1.0 INTRODUCTION

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of wild plants. The plants are the valuable natural resources which have to be protected, preserved and developed in a manner that is beneficial for successive generations. They have always been the principal and traditional form of medicines in India and presently they assure exceptional significance throughout the world, as they have curative properties. Medicinal plants are the local heritage with global importance (Fabricant and Farnsworth, 2001).

Medicinal plants have been used for treating various diseases since time immemorial. The first written records on the medicinal uses of plants appeared from Sumeriens and Akkadians in about 2600 Bc. Susruta Samhita and Charaka Samhita dates back from about 1000BC recorded documentation of Ayurvedic System (Kapoor, 1990; Samuelsson, 1999). The knowledge of medicinal plants has been accumulated in course of many centuries based on different systems of medicine such as Ayurveda, Unani and Siddha. About 25% of the medical drugs in the developed countries are made either from plants or their derivatives. It is estimated that less than 10% of the world’s genetic resources have been seriously studied as a source of medicine. A Substantial number of drugs have been developed from plants which are active against a number of diseases (Fabricant and Farnsworth, 2001; Principe, 2005; Sundaram et al., 2010; Raghavendra et al., 2011).

Medicinal plants are looked upon not only as a source of affordable medicare but also as a source of income. The herbal drug industry is all set to flourish in the
next century considering the high growth at the late 90s, which is well supported by statistical data. The world market for plant-derived chemicals alone exceeds several billion dollars per year. It is estimated that the global trade in medicinal plants is US $1100 million per year (Manish Gunjan et al., 2015). The botanical market inclusive of herbs and medicinal plants in the USA is estimated, at approximately US $ 1.6 billion per annum; China with exports of over 120,000 tones per annum; while India with 32,000 tones per annum dominate the international markets (Dhar et al., 2002). The annual export of medicinal plants from India is about 1200 million per annum (Ramakrishnappa, 2003). It is estimated that Europe, annually, imports about 400,000 tones of medicinal plants with an average market value of US$ 1 billion from Africa and Asia (Wakdikar, 2004). The major importers of medicinal plants are the European Union, Russia and the USA, while the major exporters of medicinal plants or its value added products are China, Japan and Russia. It is noteworthy that active Intellectual Property Right protection has helped countries like China to establish supremacy in the world trade. Forty five percentage of all patents on herb or herbal based or related medicine are with China followed by Japan with 28 percentage share (Gupta and Raina, 1998).

India is one of the richest sources and a varietal emporium of medicinal plants in the world. Being one of the most medico - culturally diverse countries, it is regarded as a part of time honoured tradition in the medicinal plant sector even today. Due to its uniqueness in biogeographic position, India is rich in all the three levels of biodiversity such as species diversity, genetic diversity and habitat diversity (Krishnarajua et al., 2005). The significance of Western Ghats, is one of the globally recognized biodiversity ‘hot spot’ is remarkable due to its medicinal plant diversity
India, as described earlier is a virtual treasure trove of plant species. Despite substantial pace of growth in the medicinal plant and product market, India’s share in world market of medicinal plant and products is mere 2.5 percent. A survey conducted by the All India Coordinated Research Project on Ethnobiology (AICRPE) 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 (Laloo et al., 2006). Many drugs are rich source of therapeutic agents used for the prevention of various diseases. A logical approach to the study of drugs and their activities is the basic principle behind the biochemical events leading to drug actions. (Tagboto and Townson, 2001; Evans et al., 2000 and Cragg and Newman, 2005). The most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds. These compounds have always been of great interest to scientists working on different diseases. Research to find out scientific evidence for claims of plants used for Indian Ayurvedic System of medicine has been intensified. Detailed research on chemistry and pharmacology of therapeutic products of plant origin are much essential and this may eventually lead to the discovery of medicines that can be used in the treatment of several hazardous diseases (Verma et al., 2005).

