India is one of the major countries, having 40 per cent of the global biodiversity and availability of rare plant species. Medicinal and aromatic plants constitute a major segment of the flora, which provides raw materials for use in the pharmaceuticals and drug industries. The indigenous systems of medicines, developed in India for centuries, make use of many medicinal herbs. These systems include Ayurveda, Siddha, Unani and many other indigenous practices. More than 9,000 native plants have been established and recorded for their curative properties. In one of the studies made by the World Health Organisation, it was estimated that 80 per cent of the population of developing countries relies on traditional plant based medicines for their health requirements (WHO, 1991). Even in many of the modern medicines, the basic composition is derived from medicinal plants and these have become acceptable medicines for many reasons that include easy availability, least side effects, low prices, environmental friendliness and lasting curative property.

In India, the use of herbal medicine can be traced back from the Vedic period and the first written reports are timed to 600 BC with Charaka Samhita. India is a varietal emporium of medicinal plants and it is one of the richest countries in the world as regards genetic resources of medicinal plants. The Ministry of Environment and Forest, Government of India has identified and documented over 9,500 species of medicinal plants that are significant for the pharmaceutical industry. Among them, 2,000 to 2,300 species are used in traditional medicines, while at least 150 species are used commercially on a large scale (EXIM Bank, 1997). Due to this rising international demand, many important medicinal plant species are becoming scarce and some of them are facing the prospect of extinction. Therefore, it is important to conserve the extensively traded medicinal plants in their natural environments or cultivate them in favourable environments.

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is also increasing the attention of the indigenous people. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most
of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various systems (Farnsworth and Soejarto, 1991).

India is the second largest exporter of medicinal plants in the world. Instead of exporting such a large amount of valuable resource with very low returns, we can think about developing in our its own Research and Development capabilities and to produce finished goods in the form of modern medicines and health care products derived from plant origin and based on the knowledge of alternative system of medicine (Kamboj, 2000).

Information about genetic relationships among accessions within and between the species has several important applications in plant improvement (Thormann et al., 1994). Therefore correct genotype identification of the plant material remains important for protection of both the public health and industry. Chemoprofiling and morphological evaluations are routinely used for the identification of medicinal plants. Chemical complexity and lack of therapeutic marker(s) are some of the limitations associated with morphological evaluation. Now a-days genetic polymorphism in medicinal plants has been widely studied to distinguish plants at inter- and/or intra-species level (Joshi et al., 2004).

Morphological traits are also commonly used to determine relationships but they do not provide good estimates of genetic distance because they are influenced by the environment and they are not variable enough to adequately characterize genetic differences among elite genotypes (Smith and Smith, 1992). Molecular markers have also been used to quantify genetic diversity in plants (Clegg, 1990). The advantages of using molecular markers are to allow direct comparisons of genetic similarity to be made at the DNA level (Newbury and Ford-Lloyd, 1993). They are not affected by plant development and also they are not modified by the environment and they are very abundant (Novy et al., 1994). In the last decade, molecular markers such as RFLP, RAPD, SCAR and AFLP have been used to assess the genetic variation at the
DNA level, allowing an estimation of the degree of relatedness between individuals without the influence of environmental factors (Miller and Tanksley, 1989; Pandian et al., 2000). Factors such as speed, efficiency and amenability to automation make RAPD analysis the most suitable method for effective germplasm management with respect to estimating diversity, monitoring genetic erosion and removing duplicates from germplasm collections (Virk et al., 1995).

Commercial venture in the herbal medicines increasing the demand for the medicinal plants which lead to irreplaceable loss of naturally occurring plant species. To meet the ever increasing demand for this valuable medicinal plant, it is necessary to find out the superior varieties in terms of action against diseases and disorders and to produce them in large scale. Culturing of callus tissue, cell suspensions, and isolated roots are the major tissue culture technologies employed so far for the characterization and evaluation of important secondary metabolites from plants (Rhodes et al., 1987).

During the last two decades there has been an upsurge in the search for new plant-derived drugs containing medicinally useful alkaloids, glycosides, polyphenolics, steroids, and terpenoid derivatives. Farnsworth et al. (1985) identified 119 secondary metabolites, isolated from higher plants that were being used globally as drugs.

Different environmental conditions can also affect the chemical composition of the plants (Khan et al., 2010). The biosynthesis of secondary metabolites varies among plants, even in different organs of plants and their biosynthesis depends on the environmental factors in which they grow. Intra-specific variation in phytoconstituents has been documented extensively among the plants (Chew and Rodman, 1979; Johnson and Scriber, 1994). Differences in biosynthesis can result from both genetic and phenotypic variations. Phenotypic variation is especially pronounced in the physiological responses of a plant under growth conditions. Many environmental factors like precipitation, mean temperature, soil, wind speed, low and high temperature extremes, duration of snow-cover, length of the vegetation period and the intensity of radiation also known to influence the biochemistry of medicinal plants (Korner, 1999). Moreover, study on phytochemicals of wild populations of plant at different altitudes were performed and it is not conclusive whether the
observed variations are the response of individual plants to environmental factors related to altitude or a genetic adaptation of the populations growing at different altitudes to their specific environment (Mc Dougal and Parks, 1984; Polle et al., 1992; Veit et al., 1996; Ruhland and Day, 2000; Zidorn and Stuppner, 2001; Zidorn et al., 2005). However, biochemistry of P. niruri growing in different geographical regions of India and environmental factors is fluctuating at various altitudes. In view of the importance of this species, its large scale multiplication and cultivation of quality planting material (based on the content of active ingredients) is urgently required.

