute with water to volume, mix, and filter, discarding the first 5 mL of the filtrate. Dilute this solution quantitatively, and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a solution.

Procedure: Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the iron emission line at 248.3 nm with an atomic absorptionspectrophotometer (see Spectrophotometry and Light-Scattering á 851 ñ) equipped with an iron hollow-cathode lamp and an air-acetylene flame, using 0.125 N hydrochloric acid as the blank. Plot the absorbances of the Standard preparations versus concentration, in µg per mL, quantity, in mg, of iron (Fe) in the portion of sample.

Lanthanum chloride solution: Prepare as directed in the Assay for calcium.

Magnesium standard stock solution: Transfer about 1.0 g of magnesium ribbon, accurately weighed, to a 1000-mL volumetric flask, dissolve in 50 mL of 6 N hydrochloric acid, dilute with water to volume, and mix to obtain a solution having a known concentration of about 1000 µg of magnesium per mL.

Standard preparations: Quantitatively dilute a volume of Magnesium standard stock solution with 0.125 N hydrochloric acid to obtain a standard solution having a concentration of 20 µg of magnesium per mL. To separate 100-mL volumetric flasks, transfer 1.0, 1.5, 2.0, 2.5, and 3.0 mL of standard solution. To each flask add 1.0 mL of Lanthanum chloride solution, dilute with 0.125 N hydrochloric acid to volume, and mix to obtain solutions having known concentrations of about 0.2, 0.3, 0.4, 0.5, and 0.6 µg of magnesium per mL.

Assay preparation: Proceed as directed for Assay preparation in the Assay for calcium, except to prepare the Assay preparation to contain 0.4 µg of magnesium per mL. Procedure: Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the magnesium emission line at 285.2 nm with an atomic absorptionspectrophotometer (see Spectrophotometry and Light-Scattering á 851 ñ) equipped with a magnesium hollow-cathode lamp and an air-
acetylene flame, using 0.125 N hydrochloric acid containing 0.1% Lanthanum chloride solution as the blank. Plot the absorbances of the Standard preparations versus concentration, in µg per mL, of magnesium, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, C, in µg per mL of magnesium in the Assay preparation. Calculate the quantity, in mg, of magnesium (Mg) in the portion of Tablets taken by the formula:

\[ 0.001CD, \]

in which D is the dilution factor used to prepare the assay preparation.

**Assay for Manganese:** Manganese standard stock solution: Transfer 1.00 g of manganese, accurately weighed, to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, dilute with 6 N hydrochloric acid to volume, and mix to obtain a solution containing 1000 µg of manganese per mL (Fowlis 1995).

Standard preparations: Quantitatively dilute 10.0 mL of the Manganese standard stock solution with 0.125 N hydrochloric acid to 200.0 mL to obtain a standard solution having a concentration of 50 µg of manganese per mL. To separate 100-mL volumetric flasks, transfer 4.0 mL of standard solution, dilute the contents of each flask with 0.125 N hydrochloric acid to volume, and mix to obtain solutions having known concentrations of about 2.0 µg of manganese per mL.

Assay preparation: Transfer 1 gram of sample in a crucible in a muffle furnace maintained at about 550° for 6 to 12 hours, and cool. Add about 60 mL of hydrochloric acid, and boil gently on a hot plate or steam bath for 30 minutes, intermittently rinsing the inner surface of the crucible with 6 N hydrochloric acid. Cool, and quantitatively transfer the contents of the crucible to a 100-mL volumetric flask. Rinse the crucible with small portions of 6 N hydrochloric acid, and add the rinsings to the flask. Dilute with water to volume, mix, and filter, discarding the first 5 mL of the filtrate. Dilute this solution quantitatively, and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a solution.

Procedure: Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the manganese emission line at 279.5 nm with an atomic
absorptiometer (see Spectrophotometry and Light-Scattering á 851 ñ) equipped with amganese hollow-cathode lamp and an air-acetylene flame, using 0.125 N hydrochloric acid as the blank. Plot the absorbances of the Standard preparations versus concentration, in µg permL, of manganese, and determine the concentration, C, in µg per mL, of manganese in the Assay preparation. Calculate the quantity, in mg, of manganese (Mn) in the portion of Sample

Assay for molybdenum: Diluting solution-Dissolve 40 g of ammonium chloride in 2000 mL of water. Molybdenum standard stock solution-Transfer about 1.0 g of molybdenum wire, accurately weighed, to a 1000-mL volumetric flask, and dissolve in 50 mL of nitric acid, warming if necessary. Dilute with water to volume, and mix to obtain a solution containing 1000 µg of molybdenum per mL. Standard preparations-Quantitatively dilute 10.0 mL of the Molybdenum standard stock solution with water to 100.0 mL to obtain a standard solution having a known concentration of about 100 µg of molybdenum per mL. To separate 100-mL volumetric flasks, transfer 2.0, 10.0, and 25.0 mL of the standard solution, and add 5.0 mL of perchloric acid to each flask. Gently boil the solution in each flask for 15 minutes, cool to room temperature, dilute each with Diluting solution to volume, and mix to obtain solutions having known concentrations of about 5.0, 10.0, and 25.0 µg of molybdenum per mL.

Assay preparation: Weigh and finely powder a counted number of Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1000 µg of molybdenum, to a suitable flask, and add about 12 mL of nitric acid. [NOTE-The volume of nitric acid may be varied to ensure that the powder is uniformly dispersed]. Carefully swirl the flask to disperse the test specimen. Sonicate for about 10 minutes, or until the test specimen is completely dissolved. Gently boil the solution for about 15 minutes, and cool to room temperature. Carefully add about 8 mL of perchloric acid, heat until perchloric acid fumes appear, and swirl the flask to dissipate the fumes. Repeat the heating and swirling until the fumes persist. Cool to room temperature. Quantitatively transfer the contents of the flask to a 100-mL volumetric flask with the aid of Diluting solution, dilute with Diluting solution to volume, and mix. Procedure-Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the molybdenum emission line at 313 nm with an atomic
absorption spectrophotometer (Spectrophotometry and Light-Scattering á 851 Ñ) equipped with amolybdenum hollow-cathode lamp and a nitrous oxide-acetylene flame, using a mixture of Diluting solution and perchloric acid (20:1) as the blank. Plot the absorbances of the Standardpreparations versus concentration, in µg per mL, of molybdenum, and draw the straight line bestfitting the three plotted points. From the graph so obtained, determine the concentration, C, inµg per mL, of molybdenum in the Assay preparation. Calculate the quantity, in mg, of molybdenum (Mo) in the portion of Tablets taken by the formula:

0.001CD,

in which D is the diluting factor used to prepare the Assay preparation.

Assay for molybdenum, Method 2: Sodium fluoride solution-Add 200 mL of water to about 10 g of sodium fluoride, stir until the solution is saturated, and filter. Store in a polyethylene bottle. Ferrous sulfate solution- Dissolve 498 mg of ferrous sulfate in water to make 100 mL.

Potassium thiocyanate solution- Dissolve 20 g of potassium thiocyanate in water, and dilute with water to 100 mL. 20% Stannous chloride solution-Transfer 40 g of stannous chloride to a beaker, and add 20mL of 6.5 N hydrochloric acid solution. Heat the solution until the stannous chloride is dissolved. Cool, dilute with water to 100 mL, and mix. Dilute stannous chloride solution-Dilute 4 mL of 20% Stannous chloride solution with water to 100 mL. Prepare this solution fresh at the time of use.

Standard preparation-Transfer about 92 mg of ammonium molybdate, accurately weighed, to a 500-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Quantitatively dilute 20.0 mL of this solution with water to 100.0 mL to obtain a solution having a known concentration of about 20 µg of molybdenum per mL.

Procedure: Weigh a counted number of Tablets, and grind the Tablets to a fine powder. Transfer an accurately weighed quantity of the powder, equivalent to about 40 µg of molybdenum, to a 200-mL beaker, marked I. To another 200-mL beaker, marked II, transfer 2.0 mL of Standard preparation. Add about 20 mL of nitric acid to each beaker. Cover each beaker with a water glass, and boil slowly on a hot plate for
about 45 minutes. Cool to roomtemperature, add 6 mL of perchloric acid to each beaker, cover each beaker with a water glass, and continue the heating until digestion is complete, as indicated when the liquid becomes colorless or pale yellow. If necessary, add further portions of nitric acid and perchloric acid to beaker I, and digest further. Evaporate the solutions in each of the beakers to dryness. Rinse the sides of the beakers and the water glasses with water, and add more water to each beakerto a volume of about 50 mL. Gently boil the water solutions for a few minutes. Cool to roomtemperature. Add 2 drops of methyl orange TS to each beaker, and neutralize with ammoniumhydroxide. Add 8.2 mL of hydrochloric acid to each beaker. Quantitatively transfer the content of the beakers to separate 100-mL volumetric flasks, rinse the beakers with water, transfer the rinsings to the respective volumetric flasks, dilute with water to volume, and mix. Transfer 50.0 mL each of the solutions from beakers I and II to separate separatory funnels. To each separatory funnel add 1.0 mL of Sodium fluoride solution, 0.5 mL of Ferrous sulfatesolution, 4.0 mL of Potassium thiocyanate solution, 1.5 mL of 20% Stannous chloride solution, and 15.0 mL of amyl alcohol, and shake the separatory funnels for 1 minute. Allow the layers to separate, and discard the aqueous layers. Add 25 mL of Dilute stannous chloride solution to each separatory funnel, and shake gently for 15 seconds. Allow the layers to separate, and discard the aqueous layers.

