I. INTRODUCTION

Man has been evolving and living on this planet for well over 9,000,000 years in about 50,000 generations. How did he survive so long on this planet? The basic secret is the wisdom of the human body. The healing power is the nature's gift to the man, especially from the plants. Many countries have their own system of medicine to cater the health needs of people. Among these, the one system of holistic management that existed since the dawn of man's history is the Indian system of Ayurveda. It is still in practice all over the world having an unbroken continuity (Hegde, 2000).

The ancient Indians gave their 'Science of Medicine' as Ayurveda. Ayur means life and veda, to know or attain. The preservation of health and its maintenance are the main aim of Ayurveda. It discusses the origin of diseases on scientific basis, exploring the herbal drugs useful in ailments, to attain happiness and to extinguish sorrows (Jyotirmita Acharya, 2000). There are now enough evidences to say that, this was the mother of all systems of medicine. A great Greek writer 'Pocoke' in his elaborated book stated that the "temple of Greek history has given evidence that Greek civilization was originally taken from India by Indians who migrated to Greece". Every person is supposed to have a unique humoural constitution, which represents the healthy state. According to WHO, the health is defined as a state of well being at physical, mental, social and spiritual levels and not mere absence of infirmity (Hegde, 2000).

Today man mesmerized by the reductionist scientific developments including the advances in the modern medicine is led to believe that he could control all these with its technology, modern drugs and surgical feats. In addition with the advent of biotechnology all claims of success in genetic engineering and also cloning have raised human hopes to the skies. But illness
and death without any drastic change still plague man in several countries. Even the claims of endemic rise and subsequent fall of degenerative diseases has been shown to be fallacious. The group of cancer death has not shown a tendency to come down although we have seen some improvements in certain child hood cancer.

Modern medicine has been there with all this gadgets for the last 4-5 decades, but man survived here long before that without any of those gimmicks. Modern medicine for all its breath taking advances seems to be slightly off balance like the tower of Pisa was the option of Prince Charles, the heir to the British throne. According to Hegde 2000, the modern medicine is analogues to the fire fighting system, when the house is already on fire with some parts of the house destroyed, the fire fighters try to quench the fire with water hoses. But, in biology this kind of fire fighting results in the hose being short of the fire in many instances.

The indigenous systems of medicine practiced in India are based mainly on the practice of folk, tribal and traditional medicines. All these codified streams are emerged as Ayurveda. The ancient physician, Charaka (1000 BC-100 AD) recorded the use of 2000 vegetable remedies. This system was not solely based on empiricism and it is evident from the fact that some medicinal plants that were used in ancient times still have their place in modern therapy. Thus, for example, ‘Ephedra’ a plant used in China 4000 years ago is still mentioned in modern pharmacopoeias as the source of an important drug, ephedrine. The plant Sarpagandha (*Rauwolfia serpentina*) which was well known in India as a remedy for insanity has now shown that one of its constituents, reserpine, a wonder drug today for curing cardiac ailments. Some of the most potent remedies discovered from the past are using today, for example, aspirin, codeine, pilocarpine, cocaine, atropine, etc. It is indeed fascinating that two thirds of the world population depends upon plant
resources for their primary health care needs and a fairly large number of modern drugs are derived from modeled after or discovered from natural products.

As modern science came up and technology advanced, people started looking for the active components involved in the therapy from the crude extract and with the technology available, tried to purify the active components and use them for the treatment of various diseases.

The chemical investigation of plants on scientific lines started from 1800 AD onwards and the important discoveries in this regard were those of Sertuner (1806), a German pharmacist, who discovered morphine from opium from the plant *Papaver somniferaum*, the isolation of strychnine (1818) from *Strychnos nuxvomica* and the French chemists, Pellatier and Caventou (1820), who discovered quinine from the Cinchona bark which was universally recognized as a cure for malaria. They are considered to have laid the foundation for the school of Plant Chemistry, which resulted in the discovery of many active principles of plants useful in the alleviation of human diseases (Azar Ali, et al., 2001).

Today, chemical and pharmaceutical investigations have added a great deal of status to the use of medicinal plants by revealing the presence of the active principles and their actions of human and animal system. Investigations in the field of Pharmacognosy and Pharmacology have supplied valuable information on medicinal plants with regard to their availability, botanical properties, method of cultivation, collection, storage, commerce and therapeutic uses. All these have contributed towards their acceptance in modern medicine and their inclusion in the pharmacopoeias of civilized nations.
A big turning point in the history of medicine occurred when people started using modified (semi-synthetic) isolated active drugs from living sources, which involved chemical modifications of drugs derived from plants and animals. The narcotic alkaloids of opium and the solanaceous alkaloids of the nightshade family are examples of plant prototype drugs that were subjected to exhaustive chemical modifications. Some researchers in the first half of the twentieth century attempted to increase the analgesic properties and decrease the addiction liability of morphine by synthesizing thousands of modified semi synthetic analogs of the opium alkaloid like desomorphine, diacetylmorphine (heroin), dihydromorphine, ethylmorphine (dionin), hydromorphine (dilaudid), oxymorphone (numorphan), etc. Semi-synthetic derivatives of solanaceous alkaloids (atropine, hyoscyamine, stramonine) includes anisotropine methylbromide (valpin), eucatropine, homatropine methylbromide (mesopin) and methscoplamine bromide (Ashwani, et al., 2005).