A wide range of plant species belonged to the genus Phyllanthus have been phytochemically investigated. Among the studied species, P. niruri, P. urinaria, P. emblica, P. flexuosus, P. amarus, and P. sellowianus have received the most phytochemical and biological attention. According to the available literature, research has either been focused on isolating all the substances in these plants, or on determining a specific class of natural products (Calixto et al., 1998). Intensive phytochemical examinations of this plant have been carried out by scientists belonged to several countries. Phytochemical constituents such as alkaloids, flavonoids, lignans, tannins, phenols and terpenes have been identified. However, the composition of the aqueous extract, used for medicinal purposes, has not been adequately studied. Although the specific compounds have not been precisely defined, some research results give valid credit for the therapeutic action of urinary tract stones to the phenols (Ishimaru et al., 1992; Calixto et al., 1998).

The aerial parts of P. niruri have been reported to contain phytochemical compounds as mentioned in the previous paragraph. Some of these isolated compounds have been tested for their pharmacological activities (Ishimaru et al., 1992, Calixto et al., 1998; Huang et al., 2003; Naik and Juvekar, 2003). Lignans from this plant have been studied most intensively and so far 17 different lignans have been identified. Several of these lignans were tested for cytotoxicity and other biological activities in vitro. Phyllanthin and hypophyllanthin were found to be protective against carbon tetrachloride and galactosamine induced cytotoxicity in primary cultured rat hepatocytes (Syamasundar et al., 1985). The major pharmacological active compounds are gallotannins (e.g. phyllanthusiin-D, amariin, geraniin and
corilagin (Foo and Wong, 1992; Foo, 1993) and the lignans - phyllanthin and hypophyllanthin.

Phyllanthus niruri Linn. is widespread in the tropical and temperate regions of the world. It was named as ‘stone breaker’ by the indigenous people of Africa and used as a effective remedy to completely remove gallstone and kidney stones. It has a wide number of traditional uses employing the whole plant for jaundice, gonorrhea, frequent menstruation and diabetes. The plant is topically used as poultice for skin ulcer, sores, swellings and itchiness. An aqueous extract of this plant possesses anti-hepatitis B virus surface antigen activity in both in vivo and in vitro studies (Thyagarajan et al., 1988; Calixto et al., 1998).

Since allopathic medicines can not give complete cure for the liver disorders caused by hepatitis B viruses, current research has been focused on Phyllanthus niruri as a potential plant for the treatment of this deadly dangerous diseases by suppressing the growth and replication of the virus (Venkateswaran et al., 1987; Mehrota et al., 1990; Thyagarajan et al., 1982; Yeh et al., 1993; Jayaram and Thyagarajan, 1996; Lee et al., 1996; Calixto et al., 1998).

Herbal medicines for liver disease have been used in India for a long time and have been popularized world wide by leading pharmaceuticals. Despite the significant popularity of several herbal medicines in general, and for liver diseases in particular, they have not become acceptable treatment modalities for liver diseases. The limiting factors that contribute to this eventuality are (i) lack of standardization of the herbal drugs, (ii) lack of identification of this active ingredient(s), (iii) lack of randomized controlled clinical trials (RCTs), and (iv) lack of toxicological evaluation (Thyagragan et al., 2002).

Conventional propagation of this medicinally important plant is achieved through seeds, but the viability of the seeds is limited to very few months. The percentage of germination is drastically reduced during the storage period and is completely lost with in a year. The embryos are also very small when present and most of the seeds are abortive (Anon, 1990). Based on the preliminary studies conducted on seed propagation, specific habitat conditions are required for its survival
and growth. Germination is also very slow. There is no reliable method for vegetative propagation of this plant. All such factors, coupled with unsustainable and indiscriminate harvesting from the wild, have posed threats to this species. Thus, conventional propagation through seeds is not sufficiently reliable or adequate to meet the demand for planting material. Hence, development of an in vitro propagation method will be of great importance for the production of planting material to build up the resource base of this particular species (Santos et al., 1994).

Based on these background information, the present study was justifiably designed with the following objectives for the effective utilization of Phyllanthus niruri as medicinally important one.

1. Collection of Phyllanthus niruri plant specimens and their seeds from the different locations of Tamilnadu.
2. Determination of the degree of variability in plant populations using morphometric and RAPD analysis.
3. Determination of the phytochemical compounds from all the collected plant specimens by gas chromatography and mass spectrometry.
4. Screening of the antibacterial efficiency of the plant against some important human pathogenic organisms.
5. Determination of the hepatoprotective potential of P. niruri by using animal models.
6. Standardization of the in vitro culture techniques for the micropropagation of selected superior accession of P. niruri.

The thesis encompasses six chapters including Introduction, Review of Literature, Materials and Methods, Results, Discussions, Summary and References. The research findings are spread over 16 plates, 28 tables and 14 figures. Results are statistically analysed.