Cancer is a leading cause of death worldwide. The main types of cancer leading to overall cancer mortality are: lung (1.3 million deaths/year); Stomach (almost 1 million deaths/year); Liver (662,000 deaths/year); Colon (655,000 deaths/year) and Breast (502,000 deaths/year). Death due to cancer in the world continues to increase, with an estimated 9 million people in 2015 and 11.4 million in 2030.

There are many treatment procedures available for cancer which includes Surgery, Radiotherapy and Chemotherapy. An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural chemical agents (alone or in combination) to control the development of cancer. Chemotherapy has been the mainstay of cancer treatment for the past 50 years. It is a major treatment modality used in the management of advanced stages of malignancies and also as a prophylactic against possible metastases in combination with radiotherapy.

However, chemotherapeutic drugs exhibit severe normal toxicity, resulting in undesirable side effects. Moreover, many of the potent drugs used in the cancer treatment are very expensive, mutagenic and teratogenic. Hence, there is a need to find alternative drugs, which are effective, inexpensive, affordable and also without serious side effects.

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. The role of natural products as a source for remedies has been recognized since ancient times. Despite major scientific and technological progress in combinatorial chemistry, drugs derived from natural products still make an enormous contribution to drug discovery today.

Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects. Several plant products have been screened for their anticancer potentials. Few of them which turned out to be potential anticancer drugs are Vincristine, Vinblastine, Podophyllotoxin and Taxol. Hence in the present dissertation attempts were made to screen commonly available plant sources in and around Trichy...
for their anticancer potential which will lead to the development of human compatible
anticancer drug from natural sources.

From the literature review it is observed that Asteraceae members which are
prominent in their occurrence are potential anticancer agents. Besides they are also rich
in anticancer compounds such as sesquiterpene lactones and royleanones.

In the present work species of Artemisia, belonging to Asteraceae were selected
and subjected to in-depth studies to assess their anticancer effect.

Species of Artemisia selected for the present study are

- *Artemisia nilagirica* Pamp. and
- *Artemisia parviflora* Buch. - Ham .Ex D. Don.

Anticancer studies employing Ehrlich ascites Carcinoma cell lines is one of the
effective and reliable methods and has been used by a number of researchers to
determine the chemotherapeutic potentials of plant sources. In the present study also
Ehrlich Ascites Carcinoma cell lines were used to assess the anticancer potential of the
selected Plant drugs.

*Artemisia nilagirica* Pamp. was collected from Tiruchirappalli and *Artemisia
parviflora* Buch. - Ham .Ex D. Don. was collected from Kodaikkanal hills. Both the
species were identified with the help of Flora of Presidency of Madras and authenticated
with the specimens deposited at the RAPINET Herbarium, St. Joseph’s College,
Tiruchirappalli. The aerial parts of the collected plant materials were shade dried and
coarsely powdered.

Water and Ethanol extracts of the plant materials were prepared and studied for
their cytotoxic potentials. From the *In-Vitro* cytotoxic studies it is concluded that
Ethanol extracts of both the plant materials were highly effective against Ehrlich
Ascites Carcinoma cell lines (Tryphan Blue Method). Hence ethanol extracts of the
selected plant drug sources were subjected to further in-depth studies. Besides the test
drugs/extracts extracts were standardized both botanically and chemically, which is
essential to maintain the uniformity and reproducibility of the data that will lead to
global acceptability of the selected drugs as potential anticancer agents.

**Macroscopic standards:**

*Table 73* clearly depicts the distinguishing organoleptic and macroscopic
features of both the species of *Artemisia*. *A.nilagirica* is an aromatic undershrub with a
pubescent stem and pinnatipartite leaves. The flowers are in small globose heads and
the fruits are of achene type whereas *A.parviflora* is a non-aromatic undershrub with a
linear oblong much reduced leaves; Heads in recemes with sterile disc florets and fruits
are achenes.

