INTRODUCTION

Cancer is an unconquerable giant whose lethal effects are yet to be subdued. Despite many advances in chemotherapy and other modes of treatment, an acceptable formulation for the complete cure for cancer has been eluding scientists since time immemorial. World Health Organisation in its report (WHO, 1990) stated that cancer occupied the second position in the list of killer diseases in the industrially advanced countries, while in the developing world, it ranked the fifth. However, the Indian situation is fast changing and with most of the infectious diseases under control, today cancer as a cause of death occupies a position above that of the other developing countries.

In the Indian scenario, we cannot hope to be able to introduce sophisticated technologies for cancer control in a short time, as envisaged in the advanced countries. Therefore, the most feasible approach for India and other developing countries will be to adopt measures for prevention and early detection of cancer, to find inexpensive drugs for therapy and to improve facilities for palliative care. Unlike in the advanced countries, one of the major challenges of cancer research in the developing countries is to develop cheap and easily available drugs for cancer therapy. Investigation into the natural products and traditional medicine to explore the possibility of developing potent drugs from local resources should be given priority (Umadevi, 2000).

An ideal and effective anticancer agent, free from any side effects has not yet been discovered. Therefore, cancer chemoprevention has become increasingly important in the recent years. Intensive search for cancer chemo preventive agents has been done on medicinal plants and many novel
phytochemicals have been discovered which could be successfully utilized by the pharmaceutical industry for the manufacture of drugs. Plant derived compounds play a vital role in drug discovery programmes.

The plant kingdom is a rich source of drugs that inhibit cell proliferation. During the past quarter century, particularly after 1960, interest in plant and plant products as protectants against cancer has grown tremendously, mainly due to their successful use against some forms of cancer, following the specific use of two derivatives, vincristine and vinblastine from the plant *Vinca rosea* against Hodgkins disease and acute leukaemia in children. Screening of natural isolates for anticancer activity was conducted all over the world during the last five decades by organizations like United States National Cancer Institute (NCI), Eli Lilly, University of Illinois, Research Triangle Institute, Glaxo Wellcome, Shaman Pharmaceuticals etc. The drug research and development branch of the NCI of USA, has confirmed after screening over 50,000 plant extracts, representing 8000 species, that over 1000 species could actively inhibit carcinogenesis (Nandi et al., 1998).

Chemotherapy is an effective treatment against cancers either singly or in combination with surgery and or radiotherapy. In Chemotherapy, drugs like cisplatin, carboplatin, cyclophosphamide, doxorubicin, melphalan, mitomycin-C, ferncitabine etc. have been used for the treatment of cancers (Black and Livingston, 1990a; 1990b). However, the therapeutic efficacy of most of them is limited due to the development of various side effects in the host and or the acquired drug resistance by the cancer cells (Black and Livingston, 1990b; Kortalou and Essigmann, 2001). In an attempt to abate these side effects and to find out a better remedy against various malignancies, many plant derivatives have been used with varying success (Roja and Rao, 2000). Vincristine, vinblastine, podophyllotoxin, peltatins, taxol, camptothecin and colchicine have attained wider acceptability in
chemotherapy. Phytochemicals that have undergone screening test and are in varying degrees of clinical trials are bruccantin, maytansine, colubrinol, indicine-N-oxide, tridiolide, homoharringtonine, ellipticine, bruvardin, forskolin, monocrotaline etc. (Vasantha Kumar and Kesavachandran, 2000).

Higher plants, a source of medicinal compounds have been well known to play a dominant role in the health care of human beings (Huang Paul et al., 1992). More than 50% of all modern drugs in clinical use are of natural product origin (Roja and Rao, 2000; Huang Paul et al., 1992). Many natural products have been recognized to have the ability to induce apoptosis in various tumour cells of human origin (Taraphdar et al., 2001). A variety of plant extracts i.e., turmeric (Curcuma longa) and its active constituent -curcumin, roots of tea plant (Camellia sinensis var. assamica) and betel leaf have been reported to have potential antitumour or anticarcinogenic activities (Kuttan et al., 1985; Sakagami et al., 1987; Azuine et al., 1991; Chaudhuri et al., 1998; Sur and Ganguly, 1994; Roja and Rao, 2000; Sharma et al., 2000).