Transfer the organic layers from the separatory funnels to separate centrifuge tubes, and centrifuge at 2000 rpm for 10 minutes. Concomitantly determine the absorbances of solutions from the Tablets and from the Standard preparation in 1-cm cells at the wavelength of maximum absorbance at about 465 nm, using amyl alcohol as the blank. Calculate the quantity, in µg, of molybdenum (Mo) in the portion of Tablets taken by the formula:

\[2C(A_U / A_S),\]

in which C is the concentration, in µg per mL, of molybdenum in the Standard preparation; and A_U and A_S are the absorbances of the solutions from the Tablets and from the Standard preparation, respectively.
Assay for phosphorus: Sulfuric acid solution: Cautiously add 37.5 mL of sulfuric acid to 100 mL of water, and mix. Ammonium molybdate solution: Dissolve 12.5 g of ammonium molybdate in 150 mL of water. Add 100 mL of Sulfuric acid solution, and mix. Hydroquinone solution: Dissolve 0.5 g of hydroquinone in 100 mL of water, and add one drop of sulfuric acid. Sodium bisulfite solution: Dissolve 20 g of sodium bisulfite in 100 mL of water. Phosphorus standard stock solution: Accurately weigh about 4.395 g of monobasic potassium phosphate, previously dried at 105° for 2 hours and stored in a desiccator, and transfer to a 1000-mL volumetric flask. Dissolve in water, add 6 mL of sulfuric acid as a preservative, dilute with water to volume, and mix to obtain a solution containing 1000 µg of phosphorus per mL.

Standard preparation: Dissolve an accurately measured volume of Phosphorus standard stock solution in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 20 µg of phosphorus per mL.

Assay preparation: Weigh a counted number of Tablets, and grind the Tablets to a fine powder. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of phosphorus, to a suitable flask, add 25 mL nitric acid, and digest on a hot plate for about 30 minutes. Add 15 mL of hydrochloric acid, and continue the digestion to the cessation of brown fumes. Cool, and quantitatively transfer the contents of the flask to a 500-mL volumetric flask with the aid of small portions of water. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

Procedure: To three separate 25-mL volumetric flasks, transfer 5.0 mL each of the Standard preparation, the Assay preparation, and water to provide the blank. To each of the three flasks, add 1.0 mL each of Ammonium molybdate solution, Hydroquinone solution, and Sodium bisulfite solution, and swirl to mix. Dilute the contents of each flask with water to volume, mix, and allow the flasks to stand for 30 minutes. Concomitantly determine the absorbances of the solutions from the Assay preparation and the Standard preparation in 1-cm cells at the wavelength of maximum absorbance at about 650 nm, against the blank. Calculate the quantity, in mg, of phosphorus (P) in the portion of Tablets taken by the formula:
in which C is the concentration, in μg per mL, of phosphorus in the Standard preparation; and A U and A S are the absorbances of the solutions obtained from the Assay preparation and the Standard preparation, respectively.

Assay for potassium: Potassium standard stock solution: Transfer about 190.7 mg of potassium chloride, previously dried at 105° for 2 hours and accurately weighed, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix to obtain a solution having a known concentration of about 100 μg of potassium per mL.

Standard preparations: Dilute an accurately measured volume of Potassium standard stock solution with 0.125 N hydrochloric acid to obtain a standard solution having a known concentration of about 10 μg of potassium per mL. Transfer 5.0, 10.0, 15.0, 20.0, and 25.0 mL of the standard solution to separate 100-mL volumetric flasks. Dilute the contents of each flask with 0.125 N hydrochloric acid to volume, and mix to obtain solutions containing 0.5, 1.0, 1.5, 2.0, and 2.5 μg of potassium per mL.

Assay preparation: Proceed as directed for Assay preparation in the Assay for calcium, except to prepare the Assay preparation to contain 1 μg of potassium per mL and to omit the use of the Lanthanum chloride solution. Procedure-Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the potassium emission line at 766.5 nm with an atomic absorption spectrophotometer (Spectrophotometry and Light-Scattering á 851 ń) equipped with a potassium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbances of the Standard preparations versus concentration, in μg per mL, of potassium, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, C, in μg per mL, of potassium in the Assay preparation. Calculate the quantity, in mg, of potassium (K) in the portion of Tablets taken by the formula:

0.001CD,

in which D is the dilution factor used to prepare the Assay preparation.
**Assay for selenium**: Diluting solution: Dissolve 40 g of ammonium chloride in 2000 mL of water. Selenium standard stock solution: [Caution - Selenium is toxic; handle it with care] Dissolve about 1 g of metallic selenium, accurately weighed, in a minimum volume of nitric acid. Evaporate to dryness, add 2 mL of water, and evaporate to dryness. Repeat the addition of water and the evaporation to dryness three times. Dissolve the residue in 3 N hydrochloric acid, transfer to a 1000-mL volumetric flask, dilute with 3 N hydrochloric acid to volume, and mix to obtain a solution having a known concentration of about 1000 µg of selenium per mL.

Standard preparations: Quantitatively dilute 10 mL of the Selenium standard stock solution with water to 100.0 mL to obtain a standard solution having a known concentration of about 100 µg of selenium per mL. To separate 100-mL volumetric flasks, transfer 5.0, 10.0, and 25.0 mL of the standard solution, and add 5.0 mL of perchloric acid to each flask. Gently boil the solutions for 15 minutes, cool to room temperature, dilute each with Diluting solution to volume, and mix to obtain solutions containing 5.0, 10.0, and 25.0 µg of selenium per mL.

Assay preparation: Weigh and finely powder a counted number of Tablets. Transfer an inaccurately weighed portion of the powder, equivalent to about 1000 µg of selenium, to a suitable flask, and add about 12 mL of nitric acid. [NOTE: The volume of nitric acid may be varied to ensure that the powder is uniformly dispersed] Carefully swirl the flask to disperse the test specimen. Sonicate for about 10 minutes or until the test specimen is completely dissolved. Gently boil the solution for about 15 minutes, and cool to room temperature. Carefully add about 8 mL of perchloric acid to the flask, heat the flask until perchloric acid fumes appear, and swirl the flask to dissipate the fumes. Repeat the heating and swirling until the fumes persist. Cool to room temperature. Quantitatively transfer the contents of the flask to a 50-mL volumetric flask with the aid of Diluting solution, dilute with Diluting solution to volume, and mix.

Procedure: Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the selenium emission line at 196 nm, with an atomic absorption spectrophotometer (Spectrophotometry and Light-Scattering á 851 ñ) equipped with a selenium hollow-cathode lamp and an air-acetylene flame,
using a mixture of Diluting solution and perchloric acid (20:1) as the blank. Plot the absorbances of the Standard preparations versus concentration, in µg per mL, of selenium, and draw the straight line best fitting the three-plotted points. From the graph so obtained, determine the concentration, $C$, in µg per mL, of selenium in the Assay preparation. Calculate the quantity, in mg, of selenium (Se) in the portion of Tablets taken by the formula:

$$0.001CD,$$

in which $D$ is the dilution factor used to prepare the Assay preparation.

Assay for selenium, Method 2:

Hydrochloric acid solution: Dilute 50 mL of hydrochloric acid with water to 500 mL, and mix.

50% Ammonium hydroxide solution-Dilute 250 mL of ammonium hydroxide with water to 500 mL, and mix. Reagent 1- Dissolve 4.5 g of edetate disodium in 400 mL of water in a 500-mL volumetric flask. Add 12.5 g of hydroxylamine hydrochloride, dilute with water to volume, and mix.

Reagent 2-Transfer 200 mg of 2,3-diaminonaphthalene to a 250-mL separatory funnel, and add 200 mL of 0.1 N hydrochloric acid. Wash the solution with three 40-mL portions of cyclohexane, and discard the cyclohexane layer. Filter the solution into a brown bottle, and cover the solution with a 1-cm layer of cyclohexane. This solution is stable for one week if stored in a refrigerator.

Standard stock preparation. [Caution Selenium is toxic; handle it with care.] Dissolve about 1 g of metallic selenium, accurately weighed, in a minimum volume of nitric acid. Evaporate to dryness, add 2 mL of water, and evaporate to dryness. Repeat the addition of water and evaporation to dryness three times. Dissolve the residue in 3 N hydrochloric acid, transfer to a 1000-mL volumetric flask, dilute with 3 N hydrochloric acid to volume, and mix to obtain a solution containing about 1000 µg of selenium per mL. Dilute a volume of this solution quantitatively, and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a solution having a known concentration of about 2.0 µg of selenium per mL.
Assay preparation: Weigh a counted number of Tablets, and grind to a fine powder. Transfer an accurately weighed portion of the powder, equivalent to about 20 µg of selenium, to a suitable flask. Add 10 mL of nitric acid, and warm gently on a hot plate. Continue heating until the initial nitric acid reaction has subsided, then add 3 mL of perchloric acid. [Caution—Exercise care at this stage as perchloric acid reaction becomes vigorous]. Continue heating on the hot plate until the appearance of white fumes of perchloric acid or until the digest begins to darken. Add 0.5 mL of nitric acid, and resume heating, adding additional amounts of nitric acid if further darkening occurs. Digest for about 10 minutes after the first appearance of perchloric acid fumes or until the digest becomes colorless. Cool the flask, add 2.5 mL of Hydrochloric acid solution, and return the flask to the hot plate to expel residual nitric acid. Heat the mixture for 3 minutes after it begins to boil. Cool the flask to room temperature, and dilute with water to about 20 mL.

Procedure: Transfer 10.0 mL of the Standard stock preparation to a glass-stoppered flask. Add 1 mL of perchloric acid and 1 mL of Hydrochloric acid solution, and dilute with water to about 20 mL (Standard preparation). Prepare a blank by adding 1 mL of perchloric acid and 1 mL of Hydrochloric acid solution to a glass-stopped flask, and diluting with water to about 20 mL. Treat the Assay preparation, the Standard preparation, and the blank as follows. Add 5 mL of Reagent 1 to each flask, and swirl gently to mix. Adjust the solution in each flask with 50% Ammonium hydroxide solution to a pH of 1.1 ± 0.1. Add 5 mL of Reagent 2 to each flask, and swirl gently to mix. Place the flasks in a water bath maintained at 50°, and equilibrate for 30 minutes, taking care that the flasks are covered to protect them from light. Cool to room temperature, and transfer the contents of each flask to separate separatory funnels. Transfer 10.0 mL of cyclohexane to each separatory funnel, and extract vigorously for 1 minute. Discard the aqueous layer. Transfer the cyclohexane layer to a centrifuge tube, and centrifuge at 1000 rpm for 1 minute to remove any remaining water. Concomitantly determine the absorbances of the solutions from the Assay preparation and the Standard preparation in 1-cm cells at the wavelength of maximum absorbance at about 380 nm against the solution from the blank. Calculate the quantity, in µg, of selenium (Se) in the portion of Tablets taken by the formula:
CD(A U / A S),

in which C is the concentration, in μg per mL, of selenium in the Standard stock preparation; D is the dilution factor used to prepare in the Assay preparation; and A U and A S are the absorbances of the solutions from the Assay preparation and the Standard preparation, respectively.