*Table 73: Distinguishing organoleptic & macroscopic standards of the selected drugs:*

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>FEATURES</th>
<th><em>A.NILAGIRICA</em></th>
<th><em>A.PARVIFLORA</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Taste</td>
<td>Bitter</td>
<td>Bitter</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Green</td>
<td>Dark Green</td>
</tr>
<tr>
<td>3</td>
<td>Odour</td>
<td>Aromatic</td>
<td>Non-aromatic</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>FEATURES</th>
<th><em>A.NILAGIRICA</em></th>
<th><em>A.PARVIFLORA</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stem</td>
<td>Aromatic, Pubescent</td>
<td>Non-aromatic undershrub</td>
</tr>
<tr>
<td>2</td>
<td>Leaves</td>
<td>Pinnatipartite</td>
<td>Linear oblong</td>
</tr>
<tr>
<td>3</td>
<td>Flowers</td>
<td>In Small globose heads</td>
<td>Globose head and presence of sterile disc florets</td>
</tr>
<tr>
<td>4</td>
<td>Fruits</td>
<td>Achene</td>
<td>Achene</td>
</tr>
</tbody>
</table>
**Microscopic standards:**

The following table gives the distinguishing microscopic standards of the selected plant drugs.

*Table 74: Microscopic standards of the selected plant drugs*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Features</th>
<th><em>A. nilagirica</em></th>
<th><em>A. parviflora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Midrib</td>
<td>Thick adaxial cone and wide abaxial zone.</td>
<td>Thin; adaxial cone absent, abaxial zone narrow.</td>
</tr>
<tr>
<td>2.</td>
<td>Palisade cells</td>
<td>Spongy parenchyma distinct</td>
<td>Spongy tissues not distinct</td>
</tr>
<tr>
<td>3.</td>
<td>Leaf margin</td>
<td>Not straight.</td>
<td>Straight.</td>
</tr>
<tr>
<td>4.</td>
<td>Petiole</td>
<td>Distinct, thick and circular.</td>
<td>Absent</td>
</tr>
</tbody>
</table>
Fluorescence behaviour of AN and AP powders and their successive extracts after treatment with various chemical agents were observed under day and UV light at 254nm and observations were recorded. AN Drug powder with aqueous and alcoholic acids and bases exhibited various shades of green and brown fluorescence under Day light; Green, Brown and Reddish Brown fluorescence under UV light. AN Extracts with various solvents revealed Dark green and Brown fluorescence in day light and Brown shades under UV light (*Table 5 & Table 6*). AP drug powder revealed various shades of pale green and yellow fluorescence in day and UV light respectively. Various extracts of AP revealed Dark green and orange fluorescence in day light and Brown fluorescence under UV light (*Table 40 & Table 41*).

**Purity & strength of the selected drugs:**

The physicochemical constants of AN & AP were recorded in *Table 7 & Table 42* respectively. Higher values of Total ash content and less acid insoluble ash values in both the species clearly indicated the purity of the test drugs and the presence of higher inorganic contents.

In both the species of *Artemisia* the Hexane and chloroform extractive values were found to be more when compared to extractive values with ethyl acetate, it may be due to the presences of essential oil and Terpenes (*Table 8 & 43*). Water extractive value was found to be more as compared to Alcohol extractives (*Table 9 & Table 44*).

The results of preliminary phytochemical analysis of AN & AP drug powders and extracts were determined and tabulated. From the data obtained it is noticed that AN drug powder and extracts were found to be showing the presence of Tannins, Terpenes, Sterols, Flavones, coumarins and Alkaloids (*Tables 10 & 11*). Preliminary phytochemical screening of the drug powder and extracts of AP showed the presence of Alkaloids, Terpenes, Sterols, Flavones and Quinones (*Tables 45 & 46*).
Ethanol extract of both the species of *Artemisia* showed the presence of many anticancer phytochemical constituents such as spathulenol, caryophyllene, eugenol, etc. These data along with the results of the In-Vitro cytotoxic studies prompted us to conduct in-depth analysis so as to evaluate the anticancer effect of ethanolic extracts of selected test drugs.

The quantitative analysis of important inorganic substances of AN was tabulated in *Table 12*. Calcium (5.14 %) and Iron (15.32 ppm) contents were found to be more. The amount of Potassium (3.19 %), Magnesium (3.20 %) and Manganese (5.15 ppm) were present in moderate levels whereas Boron (0.08 ppm), Molybdenum (0.06 ppm), copper (0.31 ppm), Phosphorous (0.42 %) and sodium (0.38 %) were present in lesser quantity. AP was found to be showing higher Calcium (5.63 %), Iron (15.62 ppm) and Manganese (10.26 ppm) contents (*Table 47*). The amount of potassium (2.59 %) and Magnesium (4.26 %) were moderate and Boron (0.49 ppm), Molybdenum (0.12 ppm), copper (0.34 ppm), Phosphorous (0.29 %) and sodium (0.39 %) contents were comparatively low.