The use of traditional herbal medicines and or direct use of some parts of plants against various ailments are very common among the tribes of the North-Eastern States of India (Syiem et al., 1999). India is one of the greatest emporia of medicinal and aromatic plants. References to miracle herbs or wonder drugs are often found in old literature. Medicinal plants constitute a very important national resource because India has one of the richest plant based ethnomedical traditions in the world (Rajasekharan and Ganesan, 2004). The Central Council of Research in Ayurveda and Siddha medicine has drawn a list of 243 commonly used medicinal plants having bulk demand for manufacture of gelanicals, mixtures, compound formation and patent medicines (Gupta, 1998). The global market for medicinal plants and herbal medicine is estimated to be worth US $800 billions a year. International export trade in medicinal plants has been dominated by China, which exports
1.21,900 tons a year and India, which exports 32,600 tons a year (Rajasekharan and Ganesan, 2004).

The large-scale use of medicinal plants and herbs in the preparation of drugs are increasing due to the growing concern about the side effects of chemicals and synthetic substances. The medicinal plant-based drugs have the advantage of being simple, effective and a broad spectrum of activity with an emphasis on the preventive action. Because of these factors, the demand for plant-based medicine (phytomedicines or phytopharmaceuticals) is increasing worldwide. Plants are also found to contain disease specific curative properties and extracts of such plants are increasingly being used to manufacture effective drugs.

Many traditional plant-based remedies are back in use and find increasing applications:

- as a source of direct therapeutic agents.
- as the raw base material for the elaboration of more complex semi-synthetic chemical compounds.
- as models for new synthetic compounds.
- as taxonomic markers for the discovery of new compounds.

The production, consumption and international trade in medicinal plants and phytomedicines are growing and expected to grow in future quite significantly. The progress in medicinal plant research has undergone a phenomenal growth during the last two decades.

Herbal preparations are being used in alleviating several diseases. Countries such as India and China have a vast array of traditional medicines, which are not yet explored significantly. These medicines are prescribed not
only to reduce suffering but also to prevent diseases produced by the pathophysiological changes. Eventhough cancer is one of the most difficult diseases for treatment, it is probably one of the most preventable diseases (Block et al., 1992). This prevention could be achieved by:

- avoidance of cancer causing substances.
- chemopreventive agents that can inhibit the metabolism of carcinogens or cause its detoxification.
- immunostimulators which can destroy the cancer cells by augmenting the immune response.
- inhibition of signal transduction pathway which can either inhibit the conversion of normal cells to cancer cells or reduce its growth capability and destroy the cells by increasing the recognition by the immunocompetent cells.

Several types of compounds numbering to more than 2000 chemicals, among which many of plant origin, have been known to inhibit the chemically induced carcinogenesis (Soudamini and Kuttan, 1989; Unnikrishnan and Kuttan, 1990).

Search for pure phytochemicals as drug is expensive and time consuming. A phytherapeutic approach to develop herbal anticancer agents is relevant to Indian condition. We have a rich resource of medicinal plants in traditional use. Simple animal cancer models can be used for screening anticancer activity in extracts or active fractions of medicinal plants selected based on traditional use. Search for immunomodulation and cancer cell specific apoptosis inducers among medicinal plants is promising to develop attractive drugs with minimal toxicity. Knowledge gained in the area of signal transduction in cells can also be applied to discover phytomedicine
A database was constructed with ethnopharmacological information about plants used for signs and symptoms frequently related to a variety of cancers associated with cancer cell lines available at the South American Anticancer Development Office (SOAD).

Plant materials have been used in the treatment of malignant diseases for centuries. This clearly indicates the potentiality of phytochemicals as an anticancer therapeutic agent. A large number of plants and plant parts have been screened for their antitumour properties (Herout and Sorm, 1959; Zheng, 1994). Among them, the plants that belong to the family Asteraceae play a significant role. The biological and therapeutic applications of the plants of the Asteraceae are the result of popular tradition and of systematically conducted chemical and pharmacological research. In addition to drugs known since antiquity from plants such as Chamomilla, Cynara and Sylibum, there are many other species in the family, which have found therapeutic applications due to their antihepatotoxic, choleretic, spasmolytic, anthelmintic, antibiotic or antimicrobial activity.