According to the findings of World Health Organization, most of the world’s populations depends on traditional medicines for their psychological and physical health care (Rabe and Van Staden, 2000), since they cannot afford synthetic drug products, apprehension towards their side effects and lack of healthcare facilities (Griggs et al., 2005). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain
human health. They were also aware that medicinal plants are highly effective only when used at therapeutic doses but they may not understand the science behind these medicines, (Maheshwari et al., 1986 and Van Wyk and Gericke, 2000).

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments (Sharma et al., 2008). Plant derived drugs occupy an important place in both traditional and modern medicines (Das et al., 2007). Since ancient times people have been exploring the nature, particularly plants in search of new drugs which has resulted in the use of huge number of herbal plants with curative properties to heal various diseases (Verpoorte, 2000). In the early twentieth century phytomedicine was considered as the prime healthcare system to maintain health. With the advent of synthetic drugs, phytomedicines gradually lost its popularity among peoples due to its fast therapeutic actions (Singh et al., 2007). Recently there has been a shift in universal trend from synthetic to phytomedicine, which can be said ‘Return to Nature’.

The plant chemicals are classified into primary and secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in all organisms. In higher plants, such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants are vegetable oils, fatty acids, carbohydrates etc. Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbicides, pesticides and many
pharmaceutical drugs for a long period of time, medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases (Wink et al., 2005). Secondary metabolites (compounds) have no apparent function in a plant’s primary metabolism, but often have an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against microorganisms, insects and higher predators and even other plants (allelochemicals). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites (Karuppusamy, 2009; Sathishkumar et al., 2009). In contrast to primary metabolites, they are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary metabolites such as steroids, quinones, alkaloids, terpenoids and flavonoids, which are used in drug manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents. Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as models for pharmacologically active compounds in drug synthesis. The general research methods includes proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design (Wink et al., 2005).

The medicinal plants contain many compounds with antibacterial activity (Cowan, 1999). Researchers are turning their attention to natural products and looking
for new drugs against cancer, viral and microbial infections (Ibrahim et al., 2000; Philip et al., 2009). Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds (Nitta et al., 2002; Salama and Marraiki 2010; Madduluri et al., 2013; Nair and Chanda 2006). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents (Thirumurugan, 2010). Thus plants are valuable sources for new compounds and require special attention in research strategies to develop new antimicrobials. (Shahidi Bonjar et al., 2006; Aslim and Yucel 2008).

The use of plant extracts and phytochemicals with known antimicrobial properties, are of great significance to therapeutic treatments (Nagesh and Shanthamma, 2009). Extracts of plants used for the treatment of various diseases forms the basis for the Indian systems of Medicine. However, this area is not much developed when compared to modern system of medicine, mainly because of the lack of scientific documentation in this field (Kalimuthu et al., 2010). Mostly the pharmacological activity of medicinal plants resides in its secondary metabolites which provide clues to synthesize new antimicrobial compounds that are relatively safe to man (Srinivasan et al., 2001). Antimicrobial studies have shown that Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria which may be due to the variation in the cell wall structures. More specifically, Gram-negative bacteria has an outer membrane composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Nair and Chanda, 2006; Palambo and Semple, 2001). Although
hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Hussain et al., 2011; Duhan et al., 2013; Reddy et al., 2014). Contrary to the synthetic drugs, antibacterial activities of plant origin are associated with less side effects and have an enormous therapeutic potential to heal many infectious diseases (Mahesh and Satish, 2008). The potential for developing antibacterial drugs from higher plants appears rewarding, as a number of clinically efficacious antibiotics are becoming less effective due to development of resistance which can be replaced by biomolecules of plant origin (Kumaraswamy et al., 2008).

