INTRODUCTION

1.1 Origin of phytomedicine

In the early period, primitive man went in search of food and ate at random plants or their parts like tubers, fruits, leaves, etc. As no harmful effects were observed he considered them as edible materials and used them as food. If he observed other effects by their eating they were considered them as inedible, and according to the actions he used them in treating symptoms or diseases. If it caused diarrhoea it was used as purgative, if vomiting it was used as emetic and if it was found poisonous and death was caused, he used it as arrow-poison. The knowledge was empirical and was obtained by trial and error. He used drugs as such or as their infusions and decoctions. The results were passed on from one generation to the other and new knowledge was added in the same way.

Traditional medicine has a long history of serving people all over the world. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs (De Pasqual., 1984). There is an evidence of herbs being used in the treatment of diseases and for revitalizing body systems in almost all ancient civilizations.

The Vedas form the earliest literature in India. They are Rigveda, Yajurveda, Samaveda and Atharvanaveda. There is no definite evidence that suggests their exact period of origin. The Vedic period in Indian history dates back to over 5000 years. The history of medicine in India can be traced to such a remote past. The earliest mention of the medicinal use of plants was found in the Rigveda and in the Atharvanaveda (3,500-1,500 B.C.) from which Ayurveda, the ancient indian system of medicine has developed. The Ayurvedic writings can be divided in to three main ones (Charaka Samhita, Susruta Samhita, Astanga Hrdayam Samhita) and three minor ones (Sarngadhara Samhita, Bhava Prakasa Samhita, Madhava Nidanam Samhita). Ayurveda is the term for the traditional medicine of ancient India. Ayur means “life” and veda means “the study” of which is the origin of the term. The oldest writing-Charaka Samhita- is believed to date
back six to seven centuries before Christ. It is assumed to be the most important
ancient authoritative writing on Ayurveda. The Susruta Samhita is thought to have
arisen about the same time period as the Charaka Samhita, but slightly after it
Astanga Hrdayam and Astanga Samhita have been dated about the same time and
are thought to date after the Charaka and Susruta Samhita. (Ben-Eric Van Wyk
and Michael Wink, 2004)

Since time-immemorial plants are being used in all cultures as a source of
medicine. Apart from primitive and ancient civilizations, the present
contemporary cultures all over the world are relying on herbs to reap the benefits
that Mother Nature has extended to mankind (Rakesh et al., 2006). It is estimated
that, 7,500 plants are used in local health traditions mostly in rural and tribal
villages of India. Out of these, the real medicinal value of over 4000 plants is
either little or not known to the main stream of population (Pushpangandam,
1995). Thus, the Ayurvedic database allows a drug researcher to start from a well-
tested and safe biological material. Ayurveda appears to be the source for
universal planetary principles of healing throughout the ancient world, based on
balance and memory with nature and the utilization of therapeutic diet, herbs,
rituals and various phytotherapies.

There has been an increasing awareness in the recent years in
ethnobiological studies, both on the traditional medicine and particularly on tribal
medicine. The claims of therapeutic efficacy and the lack of toxicity of many
plants have been scientifically proved in recent years. There are, however a large
number of plants of questionable value among the vast repertory of indigenous
drugs. It will be a worthwhile exercise if one tries to select the best out of them.
There are a large number of plants, which have to be examined thoroughly for
their useful activity.

Many of the modern medicines are produced indirectly from medicinal
plants, for example aspirin. Plants are directly used as medicines by a majority of
cultures around the world, for example Chinese medicine and Indian medicine.
Many food crops have medicinal effects, for example garlic. Medicinal plants are
resources of new drugs. It is estimated that there are more than 2, 50,000
flowering plant species. Study of the medicinal plants helps in understanding the plant toxicity and protects the human and animals from natural poisons. Cultivation and preservation of medicinal plants protect the biological diversity, for example metabolic engineering of plants.

1.2 Plant metabolites

The medicinal effects of plants are due to metabolites or organic compounds synthesized by plant using enzyme-mediated chemical reactions called metabolic pathways. Plant metabolites include: primary metabolites and secondary metabolites. These compounds are synthesized by plants for both essential functions, such as growth and development (primary metabolites), and specific functions, such as pollinator attraction or defense against herbivores (secondary metabolites).

1.2.1 Primary metabolites

Primary metabolites comprise many different types of organic compounds, including, but not limited to carbohydrates, lipids, proteins, amino acids and nucleic acids. They are found universally in the plant kingdom because they are the components or products of fundamental metabolic pathways or cycles such as glycolysis, the Citric acid (TCA) cycle, and the Calvin (C₃) cycle. Because of the importance of these and other primary pathways in enabling a plant to synthesize, assimilate, and degrade organic compounds, primary metabolites are essential.