The presence of high and moderate levels of Calcium, Iron, Manganese, Potassium and Magnesium may contribute towards maintaining the normal metabolic pathways. Ca constitutes a large proportion of the bone, human blood and extracellular fluid; it is necessary for blood coagulation and in the regulation of cell permeability. It also plays an important role in nerve-impulse transmission and in the mechanism of neuromuscular system. Iron is very important for haemopoiesis. Potassium is of importance as a diuretic. Rapidly growing tumour cells alters the haematological parameters and affect the haemopoetic system. Presence of large amounts of Ca, K, Mn and other inorganic constituents like Iron, Magnesium and Manganese in these plant drugs might contribute in reverting the haematological parameters to near normalcy.

Mg is required to maintain the osmotic equilibrium in the plasma and extracellular fluid. It is also necessary for many enzyme-catalysed reactions, especially in which nucleotides participate; whereas, Na along with K take part in maintaining the ionic balance of the human body and tissue excitability. Because of the solubility, Na
plays an important role in the transport of metabolites. Cu is a component of many enzyme systems such as cytochrome oxidase. Besides, all these inorganic constituents also play vital role in the free radical scavenging activity and in maintaining the antioxidant status. Both the species of Artemisia were totally free from heavy metal toxicity.

The data of the quantitative analysis of important organic phytoconstituents of AN and AP were estimated and tabulated in the Table 13 & Table 48 respectively. In the case of AN, The Total Flavonoids (0.9 mg/kg) and Lignin (0.82 mg/kg) contents were found to be more as compared to Alkaloids (0.42 mg/kg) and Tannins (0.68 mg/kg). Whereas, AP showed more amounts of Alkaloids (2.59 mg/kg) and Flavonoids (1.63 mg/kg) with a lesser amounts of other organic constituents.

TLC Profiles of the ethanol extract of AN (Fig 5) on Silica gel “G” plate using Toluene : Ethyl acetate : Formic acid (7:3:1) as mobile phase revealed 4 spots under UV (254 nm) at Rf 0.26 (green), 0.41 (Blue), 0.57 (dark green) and 0.73 (Green), 9 spots under UV (336 nm) at Rf 0.32 (Pink), 0.42 (Blue), 0.48 (Pink), 0.51 (Pink), 0.56 (Blue), 0.61 (Brown), 0.73 (Pink), 0.82 (Pink) and 0.88 (Pink). With Anisaldehyde-sulphuric acid as spraying reagent revealed 10 spots at Rf 0.13 (Brown), 0.17 (Blue), 0.26 (Brown), 0.35 (Brown), 0.44 (Blue), 0.50 (Violet), 0.61 (Blue), 0.71 (Brown), 0.81 (Violet) and 0.89 (Red) (Table 14).

TLC Profiles of the ethanol extract of the AP (Fig 27) on Silica gel “G” plate using Toluene : Ethyl acetate : Formic acid (7:3:1) as mobile phase showed 3 spots under UV (254 nm) at Rf 0.49 (Dark green), 0.54 (Green) and 0.75 (Green); 5 spots under UV (336 nm) at Rf 0.29 (Blue) 0.32 (Blue), 0.38 (Dark blue), 0.73 (Pink) and 0.77 (Pink). With Anisaldehyde-Sulphuric acid as spraying reagent revealed 7 spots at Rf 0.17 (Grey), 0.36 (Blue), 0.48 (Dark Blue), 0.55 (Pink), 0.58 (Pink), 0.74 (Blue) and 0.82 (Red) (Table 49).

