Plant materials have been used in the treatment of malignant diseases for centuries. The plant kingdom is a rich source of drugs that inhibit cell proliferation. This clearly indicates the potentiality of phytochemicals to be used as anticancer therapeutic agents. Scientists have been looking at nature and plants have been identified as a likely source of such drugs. A large number of plants and plant parts have been screened for their antitumour properties. 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. Terpenoids and certain phenolic compounds are responsible for the value of many species of Asteraceae in pharmacy and medicine.

The genus *Artemisia* is one of the largest and most widely distributed of the nearly 100 genera in the tribe Anthemidae of the Asteraceae. *Artemisia* species, widespread throughout the world are important medicinal plants, which are attracting the attention of phytochemists due to their biological and chemical diversity.

Among the different species, *Artemisia nilagirica* (C. B. Clarke) Pamp., a less exploited species was selected for the present study. Since all known *A. nilagirica* are sterile, they can be propagated vegetatively. This has prevented the production of new cultivar by plant breeding. Assessment of genetic variability is basic to any plant breeding programme. An alternative method for creating new forms of the plant is by selecting somaclonal variants from tissue culture material. Plant tissue culture has the potential to induce genetic variability in *Artemisia* genotypes through somaclonal variants, somatic hybrids or transgenic plants. The exploitation of tissue culture technique in medicinal plants for the
extraction of important chemical compounds is indeed more advantageous. Potential use of cell culture, multiple shoots and improvement of various cultivation conditions have been attempted to scale up production of secondary metabolites. 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.

The present study is an attempt to find out the effectiveness of *A. nilagirica* 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.

**Micropropagation**

Very little information was found in the literature, concerning the tissue culture of *A. nilagirica*. An efficient protocol was developed for the production of the somaclonal variant of *A. nilagirica*. Murashige and Skoog (MS) basal medium with different hormonal combinations of auxins and cytokinins were used for micropropagation. All the explants such as leaves, nodes and inflorescence axis, produced callus and multiple shoots in the medium supplemented with different combinations of BAP and NAA.

Of the three explants, the leaf explants produced cream friable callus in the medium having BAP (0.5 mg/l) + NAA (0.5 mg/l). Multiple shoot initiation was noticed after 3-4 weeks in 90-100% of the callus after sub culturing it in the same medium. The nodal cutting and inflorescence axis produced multiple shoots both directly and indirectly. Multiple shoots were developed directly from nodal cuttings in 90-100% of the medium containing BAP (0.5 mg/l). Callus formation
was noticed when nodal cutting was inoculated in the medium containing BAP (1 mg/l) + NAA (1 mg/l). 80-90% of multiple shoots were developed indirectly from the callus when subcultured in the same medium.

Inflorescence axis produced 80-90% multiple shoots directly in BAP (1 mg/l) medium and indirectly from callus in the medium with BAP (1 mg/l) + NAA (1 mg/l).

*In vitro* shoots developed roots in the medium devoid of growth hormones. However, the medium supplemented with IBA (0.5 mg/l) also developed maximum number of roots. These were later transferred to the sterilized soil and sand mixture and about 95-100% field survivals was obtained in pots.

Vigorous vegetative propagation by stem cutting was noticed in the cultured plants also. Since no remarkable morphological variation was noticed among the field-established plants, further analysis at cytological, molecular and phytochemical levels was conducted to search for the possible somaclonal variations.

**Cytological Analysis**

The cultured plant was analyzed for any karyomorphological changes and to detect the cytological basis of any variation, if present. The ploidy level of the parent plant, callus and the cultured plant was found to be different. The parent plant was found to be tetraploid (2n = 4x = 36), while the somaclonal variant was octaploid (2n = 8x = 72). The calli were of 3 types *viz.*, cells showing tetraploidy (2n = 4x = 36), octaploidy (2n = 8x = 72) and hexadecaploidy (2n = 16x = 144).
The frequency of callus cells containing the octaploid number was more when compared to the other two types of cells observed in the callus.

The number of chromosomes with secondary constriction also showed variations. The parent plant has 4 chromosomes with secondary constriction whereas in the somaclonal variant, it was 8. Chromosomes with secondary constriction were found to be 4 in tetraploid, 8 in octaploid and 16 in hexadecaploid calli.

Changes in chromosome length, disparity index, variation coefficient and total forma percentage were also noticed.

**Random Amplified Polymorphic DNA (RAPD) Analysis**

DNA was isolated from the parent plant (P) and twelve tissue cultured plants designated as number TC1 to TC12 using CTAB method.

