Chapter 1

Chapter 1: Introduction

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Chapter 1

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Animals’ continuation on this earth has been made possible only because of the vital role played by plants. Plants and animals coexist together and their dependency on each other adds meaning to breathing life. Medicinal plants existing even prior to human being made their emergence on the earth.

Each division of the world has its own way to use traditional medicines. Most of the long-established medicinal system includes herbal drugs. The major areas are Chinese, Indian and European traditions. The idea of these traditional medicines has some similarity to each other but differs extensively from present advance medicine. Herbal drugs have the advantage of being available for patients in the geographical area of the traditional medicine. In vision of the advancement of medicine not only new synthetic drugs but also herbal drugs have to fulfill the international requirements on quality, safety and efficacy. The development of herbal drugs for international use has to be different from that of synthetic drugs.

Basically every country develops its own medical system, which includes the primordial civilization of China, Egypt and India. Thus, the Indian medical system Ayurveda came into existence. The raw materials for ayurvedic medicines were typically obtained from plant sources in the
form of crude drugs such as dried herbal powders or extracts or mixture of products. Also, Siddha, Unani and Tibb are traditional health care systems which have been active for many centuries. Apart from these systems there has been a rich heritage of ethnobotanical usage of herbs by various colorful tribal communities.

Vast ethnobotanical awareness exists in India from older time. Our work over four decades, both in the field and literary studies, has resulted in a dictionary of Indian folk-medicine and ethnobotany that includes 2532 plants. India has about 45,000 plant species; medicinal properties have been assigned to several thousand. About 2000 figure frequently in the literature; indigenous systems commonly employ 500. Despite early (4500-1500 BC) origins and a long history of usage, in the last two centuries Ayurveda has received little official support and hence less attention from good medical practitioners and researchers. Much work is now being done on the botany, pharmacognosy, phytochemistry, pharmacology and biotechnology of herbal drugs. The value of ethnomedicine has been realized; work is being done on household remedies. Statistical methods are being used to judge the reliability of claims. A inspection of folk claims found 203 plants for evaluation. Less well known ethnomedicines have been identified that are used to treat intestinal, joint, liver and skin diseases. Routine random efforts are not likely to increase the desired success rate of innovation, while experience indicates that a modified collection policy offers the best chances for the
discovery and development of agents for the treatment of AIDS (acquired immune deficiency syndrome) and cancer.3

Our past, Rigveda, one of our oldest repositories of human knowledge written between 4500-1500 B.C. mentions the use of 67 plants for the therapeutic purposes and Yajurveda enlists 81 plants whereas Atharvaveda written somewhere 1200 B.C. describes 290 plants.

India incontrovertibly occupies the top position in the use of herbal drugs. It is one of the prime countries exporting plant drugs or their derivatives in home consumption too. According to Indian mythology, when the illness and diseases got wild on the earth, the sages learnt the science of healing from Lord Indra and recorded them in scriptures.4

It has been estimated that about 75,000 species of higher plants exist on the earth. A sound estimate of about 10% has been used in traditional medicine. However, perhaps only about 1% of these are acknowledged through scientific studies to have therapeutic value when used in extract form by human.5

Traditional healers and pharmacists in developing countries are in key source of information about plant sources of drugs. Only a part of the earth's natural pharmacopoeia has been analyzed with modern techniques. The threat of looming disappearance of many plant species, especially in tropical areas, makes it urgent for scientists to study as
much as possible before old remedies are forgotten or their raw materials are destroyed. This process requires the observation and recording of medical techniques, identification of plant materials and experimental investigation of the ingredients and their effects. Ethnopharmacology can also be an important element of a developing nation’s medical and economic system. Third World governments are being encouraged to seek a synthesis between modern and traditional medicine. Although developing countries are providing many of the raw materials needed in drug manufacturing, the final products are often returned as high-priced medicines. As more plants are needed for large-scale production, over harvesting has led to stock depletion. Chemists have so far been unable to reproduce the complex structure of many plant compounds. Further coordinated research into folk traditions, plant species, growing conditions and local medical needs is urged. Care must be taken however to preserve the main advantages of traditional medical care, low cost and easy access\textsuperscript{6,7}.