Assay for zinc: Zinc standard stock solution: Transfer about 311 mg of zinc oxide, accurately weighed, to a 250-mL volumetric flask, and add 80 mL of 5 M hydrochloric acid, warming if necessary to dissolve. Cool, dilute with water to volume, and mix to obtain a solution having a known concentration of about 1000 μg of zinc per mL.

Standard preparations: Dilute a volume of the Zinc standard stock solution quantitatively, and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a standard solution having a known concentration of about 50 μg of zinc per mL. Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 mL of this solution to separate 100-mL volumetric flasks. Dilute the contents of each flask with 0.125 N hydrochloric acid to volume, and mix to obtain solutions having known concentrations of about 0.5, 1.0, 1.5, 2.0, and 2.5 μg of zinc per mL.

Assay preparation: Proceed as directed for Assay preparation in the Assay for calcium, except to prepare the Assay preparation to contain 2 μg of zinc per mL and to omit the use of the Lanthanum chloride solution.

Procedure: Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the zinc emission line at 213.8 nm with an atomic absorption spectrophotometer (see Spectrophotometry and Light-Scattering â 851 nm) equipped with a zinchollow-cathode lamp and an air-acetylene flame, using 0.125 N hydrochloric acid as the blank. Plot the absorbances of the Standard preparations versus concentration, in μg per mL, of zinc, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, C, in μg per mL, of zinc in the Assay preparation. Calculate the quantity, in mg, of zinc (Zn) in the portion of Tablets taken by the formula:

0.001CD, in which D is the dilution factor used to prepare in the Assay preparation.
1 USP Units of activity for vitamins, where such exist or formerly existed, are equivalent to the corresponding international units, where such formerly existed. The USP Unit for Vitamin E has been discontinued. International units (IU) for vitamins also have been discontinued; however, the use of IU on the labels of vitamin products continues. Where articles are labeled in terms of Units in addition to the required labeling, the relationship of the USP Units or IU to mass is as follows. One USP Vitamin A Unit = 0.3 µg of all-trans retinol (vitamin A alcohol) or 0.344 µg of all-trans retinyl acetate (vitamin A acetate) or 0.55 µg of all-trans retinyl palmitate (vitamin A palmitate), and 1 µg of retinol (3.3 USP Vitamin A Units) = 1 retinol equivalent (RE); 1 IU of beta carotene = 0.6 µg of all-trans betacarotene; 1 USP Vitamin D Unit = 0.025 µg of ergocalciferol or cholecalciferol; 1 mg of dl-alpha tocopherol = 1.1 former USP Vitamin E Units, 1 mg of dl-alpha tocopheryl acetate = 1 former USP Vitamin E Unit, 1 mg of dl-alphatocopheryl acid succinate = 0.89 former USP Vitamin E Unit, 1 mg of dl-alpha tocopherol = 1.49 former USP Vitamin E Units, 1 mg of dl-alpha tocopheryl acetate = 1.36 former USP Vitamin E Units, 1 mg of dl-alpha tocopheryl acid succinate = 1.21 former USP Vitamin E Units.

In terms of dl-alpha tocopherol equivalents, 1 mg of dl-alphatocopheryl acetate = 0.91, 1 mg of dl-alpha tocopheryl acid succinate = 0.81, 1 mg of dl-alpha tocopherol = 0.74, 1 mg of dl-alpha tocopheryl acetate = 0.67, and 1 mg of dl-alpha tocopheryl acid succinate = 0.60.

Principle: b1 thiamine gets converted to thiochrome and it has fluorescence only in alkaline medium N-butanoi is more miscible in water, so separation of 2 layers is difficult. Colour development will be stable in organic layer.

Standard: 50 mg of vitamin b1 hcl + 25 ml of n/1 hcl à 250 ml with water (stock solution) à 5 ml à 100 ml à 5 ml à 100 ml with water (0.5 mcg/ml)

Test: pipette necessary quantity equivalent to 1 mg of vitamin b1 à 100 ml with water (if liquid) .dilute 5 ml à 100 ml with water (0.5 mcg/ml) (or) Weigh a quantity equivalent to 1 mg of vitamin b1 + 10 ml of 1n hcl + 20 ml of water shake well & make upto 100 ml with water (if capsule /tablets) dilute 5 ml to 100 ml with water (0.5 mcg/ml)

Extraction: take 5 ml of std in a seaprate , add 2 ml of oxidising reagent & 20 ml of iba (within 30 seconds).Shake for 1 ½ minutes & add 2 ml methanol Take the upper
layer + add anhydrous sodium sulphate. Take 5 ml of spl and treat similarly as std. (note: if label claim is mononitrate multiply the value with 0.9703)

**B2 vitamin:** Standard: 50 mg + 2.5 ml of glacial acetic acid + 100 ml of boiling water. Shake well, cool to 250 ml with water. 5 ml + 1.5 ml of 2% sodium acetate solution to 100 ml with water (10 mcg/ml). À 5 ml to 100 ml with water (0.5 mcg/ml)

Sample: pipette out necessary volume equal to 1 mg of vitamin b2 à 100 ml with water (if liquid)(or) weigh a quantity equal to 1 mg of vitamin b2 + 1 ml glacial acetic acid + 50 ml boiling water. Shake well, cool à 100 ml with water (0.5 mcg/ml)

**B6 vitamin:** Std: 75 mg of pyridoxine hcl + 25 ml 1n hcl à 250 ml with water à 5 ml à 100 ml with water (15 mcg/ml). À 5 ml make upto 25 ml Sample: Pipette necessary volume equal to that of 1.5 mg eq of vitamin b6 à 100 ml with water. (if liquid). Dilute 5ml à 25 ml with ipa (or) weigh a quantity equal to that of 1.5 mg eq of vitamin b6+ 10 ml 1n hcl + 20 ml water shake well make upto 100 ml with water (if capsule/tablet) (15 mcg/ml) dilute 5ml à 25 ml with ipa. Measure the absorbance at 620 nm.

### 3.6. Invitro Antioxidant Studies

Antioxidant activity of Bambusa arundinacea extracts were determined using invitro methods.

#### 3.6.1. DPPH Scavenging Activity

DPPH free radical scavenging activity was measured by the modified method (Dhriti et al. 2014). The reaction mixture (3.0 ml) consisted of 1.0 ml DPPH in methanol (0.3 mM), 1.0 ml methanol and 1.0 ml different concentrations of the methanolic extract was incubated in dark for 10 min, after which the absorbance was measured at 517 nm against blank. For control, 1.0 ml of methanol was used in place of extract. Ascorbic acid was used as positive control. Percentage of inhibition was calculated using the formula: Inhibition (%) = \( (A0 – A1 / A0) \times 100 \) Where, A0 is the absorbance of control and A1 is the absorbance of sample (William and Fleming 1989).
Percentage of inhibition was calculated using the formula: Inhibition (%) = (A0 – A1 / A0) × 100 Where, A0 is the absorbance of control and A1 is the absorbance of sample.

3.6.2. Hydrogen Peroxide Scavenging Activity

Scavenging activity of Hydrogen peroxide (H2O2) by the bamboo extract was determined. Plant extract (4 ml) prepared in distilled water at various concentration(50, 100, 250, 500 µg/ml) was mixed with 0.6 ml of 4 mM H2O2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using Eq. IC50 values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

3.6.3. Nitric Oxide Scavenging Activity

Nitric oxide radical scavenging activity was determined according to the method. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations prepared in ethanol and the mixture incubated at 25oC for 30 min. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. The nitric oxide radicals scavenging activity was calculated. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples. IC50 values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm (Jang et al. 2003).

3.6.4. Reducing Capacity Assessment

The reducing capacity assessment was measured by the modified method. 1.0 ml of different concentrations of methanolic extract was mixed with 2.5 ml of phosphate
buffer (200 mM, pH 6.6) and 2.5 ml of K3Fe(CN)6 (30 mM). The mixture was then incubated at 50 °C for 20 min. There after 2.5 ml of TCA (600 mM) was added to the reaction mixture and then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 (6 mM) and absorbance was measured at 700 nm against blank. Ascorbic acid was used as positive control.

3.6.5. Total Antioxidant Activity
Principle: This assay mainly based on the reduction of MO(VI) to MO(V) by the sample analyte and subsequent fraction of a green phosphate – MO (V) complex at acidic pH (Jala and Haribabu 2004). Reagents required: Sulphuric acid (0.6M) in distilled water. Sodium Phosphate-28 mM in distilled water. Ammonium molybdate – 4 mM in distilled water. Extract preparation: (in 50% DMSO) 10 mg/ml or 20 mg/2 ml. Procedure: Control: 0.1 ml DMSO + 1 ml reagent, then treated same as test = 0.8031. Blank: Distilled water. Standard: Ascorbic acid (various concentrations).

3.7. Invitro Antidiabetic Assays
Anti diabetic activity of Bambusa arundinacea extracts were determined using invitro methods.