HPTLC fingerprints of the ethanol extracts of the plant drugs were also determined and shown in Fig 6 & Fig 28. HPTLC fingerprint of EEAN revealed 15 peaks and HPTLC fingerprint of EEAP revealed 14 peaks.
ANTICANCER SCREENING

IN-VITRO STUDIES

Studies on cytotoxicity:

Trypan blue method was employed to assess the cytotoxic potential of the plant extracts under study namely EEAN & EEAP. The principle involved in this method is the inability of the plasma membrane of dead cells to prevent the entry of Tryphan blue dye. Ehrlich Ascites Carcinoma cells were treated with different concentrations of the Ethanol extract of the test drugs. Increased cytotoxicity was noticed with an increase in the concentration of the test drugs. Dead cell percentage was high in the case of EAC cells treated with 500 µg/ml of EEAN (94.94 %) when compared to EAC cells treated with 500 µg/ml of EEAP (89.90 %) (Tables 18 & 53). There might be an induction of apoptosis in the EAC cells on treatment with the plant extracts which might have caused loss of integrity of the cell membrane which is followed by an increase in the number of Tryphan blue positive (dead) cells. Other scientific evidences to support the induction of apoptosis due to the administration of test drugs will be discussed in sequel.

DNA fragmentation analysis:

Effect of EEAN & EEAP on the DNA of EAC cells were studied and recorded in Fig10 & Fig 32 respectively. Treatment with plant extracts showed loss of integrity and degradation of the chromosomal DNA in a dose dependant manner resulting in a ladder like appearance of DNA fragments. DNA fragmentation is the biological hallmark of apoptosis. This might be due to the activation of Caspases, which are cysteine proteases that play a critical role in the execution of apoptosis. Activation of caspase leads to the activation of DNase, a DNA breaking enzyme (endonuclease). Caspase-activated DNase (CAD) is associated with inhibitor of CAD (ICAD) in the cytosol. Upon caspase activation by external stimuli, CAD dissociates from ICAD and translocates to nucleus to disrupt the genetic material. Probably treatment of EAC cells with EEAN & EEAP might have activated caspase which in-turn might have activated its downstream target CAD that is responsible for the fragmentation of DNA.
These results thus indicate the possible triggering of apoptotic signalling pathway in EAC cells, due to the EEAN & EEAP administration.

**MTT assay:**

MTT assay, an important and reliable method employed to assess the cytotoxic potential of drugs under study. The cytotoxic potential of EEAN & EEAP was evaluated against Ehrlich Ascites Carcinoma cell lines and L-929 Lung Fibroblast cell lines using MTT assay. The plant extracts were found to be highly cytotoxic against EAC cells whereas moderate toxicity was noticed against L-929 Lung Fibroblast Cell lines. Cytotoxicity effects of the extracts increased with increased concentration. EEAN exhibited 68 % cell death at a concentration of 15 µg/ml with an IC₅₀ Value of 11.03 µg/ml (Table) against EAC cells. 16.23 % cytotoxicity was recorded at a higher concentration (10 µg/ml) against L-929 Lung Fibroblast cell lines whose IC₅₀ value was found to be 30.80 µg/ml (Table 19 & 20). EEAP exhibited 67.32 % of Cytotoxicity against EAC cell lines & 18.02 % of cytotoxicity against L-929 Lung Fibroblast cell lines with IC₅₀ Values of 11.14 µg/ml & 27.74 µg/ml respectively (Table 54 & 55). As per the review a drug showing an IC₅₀ value of less than 30 µg/ml is considered as an active drug²²⁸. As the plant drugs under investigation are exhibiting IC₅₀ values of less than 30 µg/ml, they could be considered as potential anticancer agents.
**IN - VIVO STUDIES**

**Study on life span:**

Treatment with various concentrations of EEAN (100, 200, 300 mg/Kg b.w.) for 14 days increased the lifespan of tumor bearing animals and the results were tabulated in Table 21. Administration of EEAN increased the life span of the tumour bearing animals in a dose dependant manner and the maximum dose 300mg/Kg.b.w. (Group V) revealed significant increase in the life span of tumor bearing animals up to 83.33 %. Increase in life span noticed in the tumour bearing animals treated with the maximum dose of EEAP is 77.77 % (Table 56). Almost similar increase in the life span was observed in the case of Group VI animals which received the Standard drug (5-FU). The reliable criteria for judging the value of any anticancer drug is the prolongation in the life span of animals. Increase in the life span of tumour bearing animals up to 83% on treatment with EEAN & EEAP suggested the anticancer effect of EEAN & EEAP.