The rich accumulation of essential oils and other terpenoids in certain Asteraceae members is responsible for the use of various taxa such as tansy (Tanacetum vulgare) and wormwood (Artemisia absinthium) for flavouring foods or liquors. Terpenoids and certain phenolic compounds are responsible for the value of many species of Asteraceae in pharmacy and medicine. Wagner (1977) in his detailed review on the pharmaceutical properties of Asteraceae, has also pointed out the eminent role played in this regard by the genus Artemisia.

The genus Artemisia is one of the largest and most widely distributed one of nearly 100 genera in the tribe Anthemidae of the Asteraceae (Mucciarelli and Maffei, 2002). Asia seems to show the greatest concentration of species with 150 accessions for China (Hu, 1965), 174 in
Russia (Poljakov, 1961) and about 50 reported to occur in Japan (Kitamura, 1939; 1940).

The geological history of the Asteraceae is strongly linked to that of the genus *Artemisia* and of particular interest here is that the most convincing early fossils of the Asteraceae include *Artemisia* pollen of the late oligocene of Central Europe, *Artemisia* fruits and seeds from Poland (middle miocene) and *Artemisia* pollen from Eastern and Western North America of the late miocene and late oligocene respectively (Mucciarelli and Maffei, 2002).

Until recently, consensus placed the origin of the genus *Artemisia* in Central Asia with subsequent migration to North America through the Bering Land Bridge (Clements and Hall, 1923; Mc Arthur and Plummer, 1978; Stebbins, 1974; Mc Arthur, 1979). However, biological evidences point Eurasia as the centre of origin. Shah (1996) describes the genus *Artemisia* as one of the largest and most difficult taxa to understand under an ethnobotanical point of view. The medicinal use of *Artemisia* species was introduced into the Indian Himalayan region by different cultural and ethnic groups who entered this region in the past, coming from the Mediterranean and Arabian regions.

The genus *Artemisia* L. was named after Artemis, daughter of Jupiter and Latona of Greek mythology. Artemis was also the virgin Goddess of Moon and of hunting, supposed to kill without pain, in allusion to the soothing but harmful properties of the plant (Nayar, 1985). Artemis was also considered as one of the names of Diana, the goddess of nature on account of it being used in bringing on precocious puberty (Hereman, 1868).

The genus *Artemisia* includes a large number of species and some have been cultivated as commercial crops with a wide diversity of uses. Some better known uses include antimalarial activity (*A. annua* or sweet
wormwood), as culinary spices (*A. dracunculus* - French tarragon), for liquor flavouring (*A. absinthium* - absinthe), as garden ornamental (*A. abrotanum* - southernwood) and as an insect repellent (*A. vulgaris* - mugwort) (Laughlin *et al.*, 2002).

Several *Artemisia* species are used medicinally and hence are of more commercial value. In Western herbal medicine, they include *A. abrotanum*, *A. absinthium*, *A. cina*, *A. dracunculus*, *A. maritima*, *A. pontica* and *A. vulgaris* (Frohne and Jensen, 1992; Evans, 1996). In traditional Chinese herbal medicine, the following *Artemisia* species are used: *A. annua*, *A. argyi*, *A. scoparia* and *A. capillaris* (Tang and Eisenbrand, 1992). In addition, *A. annua* is a source of artemisinin, which is the mother compound of a novel class of antimalarial drugs (Woerdenbag *et al.*, 1994).

Among the different species, *Artemisia nilagirica* (C. B. Clarke) Pamp., a less exploited species, was selected for the present study. It is distributed in the South Indian hills, chiefly on the Nilgiris, Khasia hills and Darjeeling in India (Agarwal, 1997).

The plants are bitter, aromatic, gregarious herbs or shrubs (Warrier *et al.*, 1994), usually characterized by much divided, oblong, lanceolate leaves with white tomentoes below (Rajan *et al.*, 2000), inconspicuous flowers and absence of pappus, a feature which is uncommon in other members of Asteraceae (Govil *et al.*, 1993).