3.7.1. α- Amylase Inhibitory Activity
Alpha-amylase activity can be measured in-vitro by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine, which gives blue colour with starch. The reduced intensity of blue colour indicates the enzyme-induced hydrolysis of starch in to monosaccharides. If the substance/extract possesses α-amylase inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of blue colour in test sample is directly proportional to α-amylase inhibitory activity. α-Amylase was premixed with the Bambusa extracts at various concentrations (20-100 μg/ml) and 0.5% starch solution was added at 37°C for 5 min to start the reaction and terminated by addition of 2 ml of 3,5-dinitrosaliclyc acid (Holecheck et al. 1982). The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an
ice bath α-Amylase activity was determined by measuring spectrum at 540 nm and IC50 value was measured. The anti-diabetic activity was determined through the inhibition of α-amylase which was expressed as a percentage of inhibition and calculated by the following equations:

\[
\text{% reaction} = \frac{\text{(maltose) test}}{\text{(maltose) control}} \times 100
\]

\[
\text{% inhibition} = 100\% \text{ reaction}
\]

3.7.2. α–glucosidase Inhibitory Activity

The inhibitory activity was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 ml with 0.2 MTris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. Thereaction was initiated by adding 1 ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540 nm (Adamovics 1990).

3.8. Anticancer activity:

Cell lines and Culture medium: Human colon cancer cell line colo320 and normal cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. It is a suspension culture and stock cells were cultured in Dulbecco’s modified Eagle medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 μg/mL) and amphotericin B (5 μg/mL) in a humidified atmosphere of 5% CO2 at 37 °C until confluent. The confluent cell suspension was centrifuged at 2000 rpm for 10 min and the cell pellet was resuspended in fresh medium. The stock cultures were grown in 25 cm2 culture flasks and all experiments were carried out in 96 micro titer plates (Tarsons India Pvt. Ltd., Kolkata, India).

MTT Assay for Cell Viability: The MTT assay is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at
37°C in humidified atmosphere with 5% CO₂. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 × 10⁴ cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the extracts (50, 100, 150µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control (Weisburger et al. 1993).

3.9. Antimicrobial Effect

Microorganisms used: The extracts were screened for antibacterial activity against human pathogens such as Enterococcus faecalis (ATCC - 29212), Staphylococcus epidermidis (MTCC-3615), Pseudomonas aeruginosa (ATCC-27853), Escherichia coli (ATCC 25922) and the fungal pathogen Aspergillus flavus were isolated from infected patients in ACS Medical college and hospital and the isolates were identified according to published guidelines (22-25). The microorganisms were inoculated into nutrient broth (Hi media Pvt. Ltd., Mumbai, India) and incubated at 37°C for 24 h and the suspension was checked to provide approximately 108 CFU/ml.

3.9.1. Anti bacterial activity

Antibacterial and antifungal activities were carried out using disc diffusion method (Preethi et al. 2010). Petri plates were prepared with 20 ml of sterile MHA (Mueller-Hinton Agar) and PDA (PotatoDextrose Agar) (Hi-media, Mumbai). The test bacterial and fungal cultures (100 µl of suspension contains 108 CFU/ml bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. Three different concentrations of the extracts (1.25, 2.5 and 5 mg/disc) were used for antibacterial activity.
3.9.2. Anti fungal activity
In antifungal activity 5mg/disc was used to load on a sterile disc and placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Streptomycin and Ketoconazole (10 µg/disc) were used as positive controls. These plates were incubated for 24 h at 37 °C. Zone of inhibition was recorded in millimetres (mm) (Madsen et al. 1987).

3.10. Anti helmintic activity
The anthelmintic activity was performed according to the method (Singh Partap et al. 2007). On adult Indian earth worm Pheretima posthuma as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Pheretima posthuma was placed in petridish containing two different concentrations (50 & 100 mg/ml) of HEBA, AEBA and HEBA. Each petridish was placed with 2 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with Reference compound Albendazole (20 mg/ml) treated samples.

3.11. Invitro antiarthritic activity
The antiarthritic activity was performed by protein denaturation method. 0.05 ml various concentrations (100, 250, 500, 750 µg/ml) of test drugs and standard drug diclofenac sodium (100, 250, 500, 750 µg/ml) were taken respectively and 0.45 ml (0.5% w/V BSA) mixed. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 255 nm (Habibur et al. 2015). The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation can be calculated as Percentage Inhibition = 100 – [(OD of test solution – OD of control)/optical density of test] × 100.
3.12. Statistics

All the analyses were carried out in triplicate and the results were expressed in mean ± SED (Descriptive statistics). Data was analyzed using one-way ANOVA.
CHAPTER 4

RESULTS

4.1. Macroscopic Characters

Bambusa arundinacea is a thorny tree, stems many, tufted on a stout root-stock, grows upto 30 meter high; culms 15-18 cm across; nodes prominent, the lower emitting horizontal almost naked shoots armed at the nodes with 2-3 stout recurved spines; internodes upto 45 cm. long. Leaves linear or linear-lanceolate, tip stiff, glabrous or puberulous beneath, margins scabrous, base ciliate, mid-rib narrow, leaf-sheath ending on a thick callus and shortly bristly auricle. It flowers gregariously once in 30-40 years. Rhizomes short, stout, knotty; culms dense, reaching 24-30m in height and 15-17cm in diameter(Fig 4.1). Flowers in large panicles, sometimes occupying the whole culm; caryopsis oblong 5-8mm long, grooved on one side. A bamboo culm consists of internodes (which is hollow for most bamboo) and a node, which is solid and provides structural integrity for the plant.

Figure 4.1.Bambua arundinacea seeds
4.2. Microscopic Characters

Caryopsis (grain) oblong, 5-8mm long, grooved on one side and covered with thick seed coat. The fruit of bambusa is known as caryopsis. It consists of a basal bowl shaped stalk called rachilla which bears two perianth lobes called lemma and palea. Enclosed within the lemma and palea occurs elliptically oblong, smooth, brown grain. There is a longitudinal groove on one side of the grain (Fig 4.2 - Fig 4.5). The rachilla is a bowl shaped cup bearing the lemma and palea enclosing the grain. The grain consists of the embryo on its lower lateral part. The embryo has folded cotyledons which appear as thin curved leaf like structures enclosing the embryo proper. Surrounding the embryo is starchy endosperm. The grain consists of a thick walled epidermal layer, followed by two layers of large, compact square shaped inner cell layers. Inner to the cell layers is the endosperm which is in the form of dense disassociated starch grains.

Figure 4.2. Caryopsis

Figure 4.3. Grain in surface view

Figure 4.4. Lemma

Figure 4.5. Palea
Figure 4.6. Embryonic chamber  Figure 4.7. Rachilla

Figure 4.8. L.S.of seed coat  Figure 4.9. Enlarged seed coat

Figure 4.10. Surface view of Periyanth  Figure 4.11. Conical trichomes
Figure 4.12. Silica and Cork cells

Figure 4.13. Trichomes echinatespines

Figure 4.14. Starch grains

Figure 4.15. Marginal trichomes

Figure 4.16. Rachilla

Figure 4.17. Fibres of Perianth
4.3. Powder Microscopy

Small pieces of epidermal cells of the perianth members of the grains are seen in the powder. In surface view the perianth members are composed of long, narrow thick walled fibres compactly arranged. On the surface are also seen diffusely distributed pairs of silica cells and cork cells. Silica cells are white and transparent and cork cells are darkly stained. There are also triangular, thick walled conical trichomes with pointed ends. There are thick long pointed trichomes seen in the powder. There trichomes have short, curved echinate spines. Starch grains are abundant in the powder. The starch grains are spherical or squarish. They are simple type. Along the margins of lemma and palea there are long dense trichomes which are directed towards the tip. These trichomes have thick, smooth walls similar type of trichomes are also seen along the margin of therachilla. The trichomes are thick walled and lignified. Long, narrow, thick walled pointed fibres are abundant in the powder. They have thick lignified walls and narrow lumen. Brachy sclereids are abundant in the powder. These sclereids are polygonal or rectangular. There are also elongated cylindrical sclereids. The sclereids have very thick lignified walls with numerous canal like simple pits. The lumen is very wide (Fig 4.6 - Fig 4.18).

4.4. Physicochemical properties

The results of the physicochemical parameters are given (Table 4.1). The average values are expressed as percentage of air-dried material. The crude powder of
B. arundinacea showed 12% loss on drying. It contained 5.8% total ash, 0.4% acid insoluble ash and 2.5% water soluble ash. The percent extractive yield of crude powder extracted in hydroethanol was 20.16%, extracted in methanol was 21.18%, extracted in hexane was 9.12% and extracted in acetone was 13.25%. The pH of hydroethanolic extract was 7.5. The extract was alkaline in nature. The melting point and boiling point of hydroethanolic extract were 80 °C and 85 °C respectively.

Table 4.1. Physiochemical parameters of Bambusa arundinacea seeds

<table>
<thead>
<tr>
<th>No</th>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total ash</td>
<td>5.8% (w/w)</td>
</tr>
<tr>
<td>2</td>
<td>Acid insoluble ash</td>
<td>0.4% (w/w)</td>
</tr>
<tr>
<td>3</td>
<td>Water soluble ash</td>
<td>2.5% (w/w)</td>
</tr>
<tr>
<td>4</td>
<td>Loss on drying</td>
<td>12% (w/w)</td>
</tr>
<tr>
<td>5</td>
<td>Methanol soluble extract</td>
<td>21.18% (w/w)</td>
</tr>
<tr>
<td>6</td>
<td>Hydroethanol soluble extract</td>
<td>20.16% (w/w)</td>
</tr>
<tr>
<td>7</td>
<td>Hexane soluble extract</td>
<td>9.12% (w/w)</td>
</tr>
<tr>
<td>8</td>
<td>Acetone soluble extract</td>
<td>13.25% (w/w)</td>
</tr>
<tr>
<td>9</td>
<td>pH of hydroethanol extract</td>
<td>7.5</td>
</tr>
<tr>
<td>10</td>
<td>Melting point of hydroethanol extract</td>
<td>80°C</td>
</tr>
<tr>
<td>11</td>
<td>Boiling point of hydroethanol extract</td>
<td>85°C</td>
</tr>
</tbody>
</table>

4.4.1. Solubility

The hydroethanol extract of B. arundinaceawas evaluated for qualitative solubility test for 8 solvents with varied polarities (Table 4.2). The extract was maximum soluble in methanol (29 mg/ml) followed by dimethylsulphoxide (24.7 mg/ml) while the extract was less soluble in petroleum ether (5 mg/ml) followed by chloroform (7.5 mg/ml). The extract was moderate soluble in other solvents such as glacial acetic acid (26.7 mg/ml), acetone (11.5 mg/ml), ethyl acetate (12.9 mg/ml) and 1, 4, dioxan (9.2 mg/ml).
Table 4.2. Determination of solubility of B. arundinacea seed