To detect the somaclonal variation at the molecular level, RAPD analysis was carried out using 15 primers of arbitrary sequences. Of the 15 primers used, only 5 successfully amplified the extracted DNA with consistent reproducible bands. The number of bands resolved per primer ranged from a minimum of one to a maximum of thirteen. RAPD fingerprints of the tissue cultured plants (TC1-TC12) differed from parent (P) with all five primers (OPA 01, OPA 02, OPB 17, OPB 18 and OPC 01).

A few bands were found to be missing in the variants when these five primers were used. Additional bands in the variants were also detected by this marker screening. The reproducibility of the genomic DNA bands of TC3 was consistent in successive repetition than other plants. Since the amplification of
TC3 plant showed more polymorphism and consistency than other plants, it was considered as somaclonal variant and used for further analysis.

**Essential Oil Analysis**

RAPD analysis revealed a notable deviation in the genetic make up of the *in vitro* plants. So the essential oils of the parent plant and the *in vitro* plants were analyzed quantitatively and qualitatively to search for biochemical variations in the secondary metabolism. The oil yield of the parent plant was comparatively low (1.1%). The *in vitro* (TC3) plant contained a higher quantity of oil (1.8%).

In the present investigation, the essential oil of *A. nilagirica* seems to belong to a terpenoid chemotype. GC-MS analysis revealed 29 components in the parent plant and 21 in the somaclonal variant. There was a clear difference between the compositions of the two oils tested. The percentage of monoterpenes present in the *in vivo* plant was 93.82 while that of *in vitro* (TC3) plant was 86.04. But the percentage of sesquiterpenes was higher in the *in vitro* (TC3) plant (13.96) than in the parent plant (5.99). The major component was mainly thujone in both the oils.

Sixteen essential oil components were found to be similar in both *in vivo* and *in vitro* (TC3) plants. Among them, the percentage of eight components like α-fenchene, sabinene, terpinen-4-ol, α-copaene, β-caryophyllene, germacrene-D, δ-cadinene and β-eudesmol were more in the *in vitro* (TC3) plant than *in vivo* plant.
The *in vitro* (TC3) plant was characterized by new components like ρ-cymene, dl-limonene, α-terpinene, farnesene and bicyclogermaclene.

The lesser value of coefficient of similitude (47.05) obtained when essential oils of both the parent plant and somaclonal variant (TC3) was compared, shows the dissimilar nature of these oils. This dissimilarity may be probably due to the variation in the biosynthetic pathway of essential oils which are genetically controlled.

**Cytotoxicity (*In vitro* studies)**

Methanol extracts of both *in vivo* and *in vitro* (TC3) plants of *A. nilagirica* produced a concentration dependent cytotoxic effect to DLA and EAC cells. *In vitro* plant extract produced 100% toxicity at a concentration of 250 µg/ml for DLA cells and 500 µg/ml for EAC cells, while that of *in vivo* plant extract caused 100% cytotoxicity only at a concentration of 500 µg/ml for both DLA and EAC cells.

**Antitumour activity (*In vivo* studies)**

**Effect on solid tumour reduction**

There was a significant (ρ < 0.01) reduction of tumour volume in the *in vivo* and *in vitro* (TC3) plant extract treated animals. The tumour volume of control animals with 250 µg/ml gum acacia on 31st day was 2.8 cm³, while that of 250 µg/ml *in vivo* and *in vitro* (TC3) plant extracts treated animals was only 0.138 cm³ and 0.097 cm³ on the same day. For 500 µg/ml gum acacia, the tumour volume was 2.6 cm³ and for 500 µg/ml *in vivo* and *in vitro* (TC3) drug treated animals was 0.074 cm³ and 0.058 cm³ respectively.

**Effect on ascites tumour development**
Life span of ascites tumour bearing mice, treated with *in vivo* and *in vitro* (TC3) plant extracts was found to be significantly increased. Control animals survived only 19 days after the tumour induction while the 250 µg/ml extract of *in vivo* and *in vitro* (TC3) treated animals survived 27 and 29 days with an increase in the life span of 39.47 % and 54.37 % respectively. For 500 µg/ml plant extracts, it was 31 and 33 days with an increase in the life span of 61.37 % and 75.42 % respectively. An enhancement of life of 25% or more was considered as an effective antitumour response.

This study revealed a shoot regeneration method for the production of somaclonal variant of *A. nilagirica*, having better essential oil profiles and antitumour activity and it also reveals the cytological and molecular basis for the phytochemical variations in them.