*A. nilagirica* is the most common species found in earlier Indian literature. It was used as a decoction and infusion for the relief of nervous and spasmodic afflictions by Himalayan people (Shah, 1996). It provides us medicinal principles for the amelioration of human suffering not only sufficient for our own but also for the purpose of export.
The plant is used to cure various human ailments such as coughs, bronchitis, cephalalgia, leprosy, anorexia, dyspepsia, flatulence, fever, colic, anaemia (Warrier et al., 1994), asthma, skin diseases and measles (Agarwal, 1997). An infusion of the leaves and flowering tops of *A. nilagirica* is administered in nervous and spasmodic affliction (Chopra et al., 1956).

*A. nilagirica* along with other plants have been used for the treatment of specific human ailments such as allergies, burns, cuts, wounds, inflammation, leucoderma, scabies, smallpox and sexually transmitted diseases (Begum and Nath, 2000). It can be used as a substitute for *Cinchona* in fevers (Govil et al., 1993; Agarwal, 1997). It is also used as an emmenogogue, diuretic, aphrodisiac, appetizer, febrifuge, alexiteric (Warrier et al., 1994), anthelmintic (Chopra et al., 1969; Agarwal, 1997; Warrier et al., 1994), expectorant and antiseptic (Chopra et al., 1969).

Roots of *A. nilagirica* are used as tonic and antispasmodic (Agarwal, 1997). Leaves and flowering tops are used for asthma (Chopra et al., 1956; 1969) and they are bitter, astringent, acrid, thermogenic, aromatic, anodyne, antiinflammatory, digestic, febrifuge and haematinic (Warrier et al., 1994).

The plant is reported to possess antibacterial (Agarwal, 1997; Samaiya and Saxena, 1986; Mehrotra et al., 1993) and antidermophytic (Kishore et al., 2001) activities. The essential oil of *A. nilagirica* is fungistatic in nature and has a broad fungitoxic spectrum (Mehrotra et al., 1993; Kishore et al., 2001). An ointment of the essential oil, prepared in polyethylene glycol showed pronounced efficiency as herbal antifungal agent against dermatomycosis induced in guinea pigs within 14 days of application (Kishore et al., 2001). Thoppil et al. (2002) reported antimicrobial properties of essential oil of *A. nilagirica*. Leeja and Thoppil (2004a) reported the cytotoxic potential of extracts of *A. nilagirica*. Essential oil composition and mosquito larvicidal
activity of *A. nilagirica* from South India has been studied (Leeja and Thoppil, 2004b).

The plant was used also for magical purposes. It was traditionally kept at front doors and under pillows to discourage evil spirits and ghosts and the aerial parts were used in festivals for worshipping or offered to the local divinity (Shah, 1996). The essential oil is also finding its place in the indigenous perfumery industry.

*Artemisia*, a herb for cancer has been studied by many workers. Many species of *Artemisia* such as *A. agri* (Seo et al., 2003), *A. princeps* (Hwang et al., 1999), *A. iwayomogi* (Koo et al., 1994) etc. are reported to possess cytotoxic and antitumour activities. Among the different species of *Artemisia*, anticancer activity of *A. nilagirica* has not been yet reported. Hence, the present study.

Since all known *A. nilagirica* are sterile, they can be propagated vegetatively, which has prevented the production of new cultivar by plant breeding. Assessment of genetic variability is basic to any plant breeding programme (Farooqi et al., 1990). An alternative method for creating new forms of the plant is by selecting somaclonal variants from tissue culture material. Somaclonal variation is a term coined by Larkin and Scowcroft (1981) to cover all types of variations, which occur in plants regenerated from cultured tissues. Plant tissue culture has the potential to induce genetic variability in *Artemisia* genotypes through somaclonal variants, somatic hybrids or transgenic plants. However, a pre-requisite to applied plant biotechnology is the development of a suitable and reproducible plant regeneration system (Jullien et al., 1998). Potential use of cell culture (Drupeau et al., 1987), multiple shoots (Constabel et al., 1982; Endo et al., 1987; Hirata et al., 1987) and improvement of various cultivation conditions (Facchini and Dicosmo, 1991) have been attempted to scale up production of
secondary metabolites. The exploitation of tissue culture technique in medicinal plants for the extraction of important chemical compounds is indeed more advantageous (Tabata, 1977). A wide variety of compounds have been shown to be produced in shoot, callus or cell suspension cultures at levels equal to or higher than the levels in the intact plant sources (Brodelins, 1988; Dodds and Roberts, 1995).