Examples of primary metabolites include energy-rich fuel molecules, such as sucrose and starch, structural components such as cellulose, informational molecules such as DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), and pigments, such as chlorophyll. In addition to having fundamental roles in plant growth and development, primary metabolites are precursors (starting materials) for the synthesis of secondary metabolites.
1.2.2 Examples for primary metabolites

α-Linolenic acid (polyunsaturated omega-3 fatty acid(C$_{18}$H$_{30}$O$_2$))

Glucose

Arginine

Phenylalanine

Methionine

Lysine

Histidine

Tryptophan

Threonine

Alanine

Aspartic acid

Asparagine

Cysteine

Glutamine

Glutamate

Glycine
1.2.3 Secondary metabolites

Secondary metabolites largely fall into three classes of compounds: alkaloids, terpenoids, and phenolics. However, these classes of compounds also include primary metabolites, so whether a compound is a primary or secondary metabolite is a distinction based not only on its chemical structure but also on its function and distribution within the plant kingdom. Many thousands of secondary metabolites have been isolated from plants, and many of them have powerful physiological effects in humans and are used as medicines. It is only since the late twentieth century that secondary metabolites have been clearly recognized as having important functions in plants.

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral (Mc Naught et al., 1997) and even weakly acidic properties. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and more rarely other elements such as chlorine, bromine, and phosphorus. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals.

Terpenes are naturally occurring hydrocarbons, based on combinations of the isoprene units. Terpenoids are compounds related to terpenes, which may
include some oxygen functionality or some rearrangement. However theses two terms are often used interchangeably.

Flavonoids (or bioflavonoids) (from the Latin word *flavus* meaning yellow, their colour in nature), are a class of plant secondary metabolites. Flavonoids were originally referred to as Vitamin P, probably due to the effect they had on the permeability of vascular capillaries, but this term is rarely used now (Mobh, Shiro., 1938).

1.2.4 Examples for secondary metabolites

- **Quercetin (flavonoid)**
- **Nicotine (alkaloid)**
- **Anthocyanidin (flavonoid)**
- **Artemisinin (terpene)**
- **Paclitaxel (terpene)**
- **Allicin (organosulfur compound)**
1.2.5 Modes of action of secondary metabolites (Ben-Eric Van Wyk and Michael Wink., 2004)

Several secondary metabolites have been used by mankind for thousands of years as dyes (e.g., indigo, shikonine), flavours (e.g. vanillin, capsaicin, mustard oils), fragrances (e.g. rose oil, lavender oil and other essential oils), stimulants (e.g. caffeine, nicotine, ephedrine), hallucinogens (e.g. morphine, cocaine, scopolamine, tetrahydrocannabinol), insecticides (e.g. nicotine, piperine, pyrethrin), vertebrate and human poisons (e.g. coniine, strychnine, aconitine) and most importantly as therapeutic agents (e.g. atropine, quinine, \('cardenolides, codeine\).

In order to be effective as a therapeutic agent, a secondary metabolite must interfere with an organ, tissue, cell and ultimately with a molecular target in the human body. Secondary metabolites usually are multifunctional compounds because most of them carry more than one pharmacologically active chemical group fig.1.1. In addition, secondary metabolites usually occur in complex mixtures. In consequence, the extract of a medicinal plant affects more than one molecular target and it is likely that several targets are affected concomitantly when phytomedicines are taken. In complex disorders the application of such extracts increases the chances of “hitting” one or several relevant targets.

In general, we find a series of related compounds in a given plant species; often a few major metabolites and several minor components, which differ in the position of their chemical groups. The profile usually varies between plant organs, within developmental periods and sometimes even diurnally. Also marked differences can usually be seen between individual plants of a single population, even more so between members of different populations. Even small
changes in chemistry can be the basis for a new pharmacological activity. This aspect is important for quality control in phytotherapeutics.

An overview of all the main molecular targets that are modulated by plant medicines are depicted in fig. 1.2. Structures of allelochemicals appear to have been shaped during evolution in such a way that they can mimic the structures of endogenous substrates, hormones, neurotransmitters or other ligands; this process can be termed “evolutionary molecular modeling”. Other metabolites intercalate or alkylate DNA, inhibit DNA and RNA related enzymes, protein biosynthesis, modulate metabolically active enzymes or disturb membrane stability. As a consequence of such interactions, plant medicines can interfere with organ malfunctions (heart and circulation, stomach and intestines, lung, liver, kidney, CNS disorders, gonads, inflammation and infections). In conclusion, phytotherapy is a traditional approach to use the right plants in the right concentrations to restore health or to relieve symptoms of disorders and disturbances.