A gradual increase in the body weight was witnessed in Group II animals (49.87 %) after inoculation of EAC cells, due to constitutive replication of EAC cells followed by an accumulation of ascites fluid in the peritoneal cavity. But, in the case of tumour bearing animals treated with the plant extracts, the percentage increase in the body weight was found to be reduced in a dose dependant manner. After 15 days of treatment with EEAN, Group V animals showed only 7.67 % increase in the body weight, which is almost similar to that of the Group VI animals (8.25 %) and in par with that of the Group I animals (10.55 %) (Table 22). A similar kind of effect was witnessed on the animals treated with EEAP as well, which showed 9.7 % increase in body weight after 15 days of treatment (Table 57). Noticeable increase in body weight was not noticed in the test drug treated group. This might be due to the death of EAC cells, and decrease in the body fluid accumulation.

**Studies on tumor growth response:**

In EAC-bearing mice, a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase
in ascitic fluid would be a means to meet the nutritional requirement of tumor cells. It may be correlated that extracts of both the species of *Artemisia* increases the life span of EAC-bearing mice by decreasing the number of tumour cells (*Table 23 & Table 58*). As growth of tumour cells is arrested, ascites tumour volume is also decreased.

**Studies on Haematological Profiles:**

Usually, in cancer chemotherapy the major problems that are being encountered are myelosuppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions. Treatment with plant extracts brought back the hemoglobin content, RBC, and WBC count more or less to normal levels (*Table 24 & Table 59*). This indicates that EEAN & EEAP possess protective action on the hemopoietic system.

On treatment with the plant extracts, restoration in the altered levels of Neutrophils, Lymphocytes and Basophils was witnessed in a dose dependent manner (*Table 25 & Table 60*). This might be due to the inhibition of tumor cell proliferation caused by the plant drugs under study.

**Studies on Biochemical Parameters:**

Hypoglycemia prevails in the Tumour bearing animals, as the fast growing cancer cells utilizes the available glucose for its nutrition. Treatment with the plant extracts reverted back the level of glucose which indicated reduction in the utilization of glucose that might be due to the prevention of growth of tumour cells (*Table 26 & Table 61*).

The presence of tumor is known to affect many functions of the vital organs, especially of the liver. A decreased level of protein, urea and liver glycogen and an increased level of uric acid & bilirubin in group II animals is a direct indication of the functional impairment of hepatic tissue. Secondary carcinoma in the hepatic tissue and functional impairment were prevented in the experimental animals which was
evidenced by the restoration of the altered levels noticed in protein, urea, uric acid, bilirubin and liver glycogen\textsuperscript{233} (Table 27 & Table 62).

**Studies on Lipid Profiles:**

The tumour bearing animals were found to be showing a decreased levels of triglycerides, cholesterol and phospholipids in the liver tissue, which is a direct indication of lipolytic activity observed in the tumour bearing mice due to excessive energy requirement of constitutively replicating tumour cells\textsuperscript{234}. These lipid levels were normal in the case of animals treated with EEAN & EEAP (Table 28, 29 & Table 63, 64). This clearly indicates that the plant extracts prevented the growth of the tumour cells there by reduced lipolysis and helped in maintaining a normal level of the lipids in serum and liver.

The level of plasma and liver free fatty acid was found to be increased in the Group II animals. This is due to the increased mobilization of free fatty acids from the adipose tissue to the liver through plasma to meet the energy needs as well as to satisfy the membrane requirements of the fast growing tumour cells\textsuperscript{235}. The mobilization of cholesterol was also found to be elevated in the tumour bearing animals. These levels were found to be almost normal in the case of animals treated with the test drugs. These effects might be due to the tumour inhibitory potential of the plant drugs over EAC cells.

**Studies on Lipoprotein Levels:**

The level of LDL and VLDL greatly increased and the HDL level decreased markedly in the animals bearing EAC cells\textsuperscript{236}. This condition is normally associated with tumour. In the case of drug treated animals the level of these lipoproteins were found to be normal indicating the tumour inhibitory potential of EEAN and EEAP (Table 30 & Table 65). The test drug might have activated the lipoprotein lipase and must have reduced the level of LDL and VLDL. The plant drugs also restored the level of HDL.
**Studies on Hepatic Marker Enzymes:**

Elevation in the level of hepatic marker enzymes was observed in the case of tumour bearing animals, which is due to the abnormal metabolic condition resulting due to severe malignancy. As liver is the immediate organ which is being infiltrated (metastasized) and affected by the fast growing tumour cells, a marked imbalance in the normal functioning of the organ was observed\(^{237}\), which resulted in the cellular destruction and leakage of ALT, AST, \(\gamma\)-glutamyl transferase and ALP into the plasma from hepatocytes. All these enzymes play a vital role in the metabolism of macromolecules.