<table>
<thead>
<tr>
<th>S.No</th>
<th>Solvents</th>
<th>Soluble in mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>29.30</td>
</tr>
<tr>
<td>2</td>
<td>Acetone</td>
<td>11.50</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum ether</td>
<td>05.80</td>
</tr>
<tr>
<td>4</td>
<td>chloroform</td>
<td>07.50</td>
</tr>
<tr>
<td>5</td>
<td>DMSO</td>
<td>24.70</td>
</tr>
<tr>
<td>6</td>
<td>1-4 Dioxan</td>
<td>09.20</td>
</tr>
<tr>
<td>7</td>
<td>DMF</td>
<td>22.30</td>
</tr>
<tr>
<td>8</td>
<td>Glacial acetic acid</td>
<td>26.70</td>
</tr>
<tr>
<td>9</td>
<td>Ethyl acetate</td>
<td>12.90</td>
</tr>
<tr>
<td>10</td>
<td>Distilled water</td>
<td>21.20</td>
</tr>
</tbody>
</table>

4.4.2. Heavy metal analysis
Lead, chromium, arsenic and cadmium were present in the methanolic extract with varying values (Table 4.3). The extract contained 3.2 ppm lead, 6.4 ppm chromium, 4.7 ppm arsenic and 0.3 ppm cadmium. Mercury was absent in the extract. Although, there was minor presence of some heavy metals but the extracts did not exceed the limit given according to the WHO guidelines.

Table 4.3. Heavy metals in HE extracts of B.arundinacea seeds

<table>
<thead>
<tr>
<th>S.No</th>
<th>Heavy Metals</th>
<th>Results (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mercury</td>
<td>Not detected</td>
</tr>
<tr>
<td>2</td>
<td>Lead</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>Chromium</td>
<td>6.4</td>
</tr>
<tr>
<td>4</td>
<td>Arsenic</td>
<td>4.7</td>
</tr>
<tr>
<td>5</td>
<td>Cadmium</td>
<td>0.3</td>
</tr>
</tbody>
</table>
4.4.3. Yield of Extracts

The percent extractive yield of crude powder extracted in hydroethanol was 7.60%; that extracted in acetone was 2.11% and extracted in hexane was 1.16%.

4.5. Qualitative phytochemical analysis

The results of qualitative phytochemical analysis of the hydroethanolic, actone and hexane extract of B. arundinacea are shown. (Fig 4.19). In the present investigation preliminary phytochemical screening of the H. ethanol, acetone and hexane extract shows the presence of flavonoids, glycosides, phenol, tannin, coumarins, quinones and phytosteroids. In all these extracts alkaloid, saponins and terpenoids were found to be absent (Table.4.4). The extracts contained maximum amount of phenols & flavonoids. In the hydroethanolic extract of B. arundinacea cardiac glycosides, tannins, were present in moderate amount.

figure 4.19. Qualitative phytochemical analysis of Bambusa seed
Table 4.4. Qualitative phytochemical analysis of B.arundinacea seeds

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Test</th>
<th>Name of the Extract</th>
<th>Hydroethanol</th>
<th>Hexane</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Quinones</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Terpinoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Phenols</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Cumarins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Steroids</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>12</td>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Protein</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

(-) = Negative, (+) = Positive

4.6. Quantification of secondary metabolites

The estimation of secondary metabolites were carried out using standard methods.

4.6.1. Total phenol and flavonoid content

The total phenol content was more than that of total flavonoid content in methanolic extract of Bambusa arundinacea seeds. The amount of total phenol was $15.17 \pm 0.69$ mg/g, while the amount of total flavonoid was $11.14 \pm 0.78$ mg/g (Table 4.5). The quantitative estimates of the B.arundinacea extracts were obtained as steroids 5.77mg, quinones 0.565mg and Tannins 1.45mg (Fig.4.21).
Table 4.5. Quantitative estimation of phytochemicals of Bambusa

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytochemicals</th>
<th>Phytochemicals</th>
<th>Amount in mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>Rutin</td>
<td>9.6 ± 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quercetin</td>
<td>1.46 ± 0.24</td>
</tr>
<tr>
<td>2</td>
<td>Phenols</td>
<td></td>
<td>15.17 ± 0.69</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td></td>
<td>5.77 ± 0.64</td>
</tr>
<tr>
<td>4</td>
<td>Quinones</td>
<td></td>
<td>0.565 ± 0.12</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td></td>
<td>1.45 ± 0.45</td>
</tr>
</tbody>
</table>

Figure 4.20. Chromatogram of Flavanoids standard rutin
Figure 4.21. Chromatogram of Flavonoid quantification of Bambusa

4.6.2. Quantification of Nutraceuticals

The analysis of seeds of B.arundinacea has shown its potential significance (Fig.4.22). It has revealed that B.arundinacea seeds are a rich source of carbohydrate 62.861gms, protein 24.735 gms, lipid 2.98gms and fibre 6.07 gms (Table 4.6).

Table 4.6. Nutraceutical composition

<table>
<thead>
<tr>
<th>S.No</th>
<th>Nutraceuticals</th>
<th>Composition/100gms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>62.861±1.23</td>
</tr>
<tr>
<td>2</td>
<td>Protein</td>
<td>24.861±0.25</td>
</tr>
<tr>
<td>3</td>
<td>Lipid</td>
<td>2.98±0.23</td>
</tr>
<tr>
<td>4</td>
<td>Fibre</td>
<td>6.07±0.45</td>
</tr>
</tbody>
</table>
4.6.3. Quantification of Amino acids

The quantity of amino acids were estimated using HPLC(Fig.4.23). It has revealed that B.arundinacea seeds are a rich source of essential amino acids such as histidine(0.403gm), isoleucine(0.898gm), leucine(0.433gm), lysine(0.783gm), methionine(0.646gm), phenylalanine(0.293gm), threonine(0.604gm), tryptophan(0.334), and valine(0.2213gm). Most of the non essential amino acids also found in moderate amount(Table 4.7).
Table 4.7. Quantification of Amino acids

<table>
<thead>
<tr>
<th>S.No</th>
<th>Amino acids</th>
<th>Amount/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aspartic acid</td>
<td>0.334±0.23</td>
</tr>
<tr>
<td>2</td>
<td>Glutamic acid</td>
<td>0.192±0.45</td>
</tr>
<tr>
<td>3</td>
<td>Asparagine</td>
<td>0.345±0.37</td>
</tr>
<tr>
<td>4</td>
<td>Serine</td>
<td>0.203±0.34</td>
</tr>
<tr>
<td>5</td>
<td>Glutamine</td>
<td>0.335±0.25</td>
</tr>
<tr>
<td>6</td>
<td>Glycine</td>
<td>0.453±0.76</td>
</tr>
<tr>
<td>7</td>
<td>Threonine</td>
<td>0.604±0.32</td>
</tr>
<tr>
<td>8</td>
<td>Arginine</td>
<td>0.293±0.34</td>
</tr>
<tr>
<td>9</td>
<td>Alanine</td>
<td>0.193±0.93</td>
</tr>
<tr>
<td>10</td>
<td>Cysteine</td>
<td>0.334±0.01</td>
</tr>
<tr>
<td>11</td>
<td>Tyrosine</td>
<td>0.193±0.34</td>
</tr>
<tr>
<td>12</td>
<td>Histidine</td>
<td>0.403±0.45</td>
</tr>
<tr>
<td>13</td>
<td>Valine</td>
<td>0.2213±0.23</td>
</tr>
<tr>
<td>14</td>
<td>Methione</td>
<td>0.646±0.12</td>
</tr>
<tr>
<td>15</td>
<td>Isoleucine</td>
<td>0.898±0.65</td>
</tr>
<tr>
<td>16</td>
<td>Phenylalanine</td>
<td>0.293±0.07</td>
</tr>
<tr>
<td>17</td>
<td>Leucine</td>
<td>0.433±0.04</td>
</tr>
<tr>
<td>18</td>
<td>Lysine</td>
<td>0.783±0.25</td>
</tr>
<tr>
<td>19</td>
<td>Proline</td>
<td>0.924±0.19</td>
</tr>
<tr>
<td>20</td>
<td>Tryptophan</td>
<td>0.334±0.47</td>
</tr>
</tbody>
</table>
Figure 4.23. Chromatogram of amino acid quantification (standard)

Figure 4.24. Chromatogram of amino acid quantification of bambusa
4.6.4. Quanitification of fatty acids

The quantity of fatty acids were estimated using Gas chromatography (Fig.4.24). It has revealed that B.arundinacea seeds are a rich source of fatty acids such as palmitic acid(0.4456gm), margaric acid(0.1023gm), stearic acid(1.224gm), oleic acid(1.094gm), linolenic acid(0.983gm) and alphalinolic acid(1.224gm) (Table 4.8).