Plant tissue culture has been extensively used to exploit the secondary metabolite it can produce. Growth of a cell in a totally controlled environment of physical and chemical factors provides an excellent system for studying changes in the production of secondary metabolites, which are always present in small quantities. The basic information has provided significant clues about genes and their functioning, leading to genetic manipulation of biosynthetic pathways to obtain desired products by either blocking a pathway or enhancing the metabolic reaction (Merillon and Ramawatt, 1999). Exploitation of possible somaclonal variation, which has been observed in *in vitro* cultures, could be used to widen the genetic pool from which to select desirable traits. Application of modern biotechnology can complement conventional breeding techniques and it helps in the development of improved varieties (Chomchalow and Sahavacharin, 1981; Vasil, 1988; Bajaj, 1981).

*In vitro* propagation can yield a large number of clonal plants for continuous plant establishment. It is also important for germplasm conservation (Kathiravan and Ignacimuthu, 1999; Kukreja and Dhawan, 2000). Variation is a ubiquitous phenomenon associated with tissue culture (Carlson and Polacco, 1975; Green, 1977). Induced variation is an alternative source to naturally occurring variability for crop improvement (Ansari and Siddiqui, 1995). Tissue culture induced variation is defined as the variation that arises de novo during the period of dedifferentiated cell proliferation that
takes place between culture of an explant and production of regenerants (Munthali et al., 1996). Plantlets derived from \textit{in vitro} culture might exhibit somaclonal variation (Larkin and Scowcroft, 1981), which is often heritable (Breiman et al., 1987). Other reports claim that useful morphological, cytological and molecular variations may be generated \textit{in vitro} (Larkin et al., 1989).

Undifferentiated and differentiated \textit{in vitro} tissue culture techniques have been recently developed, concerning the economically valuable \textit{Artemisia} species. The main efforts have been devoted to the \textit{in vitro} selection of highly yielding clones and cell lines, producing secondary metabolites with pharmacological and industrial application. In this regard, major attention has to be paid to \textit{A. nilagirica}, owing to its medicinal properties and moreover for its value as an aromatic plant employed in fragrances, perfumery and cosmetic production.

Thus, the present study aims at an attempt to develop a protocol for the regeneration of medicinally important \textit{A. nilagirica} through tissue culture for large scale multiplication and for secondary metabolite production. We also aim at developing somaclonal variants of \textit{A. nilagirica} with higher levels of secondary metabolites.

Chromosome variability is of well-known occurrence in cells of cultured tissues as well as in regenerants (Bayliss, 1973; Sacristan and Melchers, 1969). Changes can take place at the ploidy level like the production of aneuploids (Taliaferro \textit{et al.}, 1989), polyploids (Mariotti \textit{et al.}, 1984) and mixoploids (Mariotti \textit{et al.}, 1984; Taliaferro \textit{et al.}, 1989). Karyological studies can bring to light the variations in chromosome number and their size and suggest the direction of chromosomal evolution in specific taxa (Jones, 1978b). Chromosomal differences may also cause changes in the quality and composition of the essential oils (Guenther, 1949). Moreover,
Chromosome instability in cultured cells can be useful for the production of plantlet with novel genotypes including chromosomal aberrants (Larkin and Scowcroft, 1981).

Computer aided Chromosome Image Analysis System (CHIAS) is a modern technique for karyomorphological analysis. Ordinary karyotype analysis has provided a limited success in chromosome identification. Possibility of making errors is much greater in the conventional method of measuring and characterizing by visual evaluation. These difficulties can be overcome by computer aided Chromosome Image Analysis System. It allows an accurate chromosome pairing mainly in those cases where the chromosome size is very small (Fukui and Kakkeda, 1994). This technique gives a better knowledge of the cytogenetic constitution of the material under study (Fukui and Kakkeda, 1994; Fukui and Lijima, 1992; Fukui and Kamisugi, 1995).