The enzyme levels were brought back to near normalcy in the animals treated with EEAN and EEAP (*Table 31* & *Table 66*). This clearly depicts the cellular protection potentials of test drugs thereby preventing the leakage of hepatic marker enzymes.

Lactate Dehydrogenase (LDH), plays an important role in the anaerobic glycolysis, which is recognized as a potential tumor marker in assessing the progression of the constitutively replicating tumour cells. As, anaerobic glycolysis is the pathway satisfying the major energy need of the tumour cells, the level of LDH was found to be more in Group II animals (*Table 32* & *Table 67*). A low level of LDH activity was noticed in the animals treated with the plant extracts. This clearly depicts that the plant extracts blocks the energy yielding pathway of the EAC cells, thereby suppressing the proliferation and infiltration of EAC cells into the neighbouring tissues. This might be due to the direct action of the plant drugs over the expression of genes responsible for LDH as suggested by Gupta *et al.*,\(^{238}\).

**Studies on Nucleic acid Contents:**

The level of nucleic acids (DNA & RNA) were more in the disease control animals\(^{239}\). This might be due to the increased expression and activity of polymerase and other metabolic enzymes in fast growing tumour cells. Levels of these nucleic acids were found to be decreasing in the animals treated with EEAN & EEAP in a dose
dependant manner (Table 33 & Table 68), as these extracts might have lowered the synthesis and activities of the polymerase enzyme, thereby must have blocked the development of EAC cells at an early phase (G_1) of their cell cycle. The plant drugs might have also blocked the pentose phosphate pathway and the synthesis of nucleic acids in cancer cells leading to lower level of nucleic acids.

**Carbohydrate metabolizing enzymes:**

An abnormal metabolic pattern is a hallmark of fast growing tumour cells, as their energy utilization is very high. Altered carbohydrate metabolism is observed so as to suit the energy need of the tumour cells. Consequently replicating cancer cells utilize 60% of their total ATP need from glycolysis. Hence there is a decrease in the activity of Glucose-6-phosphatase, a key gluconeogenesis enzyme, in various tumour incidences. Increased activity of hexokinase and phosphoglucoisomerase has been correlated with the degree of malignancy as their activity provide necessary sugars and promote the growth of cancer cells.

In EAC bearing animals also a similar kind of alterations in the level of carbohydrate metabolising enzymes is observed (Table 34 & Table 69). In the case of animals treated with the plant extracts, these alterations are found to be minimized in a dose dependent manner. The active principles in the plant extracts might have regulated these enzymes either directly or indirectly so as to reduce the development of the tumour, by not providing necessary sugars for tumour growth.

**Studies on Glycoprotein Levels:**

Membrane associated carbohydrates are exclusively in the form of oligosaccharides covalently attached to proteins forming glycoproteins. Glycoproteins play a vital role in cell to cell recognition, intracellular processing of proteins, cell activation and in increasing the ability of cancer cells to metastasize. Alterations in the glycoprotein levels have been well exploited for the diagnosis of tumor growth. The level of an important glycoprotein of the cell membrane, Hexosamine, was found to be very high in the tumour bearing animals (Table 35 & Table 70). The Hexosamine
Discussion

Sialic acid is an acylated derivative of nuraminic acid and exists as amino acid terminal compound of the non-reducing end of glycoprotein in carbohydrate chains. Sialic acid acts as a tumor marker which could be determined from the perspective of aberrant glycosylation occurring in cancer cell membranes due to the activation of glycosyl transferase\textsuperscript{243}. The role played by Sialic acid in tumor cell metastasis is to increase the capacity of cancer cells to adhere to vascular endothelium and to decrease the ability of the host system in destroying the cancer cells.