1.1 **Herbal Wealth of India\textsuperscript{8}**

Now-a-days natural products are an integral part of human health care system, because there is popular concern over toxicity and resistance of modern drugs. India is one of the 12 leading biodiversity centers with presence of over 45,000 different plant species, 15000-18000 flowering plants, 23,000 fungi, 16,000 lichens, 18,000 bryophytes
and 13 million marine organisms. From this flora, 15,000 to 20,000 have good medicinal value. Among those only about 7,000 plants are used in Ayurveda, 600 in Siddha, 700 in Unani and 30 in modern medicines.

1.2 Introduction to Antimicrobials

Lots of diseases occur due to microbial infections. Antimicrobials are a drug moiety which either kills microbe or inhibit their growth. Even though lots of allopathic medicines are available in the market, patients need herbal treatments for many of the infectious diseases as various patients are developing resistance to many commonly available antimicrobials in to the market. Also patients are now becoming aware to the side effects of many allopathic antimicrobials. There are many conditions like pregnancy, lactation periods when patients avoid allopathic antimicrobials. Herbal remedies have been used as antimicrobials for thousands of years, yet remain effective. This suggests that bacteria, fungi and viruses have reduced ability to adapt to a plant derived antimicrobial regime. Thus need of herbal remedies as antimicrobials always present. As our traditional medicines posses various antimicrobials but many of them need standardizations. Written data is lagging. Thus role of pharmacognosist is to find out as many as antimicrobials with standardized drug system.
1.3 Introduction to *In-vitro Antioxidants*\(^{10}\)

Molecular oxygen is an essential module of all living organisms, but development of diverse reactive intermediates of molecular oxygen metabolize the cells aerobically, thus finally leading to process called oxidation. Oxidation is one of the destructive route which leads to harm various molecules. Invitro oxidation needs oxygen but biological metabolism occurs in absence of oxygen. Human body constantly engender free radicals and it posses its own defense mechanism against generated free radicals. Still free radical destruction is common. During illness when body becomes busy in recovering process free radical destruction increases which make body to look ill. Antioxidants are inhibitors to oxidative process. Many antioxidants are available with diverse nature but final role is to neutralize free radicals. Antioxidants play curtail role in pharmaceuticals as many drugs undergo oxidative decomposition inside human body. Herbal medicines have recently fascinated much awareness as alternative medicines but relatively very little data is available about their mode of action. Acetaminophen is an antipyretic analgesic drug that is available over-the-counter, and an overuse of acetaminophen can cause overproduction of reactive oxygen species during formation of N-acetyl-pbenzoquinoneimine by cytochrome P450. Many studies have confirmed that overproduction of reactive oxygen species (such as super oxide anion, hydroxyl radical and hydrogen peroxide) which further leads to oxidative stress and the result
is a injury that occurs in many developments of clinical disease processes, such as heart disease, diabetes, liver injury, cancer, aging, etc. Maintaining the balance between reactive oxygen species and antioxidant enzymes (especially superoxide dismutase, catalase and glutathione peroxidase) is therefore crucial, and could serve as a major mechanism in preventing damage by oxidative stress. This balance has been suggested to play an important role in drug toxicity, such as from acetaminophen. This mechanism has been suggested to participate in the development of oxidative stress and injury in acetaminophen-induced hepatotoxicity. Hepatotoxicity arises from infectious diseases and oxidative damages, etc.

1.4 Introduction to Anti-Inflammatory action

Inflammation is body’s defense mechanism against injury. It includes systemic and local response towards injury. The key role of anti-inflammatory agent is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid into prostaglandins. In inflammatory practice, it is essential to understand the role of chemical mediators which tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes /macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors vascular permeability,
neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Most mediators are short-lived but cause harmful effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acids (prostaglandins, leukotrienes) and cytokines (tumour necrosis factor and interleukin -1). Harmful stimuli like pathogens, irritants or injured cells set up response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue. However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhea, rheumatoid arthritis and atherosclerosis.