Table 4.8. Quanitification of fatty acids

<table>
<thead>
<tr>
<th>S.No</th>
<th>Fatty acids</th>
<th>Amount/gms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palmitic acid</td>
<td>0.4456±0.14</td>
</tr>
<tr>
<td>2</td>
<td>Margaric acid</td>
<td>0.1023±0.83</td>
</tr>
<tr>
<td>3</td>
<td>Stearic acid</td>
<td>1.224±0.09</td>
</tr>
<tr>
<td>4</td>
<td>Oleic acid</td>
<td>1.094±0.23</td>
</tr>
<tr>
<td>5</td>
<td>Linolenic acid</td>
<td>0.983±0.67</td>
</tr>
<tr>
<td>6</td>
<td>Alpha linolenic acid</td>
<td>1.224±0.56</td>
</tr>
<tr>
<td>7</td>
<td>Morotic acid</td>
<td>0.034±0.24</td>
</tr>
</tbody>
</table>
Figure 4.25. Chromatogram of fatty acid quantification (standard)

Fig.4.26. Chromatogram of fatty acid quantification of bambusa
4.6.5. Quantification of minerals

The mineral content showed the presence of calcium 65.31mg, magnesium 26mg, zinc 15.33mg, Iron 14.27mg, copper 2.03mg, sodium 145.6mg and potassium 40.91mg (Table. 4.9). Minerals like fluoride and chlorine found to be absent (Fig.4.27). Low content of pb, cd, As and Hg showed that the seed is free from toxic metals.
### Table 4.9. Quantification of minerals

<table>
<thead>
<tr>
<th>S.No</th>
<th>Minerals</th>
<th>Amount mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calcium</td>
<td>65.31±0.57</td>
</tr>
<tr>
<td>2</td>
<td>Magnesium</td>
<td>26.24±0.23</td>
</tr>
<tr>
<td>3</td>
<td>Iron</td>
<td>14.27±1.30</td>
</tr>
<tr>
<td>4</td>
<td>Zinc</td>
<td>15.33±0.39</td>
</tr>
<tr>
<td>5</td>
<td>Sodium</td>
<td>145.4±0.56</td>
</tr>
<tr>
<td>6</td>
<td>copper</td>
<td>2.03±0.15</td>
</tr>
<tr>
<td>7</td>
<td>Potassium</td>
<td>40.91±0.46</td>
</tr>
<tr>
<td>8</td>
<td>Phosphorus</td>
<td>92.24±0.34</td>
</tr>
<tr>
<td>9</td>
<td>Selenium</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>10</td>
<td>Fluoride</td>
<td>Not detected</td>
</tr>
<tr>
<td>11</td>
<td>Chlorine</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

#### Figure 4.28. Quantification of minerals
4.6.6. Quantification of vitamins
The vitamin content showed the presence of vitaminA 156.5 IU,vitaminD 0.567mcg,vitaminE 1.98mg,vitaminB6 12.33mg,vitaminB12 11.34mcg,vitaminC 5.44mg,niacinB3 13.80mg,thiamine 9.36mg,riboflavin 14.56mg and folic acid 12.59 mg( Table.4.10). Vitamin K is found to be absent.

Table 4.10. Quantification of vitamins

<table>
<thead>
<tr>
<th>S.No</th>
<th>Vitamins</th>
<th>Amount per 100 gms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vitamin A</td>
<td>156.5±0.15 IU</td>
</tr>
<tr>
<td>2</td>
<td>Vitamin D</td>
<td>0.567±0.18 mcg</td>
</tr>
<tr>
<td>3</td>
<td>Vitamin E</td>
<td>4.98±0.67mg</td>
</tr>
<tr>
<td>4</td>
<td>Vitamin K</td>
<td>Not detected</td>
</tr>
<tr>
<td>5</td>
<td>Thiamine</td>
<td>9.36±0.5mg</td>
</tr>
<tr>
<td>6</td>
<td>Riboflavin</td>
<td>14.56±0.17mg</td>
</tr>
<tr>
<td>7</td>
<td>Niacin</td>
<td>13.80±0.43mg</td>
</tr>
<tr>
<td>8</td>
<td>Pantothenic acid</td>
<td>1.4±0.2mg</td>
</tr>
<tr>
<td>9</td>
<td>Folic acid</td>
<td>28.4±0.37mcg</td>
</tr>
<tr>
<td>10</td>
<td>Vitamin B6</td>
<td>3.33±0.45mg</td>
</tr>
<tr>
<td>11</td>
<td>Vitamin B12</td>
<td>11.34±0.23mcg</td>
</tr>
<tr>
<td>12</td>
<td>Vitamin C</td>
<td>5.44±0.65mg</td>
</tr>
</tbody>
</table>

4.7. In vitro Antioxidant activity
Invitro antioxidant activity of bambusa arundinacea seed extracts were determined by using various method.

4.7.1. Determination of DPPH Scavenging Activity
DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The DPPH radical scavenging activity has been widely used as a model system to investigate the scavenging activity of
natural compounds. The reduction capability of the DPPH radical is determined by its absorbance decreases at 517 nm, as a discoloration from purple to yellow (Fig.4.29). The IC50 value (the concentration required to inhibit radical formation by 50%) of hydroethanolic, acetone and hexane extract of B. arundinacea were 58 µg/ml, 107µg/ml and 110µg/ml respectively. The IC50 value of standard ascorbic acid was 47µg/ml.

### 4.7.2. Hydrogen peroxide scavenging activity

Hydrogen peroxide is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. The model used is ascorbic acid-iron-EDTA model of •OH generating system. This is a totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radicals. The scavenging activity of hydroethanol, acetone and hexane extract of B. arundinacea and ascorbic acid are shown (Fig.4.30). In hydroethanolic extract of B. arundinacea, hydrogen peroxide was radical scavenging activity was concentration dependent and 50% inhibition was achieved at 78µg/ml concentration in the reaction mixture, while in standard ascorbic acid, 50% inhibition was achieved at 31 µg/ml concentration. The IC50 value of acetone and hexane extract of B.arundinacea 122 µg/ml and 138 µg/ml respectively.

### 4.7.3. Nitrous oxide scavenging activity

The nitrous oxide radical cation scavenging activity of hydroethanolic, acetone and hexane extract of B. arundinacea and standard ascorbic acid are shown (Fig 4.31). In hydroethanolic extract of B. arundinacea, nitrous oxide radical cation scavenging activity was concentration dependent and 50% inhibition was achieved at 95 µg/ml concentration in the reaction mixture, while in standard ascorbic acid, 50% inhibition was achieved at 45 µg/ml concentration. The IC50 value of acetone and hexane extract of B. arundinaceawere 118 µg/ml and 130 µg/ml respectively.
4.7.4. Reducing capacity assessment

The reducing capacity assessment of compounds may serve as significant indicators of its potential antioxidant activity. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of components of some plants. In the reducing power assay, the presence of antioxidants in the extract result in the reduction of Fe3+ to Fe2+ by donating an electron. The amount of Fe2+ can then be monitored by measuring the formation of blue color at 700 nm. The reducing capacity assessment of hydroethanolic, acetone and hexane extract of B. arundinaceaaand ascorbic acid are shown (Fig.4.32). In hydroethanolic extract of B. arundinacea, there was concentration dependent increase in the absorbance of reaction mixture and standard ascorbic acid. Higher absorbance of a reaction mixture indicated greater reducing power and 50% inhibition was achieved at 150µg concentration in the reaction mixture, while in standard ascorbic acid, 50 % inhibition was achieved at 50 µg/ml concentration. The IC50 value of hydro ethanol, acetone and hexane extract of B. arundinaceawere 120 µg/ml, 140µg/ml and 155 µg/ml respectively.

4.7.5. Total antioxidant activity

The antioxidant activity of B. arundinacea seed extracts was determined (Fig.4.33). In hydroethanolic extract of B. arundinacea, there was concentration dependent increase in the absorbance of reaction mixture and standard ascorbic acid. Higher absorbance of a reaction mixture indicated greater reducing power and 50% inhibition was achieved at 100µg concentration in the reaction mixture, while in standard ascorbic acid, 50 % inhibition was achieved at 50 µg/ml concentration. The IC50 value of hydro ethanol, acetone and hexane extract of B. arundinaceawere 98 µg/ml, 140µg/ml and 145 µg/ml respectively. The results of this study strongly indicate that HEEBA,AEBA,HEBA has more potent antioxidant activity.
**Figure 4.29. Determination of DPPH Activity**

**Figure 4.30. Determination of Hydrogen peroxide activity**
### Figure 4.31. Determination of Nitrous oxide activity

![Graph showing the percentage of inhibition at various concentrations of HEEBA, AEBA, HEBA, and STANDARD against Nitrous oxide activity.]

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>HEEBA-95µg/ml</th>
<th>AEBA-118µg/ml</th>
<th>HEBA-130µg/ml</th>
<th>STANDARD-45µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>26.12</td>
<td>38.23</td>
<td>51.28</td>
<td>31.45</td>
</tr>
<tr>
<td>50</td>
<td>31.45</td>
<td>43.18</td>
<td>69.34</td>
<td>52.24</td>
</tr>
<tr>
<td>75</td>
<td>51.28</td>
<td>43.18</td>
<td>69.34</td>
<td>75.56</td>
</tr>
<tr>
<td>100</td>
<td>52.24</td>
<td>75.56</td>
<td>88.24</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>88.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Figure 4.32. Antioxidant activity reducing assay

![Graph showing the percentage of inhibition at various concentrations of HEEBA, AEBA, HEBA, and STANDARD against Antioxidant activity.]

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>HEEBA-120µg/ml</th>
<th>AEBA-140µg/ml</th>
<th>HEBA-155µg/ml</th>
<th>STANDARD-40µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>26.64</td>
<td>35.6</td>
<td>31.45</td>
<td>38.12</td>
</tr>
<tr>
<td>50</td>
<td>31.45</td>
<td>38.12</td>
<td>62.63</td>
<td>44.23</td>
</tr>
<tr>
<td>75</td>
<td>57.23</td>
<td>62.63</td>
<td>75.39</td>
<td>53.21</td>
</tr>
<tr>
<td>100</td>
<td>62.63</td>
<td>75.39</td>
<td>85.56</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>85.56</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.8. In vitro Antidiabetic activity

In vitro antioxidant activity of bambusa arundinacea seed extracts were determined by using various method.

4.8.1. Alpha-amylase inhibitory activity

One of the effective method to control diabetes is to inhibit the activity of alpha amylase enzyme which is responsible for the breakdown of starch to more simple sugars (dextrin, maltotriose, maltose and glucose). This is contributed by alpha amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals. In the present study hydroethanol, acetone and hexane extracts of seeds of B. arundinacea with known antidiabetic activity were investigated for their potential to inhibit α-amylase activity. Five different concentrations viz., 20, 40, 60, 80 and 100 µg/mL of all extracts of seeds of B. arundinacea were separately tested for the inhibition of α-amylase activity (Fig.4.34). There was a dosage-dependent increase in percentage inhibitory activity against alpha amylase enzyme. It showed 50% inhibition at a concentration of
HEEBA-64µg/ml, AEBA-95µg/ml, HEBA-118µg/ml. The order of α-glucosidase inhibitory activity as Acarbose>HEEBA>AEBA>HEBA.