Elevated levels of Hexoses, Hexosamine, Sialic acid and Fucose were observed in serum and liver tissue of tumour bearing mice (\textit{Table 36 & Table 71}). This may be due to leakage of the membrane compounds from either disintegrating or dying neoplastic cell or due to consequence shedding of plasma membrane or due to neoplastic transformations. Tumour bearing animals treated with EEAN & EEAP showed restoration of the glycoprotein levels. This reduction in the glycoprotein components indicates that the plant extracts stabilizes the membrane structures and prevents the metastasis and malignancy.

\textbf{Antioxidant Studies:}

Various studies indicated that Ehrlich Ascites tumor growth can cause disturbances of antioxidant status in certain tissues. Oxidative stress as a result of overproduction of reactive oxygen species has been noticed in the pathogenesis of number of human cancers\textsuperscript{244}. Lipid peroxidation, an autocatalytic free radical chain propagating reaction, is known to be associated with pathological conditions of a cell\textsuperscript{245}. Malondialdehyde (MDA), the end product of lipid peroxidation, was reported to be higher in cancer tissues than in nondiseased organ. On treatment with EEAN & EEAP the level of MDA was found to be reduced, which might be due to the free radical scavenging property of the test drugs.
In group II (Disease control) animals a fall in the activity of Glutathione peroxidase (GPx) was observed which may be due to the lack of glutathione leading to increased production of hydroxyl radicals. Administration of test drugs effectively restored the activity of GPx and thereby decreased the production of hydroxyl radicals (Table 37 & Table 72).

Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous antioxidant system. It is found in high concentration in the liver and is known to play a key role in the protective process. Low Glutathione level was found in liver tissue of tumor bearing animals, which is mainly due to oxidation of GSH to GSSH caused by increased peroxidation. GSH level is further reduced due to the decreased activity of glutathione reductase. The level of Glutathione (GSH) was found to be increased on treatment with the plant drugs. The level of GSH was found to be increased which might be due to the liver protection action of test drugs. Hepatoprotective efficacy of the test drugs was clearly evident in the hepatic marker enzyme levels which were brought back to near normalcy on test drug administration (Table 37 & Table 72). Due to this hepatoprotective potential of test drugs, liver was well protected on test drugs administration leading to increased level of GSH. Increased level of GSH might have improved the activity of Glutathione reductase which in turn might have inhibited the lipid peroxide production leading to tumour suppression.

SOD & CAT are involved in the scavenging of superoxide and hydrogen peroxide radicals (H₂O₂). SOD catalyses the diminution of superoxide into H₂O₂, which has to be eliminated by glutathione peroxidase or catalase. It has been reported that a decrease in SOD activity in EAC-bearing mice might be due to loss of Mn²⁺ containing SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of CAT activity as a result of tumor growth was also reported. This is because super oxide radicals present in the host tissue of tumor bearing animals converts the ferrous of the catalase into ferroxy or ferryl state and makes the enzyme inactive. In the present studies also decrease in CAT activity and SOD were observed (Table 37 & Table 72) in tumour induced animals.
Treatment with the test drug restored the decreased level of SOD to near normalcy thereby protected the mitochondria and enzyme. Increase in the activity of the catalase noticed on treatment might be due to the free radical scavenging activity of the test drugs resulting in the restoration of the ferrous group and the activity of CAT & SOD.248

Thus, the lowering of lipid peroxidation and increase in levels of Gpx, GSH, SOD and Catalase in test drug treated animals clearly depicts the antioxidant potential of test drugs in EAC induced oxidative stress.

**Histopathological studies:**

Microtome sections of liver tissue (3–5 μm thickness) of EAC animals stained with Hematoxylin and Eosin clearly revealed Hepatocytes with degenerative changes such as loss of architecture, microvascular fatty changes and hepatocytes with pleomorphic condensed nuclei.249 On treatment with EEAN & EEAP an improved histoarchitecture of hepatic cells were observed with vesicular nuclei, sinusoidal dilations, mild fatty changes and granular cytoplasm. Histological observations thus confirmed the nontoxic nature of the test drugs (*Plate 11 & Plate 19*).
In-Silico Studies:

Attempts were also made to understand the mechanism of action of test drugs employing In-Silico methods using the compounds identified in GC-MS analysis such as, Eugenol, Spathulenol and α-Terpineol (Table 16 & Table 51).