Taking enough experience from the use of natural medicines or their modified versions people started synthesizing medicines chemically. During the decade of the 1950’s, the accelerated development of synthetic drugs began and today almost all of the currently used drugs are obtained from pharmaceutical plants. The selected categories of drugs include, alkylating agents, angiotensin II antagonists, bile acid sequestrants, benzodiazepines, calcium blockers, carbonic anhydride inhibitors, cox-2 inhibitors, fluoroquinolones, gastric acid pump inhibitors, mast cell inhibitors, phenothiazines, prostaglandin antagonists, serotonin receptor agonists, serotonin reuptake inhibitors, sulfonamides and thiazide diuretics etc. However, many drugs like antibiotics, digoxin, insulin and the opioid narcotics like morphine and atropine still obtained from plants and microbes because chemistry has failed to replace them.
No doubt, these purified and highly concentrated chemically synthesized medicines showed pronounced effects because of their increased potency, but in most of the cases when used for a long duration and in certain cases showed drastic side effects. Further, most of the modern synthetic medicines used to treat some common diseases have been reported to cause a number of undesirable side effects, which sometimes can be lethal. On the contrary, the traditional medicines are known to cure the diseases with comparatively lesser side effects (Ashwani, et al., 2005).

In spite of tremendous developments in the field of allopathy during the 20th century, plants still remain one of the major sources of drugs in modern as well as traditional systems of medicine throughout the world. Approximately one-third of all pharmaceuticals are of plant origin, wherein fungi and bacteria are also included. Over 60% of all pharmaceuticals are plant based.

Out of nearly 3 Lakh species of higher plants available, only a small proportion has been investigated for medicinal purposes and a still smaller number yield well defined drug. The same is the case with lower plants and with plants of the sea. Thus, the knowledge of plant constituents gained so far is still meager, considering the huge number of species available in the world. Approximately, 10% of the organic constituents of plants are discovered, rests of them are yet to be explored.

In Indigenous system of medicine several plants and plant products are being used to cure serious ailments. The knowledge of our ancient physicians was very vast and their vegetable material medica was extensive. It is curious to note that they picked up specific herbs growing in natural conditions for treating wounds and particular disorders.
Many tribal and traditional practitioners were capable to cure the serious ailments in light of their practical experiences. There exits a great demand for the plant based drugs, and has given rise to large-scale collection of medicinal plants. Modern system of medicine has a very limited application in alleviation of symptoms like jaundice whereas the herbal medicines have worked wonder in curing liver disorders. Thus a careful and extensive study of medicinal plants is necessary to identify newer plant products, isolation of purified compounds and this intern may help us to combat the dreaded problem of liver disorders.

Liver disorders are one of the common problems in a country like India. Liver being one of the most vital organs of the human system, performing diverse function, a healthy liver is one of the prerequisites for a normal and healthy life. If we take a look at the medical statistics, we find there are about 20,000 deaths globally due to cirrhosis of the liver. Liver carcinoma is one of the common forms of cancer, which is prevalent in the world today and almost 2,50,000 cases of liver carcinoma are detected every year (Chattergee, 1996). To day, there is an increasing trend in liver disorders and this may be attributed an alarming increase in population levels, increased consumption of alcohol, indiscriminate and irrational use of drugs, particularly antibiotics and due to viral infections. The liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory and neoplastic insults. The dominant primary diseases of the liver are viral hepatitis, alcoholic liver disease and hepatocellular carcinoma.

The major viral agents involved in hepatitis are hepatitis A, B, C, D, E, F and G. They are grouped into two major groups namely, infective hepatitis and serum hepatitis. The clinical features of these two types of hepatitis are almost similar. However, they differ in their mode of transmission and incubation period. The first symptom of viral hepatitis is jaundice, which occurs when the bile pigment bilirubin accumulated in the blood. This may be
caused by the inability of liver to metabolize bilirubin. Icterus is defined as a condition characterized by the yellow discoloration of sclera, mucous membrane and cornea of the eye. Normally bilirubin is formed by the breakdown of senescent red blood cells. Bilirubin is insoluble in water but solubilize by conjugation with glucoronic acid, namely, bilirubin diglucorronide. This conjugation exclusively occurs in liver. In hepatitis condition the liver cells are damaged and fail to conjugate bilirubin. Hence, excess of bilirubin is accumulated into the blood stream (Israels, 1959).