4.8.2. Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitors delay the action of alpha-glucosidases to break complex carbohydrates into simple sugars, thereby lowering the absorption of glucose. In the present study, hydroethanol, acetone, and hexane extracts of seeds of B. arundinacea with known antidiabetic activity were investigated for their potential to inhibit α-amylase activity. Five different concentrations viz., 20, 40, 60, 80 and 100 µg/mL of all extracts of seeds of B. arundinacea were separately tested for the inhibition of α-glucosidase activity (Fig. 4.34). There was a dosage-dependent increase in percentage inhibitory activity against alpha amylase enzyme. It showed 50% inhibition at a concentration of HEEBA-50µg/ml, AEBA-78µg/ml, HEBA-85µg/ml. The order of α-glucosidase inhibitory activity as Acarbose>HEEBA>AEBA>HEBA.

![Figure 4.34. Alpha amylase inhibitory activity](image-url)

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4.9. Antimicrobial activity

Antibacterial and antifungal efficiency of HEEBA, AEBA, HEBA were examined using Disc diffusion method against human pathogens such as Enterococcus faecalis, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli and Aspergillus flavus. All the extracts showed significant activity against all pathogens. The activity was quantitatively assessed on the basis of inhibition zone (Table.11). HEBA and AEBA extracts showed maximum antibacterial activity for all bacterial strains tested. In the presence of HEBA the maximum inhibition zone diameter at 5 mg/ml was obtained, that is 23 mm in E. faecalis and 16 mm in E. coli (Fig.4.36). Similarly, AEBA extract showed maximum inhibition zone with a diameter of 22 mm in E. faecalis (Fig.4.36). The HEEBA (12-15 mm) showed restrained and minimum activity. More specifically, all bacterial strains showed higher susceptibility to HEEBA. For the antifungal activity, HEEBA, AEBA, HEBA showed lowest inhibition zone with diameter ranged between 8-12 mm against A. flavus strain (Fig.4.37).
Table 4.11. Antibacterial activity of Bambusa arundinacea extracts

<table>
<thead>
<tr>
<th>Name of the Pathogen</th>
<th>Antibacterial activity (Disc Diffusion Method)</th>
<th>Strep tomycin (S10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone of Inhibition (mm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentration of Sample (mg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>Acetone</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>5</td>
</tr>
<tr>
<td>Escherichia coli (ATCC 25922)</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Enterococcus faecalis (ATCC - 29212)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus epidermidis (MTCC-3615)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (ATCC-27853)</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

(-) no inhibition, Streptomycin – Std
Figure 4.36. Antibacterial activity of Bambusa arundinacea

a&b - Staphylococcus epidermidis (MTCC-3615)
c&d- Enterococcus faecalis (ATCC - 29212)
e&f - Pseudomonas aeruginosa (ATCC-27853)
g&h- Escherichia coli (ATCC 25922)
Figure 4.37. Antifungal activity of Bambusa arundinacea extracts

Hex- Hexane extract; Acet- Acetone extract; H. eth- Hydro ethanol extract; Keto- Ketoconazole ; A.F- Aspergillus flavus

Table 4.12. Antifungal activity of Bambusa arundinacea

<table>
<thead>
<tr>
<th>Name of the Pathogen</th>
<th>Zone of Inhibition (mm)</th>
<th>Name of the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hexane (5mg/disc)</td>
<td>Acetone (5mg/disc)</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

4.10. Anti helminthic activity

The hydroethanol, acetone and hexane extracts of the seeds of Bambusa arundinacea produced a significant anthelmintic activity on Pheretima posthuma in a dose dependent manner as shown (Fig.4.38). The peak anthelmintic activity exhibited by the hydroethanol extract at highest concentration (100 mg/ml) which takes $42.61 \pm 0.12$ minute for paralysis and $95.28 \pm 0.11$ minute for death of the worms, followed by acetone extract which includes $68.44 \pm 0.11$ for paralysis and $97.15 \pm 0.21$ minute for
death of the worms. Hexane extract exhibit less activity in comparison to hydroethanol and acetone extracts at all the tested doses. Potency of the extract was inversely proportional to the time for paralysis (vermifuge) and death (vermicidal) of the worms. (Fig.4.39).

![Standard](image1)

**Test 1 (10 mg/ml)**

![Test 2 (20 mg/ml)](image2)

**Test 3 (50 mg/ml)**

**Figure 4.38. Antihelmintic activity of Bambusa arundinacea**
Figure 4.39. Antihelminthic activity of Bambusa arundinacea

4.11. In vitro Anti arthritic activity

In vitro anti-arthritic activity was performed using most popular methods such as inhibition of protein denaturation activity. Concentrations ranging from 100-750 μg/ml were tested to find out the percentage inhibition (Fig.4.40). In inhibition of protein denaturation assay it was found that the standard at concentration of 100 μg/ml it offered a percentage inhibition of 59.67 % and in 250 μg/ml it has increased further to 78.51% and likewise at 500 μg/ml it was 87.27 % and in 750 μg/ml it could offer 93.75 % activity. Hydroethanol at concentration of 100 μg/ml it offered a percentage inhibition of 37.23 % and in 250 μg/ml it has increased further to 52.34 % and likewise at 500 μg/ml it was 62.14 % and in 750 μg/ml it could offer 71.34 % activity.
4.12. Anti cancer activity

The effect of HEEBA on proliferation of human colon cancer cell lines (COLO-320) and normal cell lines was investigated using MTT assay. In the MTT assay, a mitochondrial enzyme in living cells, succinate dehydrogenase, cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan and the amount of formazan produced is directly proportional to the number of viable cells (Fig.4.41). It illustrates the effect of HEEBA on the proliferation of COLO-320 and normal cell lines. HEEBA exhibited significant reduction in proliferation of COLO-320 cell lines (Fig. 4.42). Results shows that HEEBA decreases cell viability 33.37% at a concentration of 150µg/ml. The cytotoxicity effect of HEEBA was compared to the amount of cell viability after treatment with standard anticancer drug cyclophosphamide (Fig 4.43).
Figure 4.41. Control cells  Figure 4.42. HEEBA treated cells

Figure 4.43. Anticancer activity of Bambusa arundinacea
CHAPTER 5

DISCUSSION

Natural products provide a significant source of potential drugs from which humankind has identified not only phytomedicines and herbal remedies, but also most of our current anticancer and antibiotics drugs. The evaluation of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacognostical parameters and standards must be established. Therefore some diagnostic features have been evaluated to identify and to differentiate B. arundinacea from other crude drugs and adulterants. On the surface are also seen diffusely distributed pairs of silica cells and cork cells. Silica cells are white and transparent and cork cells are darkly stained. There are also triangular, thick walled conical trichomes with pointed ends. There are thick long pointed trichomes seen in the powder. There trichomes have short, curved echinate spines. Strach grains are abundant in the powder. The starch grains are spherical or squarish. They are simple type. Along the margins of lemma and palea there are long dense trichomes which are directed towards the tip. These trichomes have thick, smooth walls similar type of trichomes are also seen along the margin of the rachilla. These were some typical characteristics which may be used for its identification and prevention of drug adulteration.

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of dry powder of seeds of B. arundinacea was 12 % which is not very high, hence it would discourage bacteria fungi or yeast growth. The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods viz. total
ash, acid-insoluble ash and water-soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both _physiological ash_ which is derived from the plant tissue itself, and _non-physiological ash_, which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards. Low amount of total ash, acid insoluble ash and water soluble ash indicates that the inorganic matter and non-physiological matter such as silica is less in seeds of B.arundinacea. The variation in extractable matter in various solvents is suggestive of the fact that the formation of the bioactive principle of the medicinal plant is influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive value reveal the presence of polar substance like phenols, tannins and glycosides.

The pH is an important parameter for formulations. The pH of powder of seeds was alkaline which implies that it may be helpful in treating gastrointestinal tract disorders like ulcer. The solubility of the hydroethanolic extract of B.arundinacea was more in methanol followed by dimethylsulphoxide and less in petroleum ether. There was minor presence of some heavy metals in the hydroethanolic extract. The permissible limit of lead, cadmium, arsenic and mercury in the dietary contents as per the WHO (1998) is 10 ppm, 0.3 ppm, 10 ppm, 1 ppm. Although WHO has not yet decided the permissible limits of chromium, zinc and other metals because they are considered as micronutrients. All these parameters are useful for the compilation of a suitable monograph for it properidentification.

The seeds of Bambusa arundinacea were made coarse powder and extracted with using hydroethanol,n-hexane and acetone as solvent using standard procedure. The behavior of various extracts like texture and colour and extractive yield were calculated. It is found that percentage yield for hydroethanol extract is more than other solvent extract. The various extracts of B.arundinacea were tested for different phytoconsituents like alkaloids,
glycosides, saponins, tannins, terpinoids, reducing sugars, phenolic compounds, flavanoids, protein, carbohydrates and volatile oils. B. arundinacea seeds were rich in flavonoids, phenols, tannins, quinines and glycosides while alkaloids and phlobatannins were absent. The Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances and to screen for biological activities. The phenolic and flavanoids are widely distributed secondary metabolites in plants having antioxidant activity and have wide range of biological activities as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants in-vitro than vitamins E or C and thus might contribute significantly to the protective effects in vivo.

Secondary metabolites may be used for the preparation of drug in a systematic way which may lead to the cure of many ailments in the future. The extract had high total phenol and flavonoid content. Plant phenolics are one of the major groups of compounds acting as primary antioxidant free radical terminators. These compounds possess a wide spectrum of chemical and biological activities including radical scavenging properties. Several studies have described the antioxidant properties of medicinal plants, foods and beverages which are rich in phenolic compounds.