Genomic analysis is a prerequisite for establishing the genetic stability and uniformity of a desired clone (Ikeda and Ono, 1967). Several strategies can be used to assess the genetic fidelity of in vitro derived clones but most of them have limitations. Karyological analysis cannot reveal alterations in specific genes or small chromosomal rearrangements (Isabel et al., 1993). The Polymerase Chain Reaction (PCR) (Saiki et al., 1988) has been the basis of a growing range of newer techniques. PCR allows the specific amplification of DNA sequences making it ideal for the identification of plant genotypes. Amplification of a genotype-specific sequence can take advantage of some of the many features of PCR like speed, simplicity, specificity, sensitivity and cost (Henry, 1997). Molecular markers such as Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD) (Welsh and Mcclelland, 1990; Williams et al., 1990) appears to be good, but when compared to RFLP, RAPD appears to
provide a better basis for genetic characterization because of simplicity of the necessary procedures (Baird et al., 1992).

The approach of using molecular markers including RAPD profiles is a powerful tool not only for the identification of genotypes but also to quantify the extent of genetic variation in any given population. While on one hand the approach of RAPD profiling has been useful in tissue culture methods for detection and selection of somaclonal variants (Munthali et al., 1996). The molecular technique, with the same logic, is directly utilizable for assessing the population of micropropagated clones from any given explant for genetic uniformity. Using PCR with short primers of arbitrary sequences, RAPD markers were recently shown to be sensitive for detecting variations among individuals between and within species (Carlson et al., 1991; Roy et al., 1992). This is an alternative approach for finding new DNA based polymorphic markers among closely related genotypes (Welsh and McClelland, 1990; Nymbom et al., 1990; Lindout et al., 1999). RAPD analysis using PCR with arbitrary oligonucleotide primers (Williams et al., 1990) has the advantage of being non-radioactive, rapid and is a convenient assay of polymorphism that requires only a small amount of crude DNA. Today, RAPD technique has been adopted most widely.

The main issues associated with the use of these techniques are the problem of ensuring reproducibility of amplification profiles. The nature of the amplification process with short primers is such that many sites in the genome are potential templates and the profile obtained may be influenced by any variation in the method used to prepare the DNA template and the exact reaction composition and conditions used in the PCR (Muralidharan and Wakeland, 1993). Obtaining reliable results depend upon standardizing the conditions or identifying combination of conditions that give consistent results, even when variations in the key variables are encountered. A key
requirement for reliable and reproducible RAPD results is a consistent approach to sample preparation and DNA isolation. Both the quality and quantity of the template DNA preparation have the potential to substantially influence the result.

Polymorphism results from either base changes at the primer binding site (point mutation) or chromosomal changes in the amplified regions (insertions, deletions or inversions) which alter the size or prevent the successful amplification of a target DNA. Southern hybridizations are not required and polymorphisms can also be detected in fragments containing highly repeated sequences, which are recalcitrant to RFLP analysis. The extent of polymorphism detected by RAPDs is therefore greater than that is observed by RFLPs (Williams et al., 1990).

Plants represent an unlimited source of phytochemicals such as the metabolites of primary and secondary metabolism. Secondary metabolites are compounds that are biosynthetically derived from the primary metabolites and their distribution in the plant kingdom is restricted. These compounds are generally detected in a lower volume compared to the primary metabolites and possess significant biological activities. Therefore, they are also termed as the higher value - lower volume products or speciality chemicals (Roja and Rao, 1998).

Secondary metabolism in a plant not only plays a role for its survival by producing attractants for pollinators, chemical defense against predators and diseases but also is an important trait of our food, taste, colour and scent. Others such as alkaloids, anthocyanins, flavonoids, quinines, lignans, steroids and terpenoids have a commercial application in the pharmaceutical and biomedical fields and are part of drugs, dyes, flavours, fragrances and insecticides (Veerpoorte et al., 2002).
Volatile oil containing drugs and essential oils has been used for a long time both in folk medicines and in therapeutics, both traditional and alternative. Essential oils, the volatile secondary metabolites responsible for the odours of aromatic plants are used in perfumery, as aroma products, flavouring agents in food and beverages, in cosmetic products and as drugs. There is an increasing global trend in the consumption of self-prescribed herbal and natural products for treating numerous ailments such as cancer and even by healthy individuals as a preventive (Teixeira da Silva, 2004).