Selection of target:

During mitogenic stimulation, growth factors bind to their receptors and promote their dimerization and autophosphorylation. This leads to the activation of SH2 domain containing proteins such as, PLCγ, PI3K, the oncoproteins Ras and Src and, in turn, the MAP kinase cascade. End products of these cascades, MAPK, p38, and JNK are translocated to the nucleus where they phosphorylate and activate many substrates including transcriptional factors such as Jun, Ets1, Ets2, Tcf, etc. This causes activation of other transcription factors. Similar reactions are also observed on binding of integrins with ECM proteins which promote the autophosphorylation of FAK. This results in the binding of FAK to the SH2 domain of the Src proto- oncoprotein followed by recruitment of the adapter Grb2 protein, and activation of Ras and MAP kinase cascades.\(^{250}\)

The major consequence of the MAP kinase activated transcription factors is the increase in expression of the oncoprotein cyclin D1 and Myc, which increase the activity of cyclin-dependent kinases operating in G1 phase (cyclin D–Cdk4 and cyclin E–Cdk2). These phosphorylate the tumour suppressor protein pRb. Phosphorylation of pRb and its binding to a number of viral oncoproteins induces release and activation of transcription complexes E2F/DP, which increase expression of genes whose products are necessary for passage of the cell to S phase of the cell cycle leading to cancer cell growth.\(^{251}\)

Hence, The MAPK (Mitogen Activated Protein Kinase) cascade forms an important pathway to carry the signals of cell division. MAP Kinase is the first enzyme protein of this cascade which is being activated by various mitogenic stimuli. This MAPK has been over expressed in a number of cancer incidences. Over expression of
this MAPK enzyme promote constitutive replication of cancer cells. Hence the MAPK protein is selected as the target for the In-Silico studies and docking were carried out with the ligands present in the Ethanol extracts using the Software - AutoDoc 3.05.

**Docking Studies:**

Following compounds identified in ethanol extract of test drugs were subjected to docking studies

Eugenol present in the extract effectively docked with the target by three hydrogen bonds with a bonding energy of -5.23 kcal/mol (Fig 52).

Spathulenol docked with the target protein with one hydrogen bond and the final docked energy was found to be -7.60 kcal/mol (Fig 53).

α-Terpeneol was found to be docked with the target protein with two hydrogen bonds and the final docked energy was -6.26 kcal/mol (Fig 54).

The bonding energy scored observed in the docking studies of the ligands present in test drugs clearly revealed that the hydrogen bonds formed between the ligand and MAP kinase protein is a stable one there by suggested that the Test drug might prevent the cancer cell entering into the S phase by deactivating the MAPK cascade and inhibiting the expression of oncoprotein as well as cancer cell growth.

Thus the data of the results of the In-Silico, In-Vitro and In-vivo studies carried out in the present work clearly depicted that both EEAN & EEAP possess anticancer/antitumor activity against Ehrlich Ascites Carcinoma. The data of the results were in par with that of the standard drug 5-fluorouracil (20mg/kg bw). It is observed that the 300mg/Kg.bw. dose level of EEAN and EEAP is the most effective dose. When compared, among the extracts selected for the study EEAN was found to be more effective than that of EEAP.
Probable mechanism of action of the selected plant drugs may be attributed to the below mentioned efficacies and observations recorded in the present work.

- Increase in the Life span of Tumor bearing animals
- Inhibition of the Ascites fluid volume and viable cell counts
- Reversal of the altered biochemical parameters to near normalcy
- Decrease in Lipolysis
- Normalization of Carbohydrate metabolism
- Cytostabilisation
- Suppressed malignancy and metastases
- Maintenance of Antioxidant status
- Restoration of Hepatic Architecture to near normalcy
- Activation of Apoptotic Pathway in EAC cells supported by the Membrane disturbance & DNA Fragmentation.
- Inhibition of signalling pathway of cell division
- IC$_{50}$ values less than 30 µg/ml.
- Presence of proven anticancer compounds such as Eugenol, Spathulenol, Terpeneol and Caryophyllene.
- Docking studies revealed encouraging energy scores and hydrogen bond formation between the ligands identified in the GC-MS analysis and MAP Kinase protein.