The other symptom in acute hepatitis includes confusion, flopping movement of hand, fever, giddiness, and lack of co-ordination. In this condition digestion of fat is impaired and fat lobules are accumulated in the hepatocytes. Although, preventive measures are available for infective and viral hepatitis there is no specific allopathic treatment. The treatment in most of the instances consists of restriction of physical activity and adequate diet (Rowe, et al., 1988).

In Indian system of medicine several plants and plant products are known to act as potent hepatoprotective principles for healing infective hepatitis (Chopra, 1965). A rapid progress has been noticed in the clinical study of hepatoprotective plants, such as, *Wedelia chinensis* (Young, et al., 1986), *Boerhaavia diffusa* (Gulati, et al., 1991), *Ocimum sanctum* (Chattopadhya et al., 1992), *Cassia angustifolia* (Ilivarasan, et al., 2001), *Entada purseatha* (Vidya, et al., 2004), *Vitex negundo* (Srinivas, 2004), *Boerhaavia rependa* (Krishna and Shanthamma, 2004) *Diospyrus cardifolia*, (Krishna, et al., 2005), and many works have been carried out on the effect of isolated constituents on hepatoprotective activities, such as, wedelolactone from *Wedelia calendulacea* (Wagner, et al., 1986), irridoid glycosides from *Picrorhiza kurrooa* (Dwivedi, et al., 1991, Saraswath, et al., 1993), flavonoids from *Andrographis alata*
(Nagaraja and Krishna, 2003), triterpenes, ursolic acid, lupeol, betulin and betulinic acid from *Diospyrus cardifolia* (Krishna, *et al.*, 2005) and so on.

In India Karnataka state is endowed with rich natural resources especially along the Western Ghats ranges. The good climatic conditions and altitudinal gradients of this belt have resulted in the developments of a variety of floras. Floristically this region harboring as many as 4,865 species of flowering plants, of which, 56 genera and 2100 species are endemic (Tewari, 1995). The anthropogenic activities in the core forests of the Western Ghats have resulted in the dwindling of natural vegetation. More than seventy plants of this region have red listed in Red Data Book of India. (Nayar and Shastry, 1982). In many cases, as the important plant parts used are roots or the entire plant, the plant collectors engaging in destructive collection, resulting many plants becoming extinct or being listed as threatened. In nature they are found scattered and it is difficult as well as uneconomical to collect and process them for medicinal purposes.

Plant tissue culture technique is a promising area of research in the conservation of medicinal plants. It offers quick and efficient methods to exploit medicinal plant meaningfully to meet the measuring demands of the pharmaceutical industries. Tissue culture technology can be used to get more medicinal components without destroying the nature plants. Similar to *in vivo* plants the *in vitro* derived calli are also able to synthesize medicinal components such as alkaloids, glycosides, steroids etc. and it is advantageous over intact plants in the production of secondary metabolites (Kamada, 1985 and Yamato and Yamada, 1986).

Plant tissue culture is a process by which parts of plants and also single cells can be grown *in vitro* in sterile culture in a medium supplemented with carbon source, minerals, growth factors and growth regulators such as
hormones. The totipotency of plant cells, which confers ability to differentiate into shoots and roots, has greatly contributed to progress in this area. Tissue culture is of great value, particularly in speeding up conventional breeding and propagation procedures.

The term micropropagation, as applied to plants means the generation (reproduction) of plants by asexual means. Methods of conventional propagation involve taking portions of a plant inducing each portion to form roots and/or shoots, thus forming a new plant. Compared to conventional propagation, micropropagation has the advantage of allowing rapid propagation in a limited space, it allows complete control of growth conditions, and the plants produced are microbiologically sterile and thus exempt from quarantine regulations that may apply to export/import of normal plants. This procedure increases the efficiency of obtaining virus free plants from infected plants. Micropropagation industry has now emerged in many countries. As this industry is labour intensive and needs a large number of trained workers with expertise in aseptic handling, developing countries such as India with a lot of trained manpower have potential to develop this industry.


In the present investigation a moist deciduous cauline herb Elephantopus scaber Linn. has been selected. Traditionally, the plant claims to be useful for various ailments such as wound healing, hepatoprotective anti-inflammatory, diuretic, tumor, analgesic, antipyretic, cardiovascular etc. The therapeutic efficacy of this species on different ailments might be due to a specific single constituent or may be due to additive effects, which may be responsible for different activities. Hence, the present investigation is focusing on the derivation of in vitro, protocol for rapid propagation of Elephantopus scaber Linn. using different explants, isolation of a sesquiterpene lactone and comparative pharmacological screening of different solvent extracts and the constituent on wound healing, hepatoprotective and anti inflammatory models.

**The main objectives:**

1. To standardize protocols for in vitro regeneration of the plantlets using different explants.
2. To know the effect of various factors affecting callus initiation, growth and differentiation.
3. To identify the phytochemical groups from different solvent extracts.
4. To isolate the sesquiterpene lactone compound from the extracts.
5. To evaluate the efficacy of the different extracts and isolated compound on wound healing, hepatoprotective and anti-inflammatory models on experimental rats.