Fig.1.1 The diversity of natural products in their biological properties
1.3 Objective and scope of work

The potential use of plants as a source of new drugs is still poorly explored. In terms of their pharmacological properties out of the estimated 2, 50,000-5, 00,000 plant species, only a small percentage has been investigated phytochemically and even small percentage has been properly studied. In most cases only the pharmacological screening or phytochemical analysis has been studied for their medicinal use (Mabberley D.J., 1997).

As natural products research continues to be an important part of the drug discovery, the author developed interest in taking up the phytopharmacological investigation (Phytochemical screening, acute toxicity studies, in vitro free radical-scavenging activity, in vivo hepatoprotective activity, in vivo anti-inflammatory activity, in vitro anti-microbial activity) of selected plant species.
1.4 Selection of plant Species

Due to the high cost of this type of research selection of the specimen to be analyzed is one of the critical points. Any inappropriate selection can result in wasting of time and resources.

According to Elisabetsky and Moraes (1988) there are three different ways of approach for selection of medicinal plants.

1. Randomized, i.e., which does not use any criteria, and the investigation takes an arbitrary course, every time a specimen is available.

2. Chemotaxonomical or phylogenitical, where the species are selected according to a given chemical category of substance in a genus or family.

3. Ethnopharmacological, in which selection of plant is based on their therapeutic use by an ethnic group.

There is another aspect which everyone agrees. If the selection of plants is made on the grounds of their traditional use, the chance of research success is greater (Trotter R.T et al., 1982; Elisabetsky, E and Wannmacher L., 1993).

After analyzing the above aspects, the author has considered ethnopharmacological uses for the selection of *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* in this research project.

There is a strong belief among the tribal people of Ankannagudem (Jellugumelli mandal, West Godavari Dist, Andhra Pradesh, India) forest region, in which they were using *Elephantopus scaber* for veterinary wound healing and joint pains. The literature survey indicated that plant extracts of *Celosia argentea* and *Catharanthus pusillus* have shown anti-tumour activity. With a view to prove the folkloric claims of these three plants and make them useful to the human community, the author felt that these three plants are worth to considered for this project.
1.5 Plan of the proposed work

The purpose of this research work is to investigate the pharmacological activities and phytochemistry of these plants in a scientific manner. The different steps adopted are given here under.

1.6 Collection of plant materials and extraction

The freshly collected plants were shade dried and powdered. The powdered materials were then subjected to maceration. Extracts obtained were concentrated under vacuum at temperature of 43 °C by using rotary evaporator and fractionated by using separating funnel.

1.7 Preliminary Phytochemical Screening

The fractions were tested for preliminary phytochemical screening and quantitative estimation of total phenols and total alkaloids.

1.8 Acute toxicity studies

Toxicity studies were conducted as per accepted protocol drawn under OECD-425 guidelines for rats. The acute toxicity study was aimed at establishing the therapeutic index i.e., the ratio between the pharmacologically effective dose and the lethal dose, and also to perform the primary screening.

1.9 Evaluation of Antioxidant activity

Sometimes the endogenous antioxidants and the protective mechanisms are found to be insufficient. Hence, the search for exogenous antioxidants is being continued. Recently, intensive research has been carried out to characterize the antioxidant properties of extracts from several plant materials. Hence the plant extracts were studied for in vitro free radical scavenging activity against superoxide, hydroxyl and DPPH radicals.

1.10 Screening for hepatoprotective activity

All the fractions were screened for the hepatoprotective nature against CCl₄-induced hepato-toxicity in rats, to know the hepatoprotective potential of the
crude extract and in order to know the fraction/ phytoconstituent responsible for the hepatoprotective activity.

1.11 Assessment of anti-inflammatory potential

All the fractions were screened for anti-inflammatory activity using carrageenan-induced rat paw oedema method, to assess the folk claims and to find out the active constituents responsible for anti-inflammatory activity.

1.12 Investigation of in vitro anti-microbial activity

All the fractions were screened for in-vitro antimicrobial activity using cup plate method. To assess the folk claims and to find out the active constituents responsible for antimicrobial activity.

1.13 Documentation of results

The results were tabulated and the statistical significance (significance: *P<0.05, **P<0.01, ***P<0.001) of the results was carried out by using unpaired student’s t-test.