Essential oils are frequently referred to as the "life force" of plants. Unlike fatty oils, these essential oils are volatile, highly concentrated substances extracted from flowers, leaves, stems, roots, seeds, bark, resin and fruit rinds. The amount of essential oils found in these plants can be anywhere from 0.01% to 10% of the total. That is why tons of plant material are required to obtain a few hundred pounds of oil. These oils have potent antimicrobial factors, having 200-300 therapeutic constituents. Essential oils cannot be substituted with synthetics. Only pure oils contain a full spectrum of compounds that cheap imitations simply cannot duplicate. Essential oils have unique properties that are prized worldwide for thousands of years, being used therapeutically in early Roman, Greek, Egyptian, Indian and Chinese civilizations (1http).

Essential oils are effectively used in aromatherapy. Aromatherapy is the use of pure essential and absolute oils for psychological and physical well being. Essential oils are believed to stimulate the olfactory nerves and exert influence on the brain centre that controls emotion (Mabey, 1988). They are used as natural rejuvenating and antiwrinkle agents in aromatherapy (Varshney, 1991). It is suitable in the treatment of pain, psychological disturbances, allergies, skin diseases, gastrointestinal disorders,
cardiovascular problems, urinary disorders, gynaecological disturbances, cancer etc. (Jamil, 1997).

The versatile use of several aromatic plants in food, cosmetic and pharmaceutical industries demand an extensive screening of essential oils and their components. Individual chemicals isolated from essential oils are more often used than the oils (Brud and Gora, 1989). Therefore, identification of trace components is very helpful to reveal the quality of the oil. Analysis of the essential oils can be easily done using the technique of Gas Chromatography and Mass Spectrometry (GC-MS). GC is a tool for separating the volatile components while analysis depends upon retention characteristics under standard conditions. The mass spectrometer can be used as a detector for gas chromatography in which, the high degree of specificity of the mass spectrometer is an aid to the identification of the sample. The large number of spectra obtained in a short time from the GC-MS technique and the routine nature of much of the data obtained make the computer a very useful accessory to the GC-MS unit. With the help of GC-MS technique, it has now been possible to analyze directly the fragrances of natural or artificial materials without the use of heat or solvents and directly by the use of head space analysis (Thappa et al., 1982). GC-MS differs from other types of spectral analysis in that the sample does not absorb radiation from the electromagnetic spectrum. It is highly sensitive and only a small quantity of the sample is required. When coupled with separation techniques like GC or HPLC (High Performance Liquid Chromatography), it is a highly specific way to identify organic compounds (Smith and Busch, 1999). A GC-MS machine with computerized library search discs is regarded as the best tool for essential oil analysis (Jose and Rajalakshmi, 2005).

Albeit, a common plant in the country, a purview of literature on A. nilagirica reveals that its potentials are yet to be exploited. The present study
is an attempt to find out the effectiveness of this plant in the field of cancer treatment and to generate somaclonal variants of *A. nilagirica* by *in vitro* techniques, that differ from the parent plant in quality and quantity of the essential oil and to reveal the genetic basis of variation in them by using the Chromosome Image Analysis System and RAPD technique.

Thus, the present study aims to fulfil the following objectives:-

* To find out the efficacy of *Artemisia nilagirica* in the field of cancer treatment and research.

* To establish a protocol for the regeneration of medicinally important *A. nilagirica* through tissue culture for large scale multiplication and for secondary metabolite production.

* To develop a somaclonal variant of the medicinal and aromatic plant, *A. nilagirica* with higher levels of secondary metabolites.

* To analyse the possible variations of somaclonal variant from the parent plant by comparing various aspects such as :-

  - Cytological and Karyomorphological analysis
  - Random Amplified Polymorphic DNA (RAPD) analysis
  - Essential oil analysis
  - Cytotoxic assays
  - Antitumour assays