1.5 Introduction to Analgesics

Pain is irritating and sometime life threatening feeling of human body. In today’s fast food life pain is commonly occurring complaint among all age groups. Pain can range from mild, localized to agony. Analgesics are the painkillers. That can be steroids or nonsteroids. Allopathic treatment for pain has many side effects as lesion due to steroids, tolerance and dependence due to opiates etc. Need for the herbal analgesic is high as it is required by day today life.
1.6 **Hepatoprotectives**

Damage to liver is called hepatotoxicity. Ability to prevent any kind of hurt to liver is hepatoprotection. Liver is the vital organ of central importance involved in the preservation of metabolic functions. Liver also involved in detoxification of the endogenous and exogenous challenges like xenobiotics, drugs, viral infections etc. various homeostatic mechanisms are affected if liver normal function is impaired with serious consequences. Most of the allopathic drugs show its side effect on liver eg. Paracetamol. Herbal drugs comparatively show fewer side effects.

Therefore in the present study it is planned to assess antimicrobial, antioxidant, analgesic, anti-inflammatory and hepatoprotective potential of selected plant extracts. Hence from all this it is thought that the present study is highly justifiable and more needful. Several studies relate antimicrobial, antioxidant activity to anti-inflammatory and analgesic activities.

In view of the potential use of medicinal plants, as a source of active constituents in eliciting novel drug molecules, the medicinal plants *Terminalia pallida* and *Boswellia ovalifoliolata* were selected to examine chemically and biologically.
1.7 Objective

The objective of the study is divided into two parts. The first part is to isolate and identify the marker compound in the methanolic and n-Hexane extracts of *Terminalia pallida* and *Boswellia ovalifoliolata*. And the other part is to evaluate the hepatoprotective, antioxidant, anti-inflammatory, analgesic and anti-microbial activity of the methanolic and n-Hexane extracts of *Terminalia pallida* and *Boswellia ovalifoliolata* in different validated experimental animal models.

The objective is subdivided as follows.

- Selection and collection of the plants and plant parts on their ethnomedical use.
- Collection and authentification of the plant resources.
- Preparation of plant extracts.
- Screening of phytochemical profile.
- Screening the selected extracts for antimicrobial and antifungal activity.
- Screening the selected extracts for antioxidant property using *in vitro* method.
- Determination of anti-microbial activities for the pure molecules isolated from plants.
- To screen the potent plants extracts for their anti-inflammatory, analgesic and hepatoprotective activities.
1.8 Plan of work

**Phase-I:** Collection and authentication of the plants materials.

**Phase-II:** Phytochemical studies

- Preparation of extracts by using methanol and nHexane in Soxhlet apparatus.
- Isolation pure compounds selected plants extract.

**Phase–III:** Pharmacological screening of the active extracts of selected plants.

- Antimicrobial activity
- *In-vitro* Antioxidant Studies
- Acute toxicity study: (Determination of LD$_{50}$).
- Anti-Inflammatory Activity
- Analgesic Activity
- Hepatoprotective Activity

1.9 Antimicrobial activity

- Cup-plate method
- Turbidomeric analysis (For MIC determination)

1.10 *In-vitro* anti-oxidant activity

- Parameters to be studied

  A. Catalase (CAT)
B. Superoxide dismutase (SOD)

1.11 Anti-Inflammatory Activity

A. Acute Anti-inflammatory activity
   ✤ Formalin-induced paw oedema in rats

B. Chronic Anti-inflammatory Activity
   ✤ Formalin induced paw oedema

1.12 Analgesic Activity:
   ✤ Acetic acid induced writhing in mice

1.13 Hepatoprotective Activity

   ✤ D-galactosamine/Lipo-polysaccharide induced hepatitis
   ✤ Ethanol induced hepatotoxicity

Parameters to be studied

   ✤ Determination of SGOT, SGPT and ALP
   ✤ Lipid profile (TGs, TC, HDL-C, LDL-C, VLDL-C)
   ✤ Total and direct bilirubin
   ✤ Liver weight and volume
   ✤ Total serum protein
   ✤ Histopathological studies