The antioxidant reactions involve multiple steps including the initiation, propagation, branching and termination of free radicals. The antioxidants which inhibit the formation of free radicals from their unstable precursors are called preventive antioxidants, and those which interrupt the radical chain reaction (propagation and branching) are the „chain-breaking“ antioxidants. Generally, there are various methods for determination of antioxidant activities. The measurement of radical scavenging activity of any antioxidant is commonly associated with the using of DPPH method because it is quick, reliable and reproducible method. It is widely used to test the ability of compounds as free radical scavengers or hydrogen donors.
and to evaluate the antioxidative activity of plant extracts. In the DPPH assay, the antioxidants reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydrazine which has maximum absorption at 517 nm.

The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC50) is a parameter widely used to measure the antioxidant activity. A lower IC50 value indicates a higher antioxidant power. In the present study, the IC50 value of hydro ethanolic extract was ≤ 500 µg/ml. Higher absorbance of a reaction mixture indicated greater scavenging activity and 50% inhibition was achieved at 150µg concentration in the reaction mixture, while in standard ascorbic acid, 50 % inhibition was achieved at 50 µg/ml concentration. It indicates that this hydro ethanol extract was a good scavenger of DPPH radical. Similarly moderate and low DPPH activity was reported in acetone and hexane extract respectively.

In hydroxyl radical scavenging assay, free radicals were generated by Fentons reaction, which react with deoxyribose and produce MDA. MDA on being incubated with TBA produces a pink chromogen. When hydroethanolic extract was added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. The IC50 value of hydro ethanolic extract was 78µg/ml which indicates that hydroethanolic extract had capacity to inhibit hydroxyl radical-mediated deoxyribose degradation in Fe3+-EDTA -ascorbic acid and H2O2 reaction mixture, to a certain level.

Nirous oxide anion radical is a potent oxidant but it gives rise to the generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. The IC50 value of hydro ethanolic extract was 95 µg/ml which again indicated a moderate nirous oxide anion scavenging activity. Compounds with reducing power indicate that they are electron donors, and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant
extracts. The hydroethanolic extract showed reducing capacity though it was little less than that of standard ascorbic acid which suggests that methanolic extract had a potency to donate electron to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction but to a limited extent.

HEEBA, HEBA, AEBA were detected and compared with Ascorbic acid. The IC50 values for DPPH assay for hydroethanol extract was found maximum followed by acetone extract and for hexane extract was minimum. Though the extracts showed good DPPH scavenging activity but it was less effective than standard Ascorbic acid. The hydroethanol extract showed highest reducing power followed by acetone and then hexane extracts. The increased reducing power in the extracts indicated that some components in the extract were electron donors that could react with the free radicals to convert them into more stable products to terminate radical chain reaction.

Hydrogen peroxide (H2O2), a biologically relevant, non-radical oxidizing species, may be formed in tissues through oxidative processes. Hydrogen peroxide (H2O2) which in turn generate hydroxyl radicals (•OH) resulting in initiation and propagation of lipid peroxidation. The IC50 values for hydrogen peroxide scavenging activity of for HEEBA extract was found maximum followed by AEBA extract and for chloroform HEBA was minimum. Though the extracts showed good hydrogen peroxide scavenging activity but it was less effective than standard Ascorbic acid. The ability of the extracts to quench OH_ seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction.

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological
systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities.

The IC50 values for nitric oxide scavenging activity of for HEEBA extract was found maximum followed by AEBA extract and for HEBA extract was minimum. Though the extracts showed good nitric oxide activity.

The scavenging capacity of the DPPH radical by the extract was found to be much higher than that of hydroxyl radical scavenging activity and superoxide radical scavenging activity as well as reducing power. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extract to react and quench different radicals. This suggests that the hydro ethanolic extract of B. arundinacea had the capability to scavenge different free radicals differently in different systems, indicating that it may be useful as a therapeutic agent for treating some radical-related pathological damage. This study also proves our earlier conclusion that it is very essential to evaluate more than one antioxidant assay when natural plant extracts are being evaluated for their antioxidant potential and one plant may show good activity in one assay but poor in another assay.

In-vitro antioxidant studies are widely carried to screen various plant containing phenolic and flavanoids constituents. Plant derived antioxidant compounds, flavonoids and phenolics have received considerable attention because of their physiological effect like antioxidant, anti-inflammatory, antitumor activities and low toxicity.

The Total phenolic contents in HEEBA,AEBA,HEBA were estimated using standard Gallic acid equivalent of phenols. The HEEBA extract was found to have maximum phenolic components and which may be one the reason of its to posses maximum antioxidant activity then other two extracts. As previously, it was reported that Polyphenolic compounds contribute significantly to the total antioxidant capacity of plants.
Alpha-amylase catalyses the hydrolysis of alpha-1,4-glycosidic linkages of starch, glycogen and various oligosaccharides. Alpha-glucosidase further breaks down the disaccharides to simple sugars, readily available for intestinal absorption. The inhibition of their activity in the digestive tract of humans is considered to be effective tool to control diabetes. The Hydroethanol extracts of Bambusa arundinacea seeds have exhibited potent inhibition of alpha-amylase and alpha-glucosidase enzyme activity. In addition, HEEBA was able to inhibit both the enzymes at lower concentration.

In the present investigation, the inhibitory effect of hexane, acetone, hydro-ethanol extracts of seeds from B.arundinacea were evaluated against both bacterial and fungal strains. The antimicrobial activity was determined using disc diffusion method. The activity was quantitatively assessed on the basis of inhibition zone. Hexane and acetone extracts showed maximum antibacterial activity for all bacterial strains tested. In the presence of hexane extract, the maximum inhibition zone diameter at 5 mg/ml was obtained, that is 23 mm in E.faecalis and 16 mm in E.coli. Similarly, acetone extract showed maximum inhibition zone with a diameter of 22 mm in E.faecalis. The hydroethanolic extract (12-15 mm) showed restrained and minimum activity. More specifically, all bacterial strains showed higher susceptibility to hydro-ethanol extract. For the antifungal activity, hexane, acetone and hydro-ethanol extract showed lowest inhibition zone with diameter ranged between 8-12 mm against A. flavus strain. No significant antifungal activity was seen in the hexane, acetone and hydro-ethanol extracts.

In our studies the crude extracts of Bambusa arundinacea showed moderate and strong antibacterial activity against the pathogen. Tannins were found to be a component of plants that showed antibacterial activity. It could be one of the components responsible for the antibacterial activity. The data revealed that the HEEBA extract showed antihelmintic activity at a concentration of 100 mg/ml, whereas the AEBA and HEBA showed paralysis and death at similar concentrations. The other test concentrations of both the extracts showed marked degree of antihelmintic activity. The antihelmintic effect of extracts is comparable with
that of the effect produced by the standard drug albendazole. The activity was concentration dependent of the different extracts. The activity of the extracts was found to be inversely proportional to the time taken for paralysis / death of the earthworms.

Anti cancer activity against colo 320 cell line Results shows that HEEBA decreases cell viability 33.37% at a concentration of 150µg/ml. The cytotoxicity effect of HEEBA was compared to the amount of cell viability after treatment with standard anticancer drug cyclophosphamide.

The percentage growth inhibition was found to be increasing with increasing concentration of test compounds. The hydroethanol extract obtained from B.arundinacea exhibit an excellent in-vitro anti-arthritic activity when tested using inhibition of protein denaturation method. However, treatment with plant extracts although may be have some unpredictability in the effectiveness; being non-toxic, side effect less alternative, purified plant extracts and their isolated phytoconstituents can be very useful against rheumatoid arthritis.

The seeds possess good amount of bioflavonoid compounds such as quercetin and rutin. May be the presence of quercetin and rutin the seed extracts exhibits good anticancer, antioxidant and antiarthritis activity. Both quercetin and rutin are used as medication for various diseases such as cancers, diabetes, heart diseases, arthritis, hemorrhoids, varicosis, microangiopathy and are ingredients of numerous multivitamin preparations. As the seeds of Bambusa arundinacea possess all the essentials phytoconstituents, amino acids, oils, vitamins and minerals it exhibits great medicinal value. Being non-toxic, having high nutraceutical values when compared with rice and wheat we can develop the seeds of Bambusa arundinacea as an alternative food. However, treatment with plant extracts although may be have some unpredictability in the effectiveness, side effect less alternative, purified plant extracts and their isolated phytoconstituents can be very useful against many diseases.
CHAPTER 6

CONCLUSION

In pharmacognostic study of Bambusa arundinacea seeds, standards like macroscopic and microscopic characteristics, ash values, extractive values, heavy metals analysis, pH, solubility, qualitative and quantitative phytochemical analysis were done which could be useful for the compilation of a suitable monograph for its proper identification. Pharmacognostic investigation revealed presence of Strach grains are abundant in the powder. The inorganic matter and non-physiological matter such as silica was less in seed of B.arundinacea. The pH of Hydroethanolic extract was acidic. The extract was free from heavy metal contamination. The solubility of extract was more in polar solvents than non polar. Qualitative phytochemical analysis revealed that B.arundinacea were rich in Flavonoids, phenols, cardiac glycosides, quinines and tannins, Quantitative phytochemical analysis revealed that B. arundinacea seeds had more phenol and flavonoids content. The chemical composition of Bambusa arundinacea shows it can be a potential source of nutraceuticals. They were found to be very good source of Phosphorus, Calcium, Magnesium and Iron. Seeds of B.arundinacea are potential source of, phytochemical, tocopherols, essential fatty acids like oleic acids, linoleic acid and linolenic acid. Evidently, the seed is a rich source of bioactive compounds and may be used to develop value added products and other food applications to enhance the health benefits. The obtained extracts have potent antioxidant, anti diabetic, anticancer, antiarthritic properties and may play an important role in drug development and health supplement. Thus, there is enormous scope for future research and further pharmacological investigation on Bambusa arundinacea seeds.
Further work should be carried out on the characterization of specific antioxidant, antidiabetic, anticancer, antimicrobial and antiarthritic components of Bambusa arundinacea and evaluation of their therapeutic significance in prevention of diseases *In vivo.*
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