Fungal diseases represent a critical problem to health and are one of the main causes of morbidity and mortality worldwide (Portillo et al., 2001). Due to the increasing development of drug resistance in human pathogens and the appearance of undesirable effects of certain antimicrobial agents, there is a need to search for new antifungal agent without toxicity and side effects (Fan et al., 2008). The activity of plant extracts on fungi has been studied by a very large number of researchers in different parts of the world (Van Vuuren and Naidoo, 2010; Duraipandiyan and Ignacimuthu, 2011). As a result, antifungal therapy is playing a greater role in health care and the screening of traditional plants in search of novel antifungals is now more frequently performed (Goulart et al., 2005). Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information (Puupponen-Pimia et al., 2001; Parekh and Chanda, 2008; Joshi et al., 2011) and a few attempts were made on understanding the inhibitory activity against certain pathogenic bacteria and fungi. Plant extracts or plant-derived compounds are likely to provide a valuable source of new medicinal agents (Ncube et al., 2008).
Free radicals play a crucial role in the development of tissue damage in various human diseases such as cancer, ageing, neurodegenerative diseases, malaria and arteriosclerosis and in pathological events in living organisms (Gutteridge, 1994). 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 \textit{in vivo} and \textit{in vitro} and create oxidative stress which results in numerous diseases and disorders (Rackova \textit{et al.}, 2007; Chanda and Baravalia, 2010; Reuter \textit{et al.}, 2010). 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 world’s population 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 (Craig, 1999). 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 and the results revealed that, the activity is due to several secondary metabolites especially, phenolic compounds (tannins, flavonoids, anthrocyanins, chalcones, xanthones, liganans, depsides, and depsidones), terpenes (sesquterpenes and diterpines), alkaloids, and organic sulfur compounds (Krishnaiah \textit{et al.}, 2011; Jabri-Karoui \textit{et al.}, 2012). The antioxidant effects of plant derived compounds, plays a vital role in the prevention of diseases which are safe (Koleva \textit{et al.}, 2002; Bandoniene \textit{et al.}, 2002 and Couladis \textit{et al.}, 2003), whereas, several
synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene are available but they are quite unsafe, and their toxicity is a problem of concern (Botterweck et al., 2000). So, considerable attention has been directed towards identification of natural antioxidants in recent years. Natural antioxidants are widely used as dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart diseases, etc (Hercberg et al., 1998).

Cancer is one of the life-threatening diseases, which still remains an aggressive killer worldwide. Ovarian cancer has emerged as one of the most common malignancies affecting women in India. The Age Specific Incidence Rate (ASIR) for ovarian cancer revealed that the disease increases from 35 years of age and reaches a peak between the ages 55-64 (Quirk and Natarajan, 2005). Several novel synthetic chemotherapeutic agents have not succeeded in fulfilling expectations, despite the considerable cost of their development. Therefore there is a constant demand to develop new, effective and affordable anticancer drugs (Coseri, 2009). Natural products have received increasing attention over the past several years for their potential cancer preventive and therapeutic agents (Newman, 2008). Compounds which have been identified and extracted from terrestrial plants for their anticancer properties which are secondary metabolites and their semi-synthetic derivatives include polyphenols, brassinosteroids and taxols. Polyphenolic compounds include flavonoids, tannins, curcumin, resveratrol and gallacatechins and are considered to be anticancer compounds (Azmi et al., 2006). Resveratrol can be found in foods including peanuts and grapes and red wine, Gallacatechins are present in green tea, presence of polyphenols in a person’s diet can improve health and reduce risk of cancers by being natural antioxidants (Apostolou et al., 2013).
Various plants such as fern species and plants used in traditional Chinese medicines like the litchi leaf have been investigated for their flavonoid contents and how these compounds affecting cancer cells (Wen et al., 2014). Cao et al. (2013) identified and investigated the anticancer effects of flavonoids on human lung cancer cells (A456 cell line) from the fern species *Dryopteris erythrosora*. Sixty percentage of currently used anticancer agents are derived in one way or another from natural sources (Cragg and Newman, 2005).

DNA barcoding is a tool for species identification and the term ‘DNA barcode’ was first coined by Hebert et al. (2003). Plant DNA barcoding research is aimed at the search of unique candidate gene for identifying all the plant species (Kress et al., 2005), which included both coding and non-coding regions. The plant DNA barcoding studies were initially restricted to the chloroplast genome to understand the variation of its gene sequences of coding (*matK*, *rbcL* and *rpoC1*) and non-coding (*ITS* and *psba-trnH*), which has been summarized by Chen et al. (2010). In the 3\textsuperscript{rd} International Barcode of Life Conference (2009), held in Mexico, considered *matK* and *rbcL* as the universal plant DNA barcodes. In the 4\textsuperscript{th} International Barcode of Life Conference (2011), held in Adelaide, cosidered *matK*, *rbcL* and *ITS* gene as standard universal barcode candidates. Earlier, the quantitative measurement to compare the barcode candidate was based on simple statistics calculation, PCR universality and sequence divergence. These analyses should allow direct comparison between putative DNA barcode markers. The two factors that are important for the barcode as marker are database design and sequence search strategies (Kress and Erickson, 2007). The Consortium for the Barcode of Life (CBOL) plant-working group recommended the 2-locus combination of standard
plant barcodes as ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit (\textit{rbcL}) and maturase K (\textit{matK}) based on assessments of recoverability, sequence quality and levels of species discrimination (Group \textit{et al.}, 2009). It relies on the information encoded in the nucleotide sequences of a standard region of the genome. These regions of chloroplast DNA were chosen based on the criterion such as efficient recovery of good-quality sequences and high levels of species discrimination (Burgess \textit{et al.}, 2011).

DNA barcodes allow the identification of species including individuals of the same species by ecology (Chase \textit{et al.}, 2005; Kress and Erickson 2008). A major advantage of DNA barcoding is the possibility of identifying cryptic species and it separates the species based on the amount of genetic distance among samples (Stevens \textit{et al.}, 2011). The genetic distances undergo the limit values of two individuals which are considered as the same species, while outside these limits they should be considered as two different species. It was observed that a value of genetic distance between two DNA-barcode sequences equal to or higher than three per cent (\(D \geq 0.03\)) is considered as distinct species (Witt \textit{et al.}, 2006).

The selected plant \textit{Cissampelos pareira} var.hirsuta is a flowering plant belongs to the family Menispermaceae. It is known as abuta or laghupatha in ayurvedic medicine. In Tamilnadu it is called ponmusutai and it is a good medicinal plant for various treatments. \textit{C. pareira} is commonly found in orchards, parks and gardens (Amresh \textit{et al.}, 2008). It possesses antibacterial, anti-inflammatory, antihistamine, antioxidant, antispasmodic, diuretic, hypotensive, muscle relaxant, uterine relaxant, antiseptic, aphrodisiac, analgesic, ant hemorrhagic, cardio tonic,
diaphoretic, expectorant, febrifuge, hepatoprotective stimulant and tonic activities. It is mainly used to stop bleeding, balances menstruation, relieves pain, reduces spasms, relaxes muscles, stops inflammation, increases urination, prevents convulsions, reduces fever, balances hormones, prevents menopausal libido loss, controls hormonal acne, control premenstrual syndrome, ease childbirth and protects liver, and prevents heart problems (irregular heartbeat, high blood pressure etc). Their roots are used in tropical countries to prevent a threatened miscarriage (Cissampelos pareira, 2012).
AIM AND OBJECTIVES

The present study is aimed at molecular characterization, structural elucidation and evaluation of pharmacological potential of *Cissampelos pareira* L var. hirsuta (Buch.-Ham.ex DC.) Forman.

The study was carried out in the following scheme and order:

- Molecular characterization of *C. pareira* by using potential barcode candidates such as *rbc*L and *mat*K for species homology identification and also for the authentication of evolution of the species using phylogenetic tree construction for better taxonomic understanding and inter-relationship of the species.
- To study computational gene expression on the barcode sequence of the selected plants to estimate their unique molecular characters.
- To determine the phytochemical characterization of different parts of the selected plant in five different solvents using standard protocols.
- To investigate the biochemical and pharmacological activities such as antimicrobial assay and antioxidant assay of the selected active plant extracts.
- Isolation of bioactive compound from the active plant extract by silica gel column chromatography and the characterization and structural elucidation of the isolated compound by using NMR, FT-IR spectroscopy and mass spectrometry.
- To evaluate *in vitro* anticancer and cytotoxicity activity of isolated bioactive compounds from the plant extract using MTT assay.