Summary and Conclusion
Cancer is one of the deadliest genetic as well as induced disease resulting due to the defects in the genetic material. It is characterized by uncontrolled and unwanted growth of cells.

Plants and plant derived products have been widely accepted as good therapeutic agents with lesser side effects particularly in the management of cancer. Since Time Immemorial plant materials have been used in the treatment of malignant diseases. Throughout medical history, plant products have been proven to be valuable sources for developing novel anti-cancer drugs. Few examples are Vincristine and Vinblastine from *Catharanthus roseus*, Taxols from *Taxus baccata* and Camptothecins from *Camptotheca acuminata*.

These well established anticancer plants and plant molecules prompted us to select plants and carry out in depth anticancer screening studies against Ehrlich Ascites Carcinoma cell lines.

Based on the literature review two plant sources *Hyptis suaveolens* Poit and *Leonotis nepetaefolia* R.Br. belonging to the family *Lamiaceae* common in and around Trichy were selected. Ethanolic extracts of selected plants were screened against Ehrlich Ascites Carcinoma cell lines employing In-vitro and In-vivo methods. Lights were also thrown to understand the mechanism of action through biochemical and bioinformatic approaches. As standardization is essential for herbal medicines, botanical and chemical standardization, studies on selected plants were also carried out. Parameters selected to determine the standards are Macro & Microscopic features, Tests for Identity, Purity and Strength, Quantification of Inorganic and organic contents, TLC and HPTLC profiles.
Summary and Conclusion

The Botanical, Analytical and chemical standards determined in the present work can contribute in the identification and authentication of these plants in dry condition in which ever form they exist in the raw drug market.

In-vitro Cytotoxic studies were carried out against Ehrlich Ascites carcinoma cell lines employing trypan blue dye method and MTT assay. Both the extracts (EEHS and EELN) showed potent Cytotoxic effect with IC50 value $10.63 \mu g/ml$ and $4.94 \mu g/ml$ respectively (MTT Assay)

Two different concentrations of Ethanolic extract of the plant drugs incubated to DNA of Ehrlich Ascites Carcinoma cell lines revealed distinct DNA fragments. DNA fragmentation is the Hallmark of apoptotic pathway and the present study clearly depicted apoptosis inducing effect of the test drugs.

Ehrlich Ascites Carcinoma cell lines were induced at the dose level of $1 \times 10^6$/mouse intraperitonially to Swiss Albino mice. After 24 hours of tumor inoculation, ethanolic extract of selected plants were administered at the dose level 100, 200, 300mg/ Kg. bw to the tumor bearing animals orally for 14 days and to the standard control 5-Fluorouracil was administered at the dose level of 20mg/Kg bw. Body weight of the animals were recorded regularly at 3days interval during the experimental period. After the experimental period the Ascites fluid was collected, the fluid volume, PCV viable and non-viable cell counts were determined. Hematological and various biochemical markers such as glucose, urea, lipid profiles, lipoprotein contents and hepatic marker enzymes such as AST, ALT, ALP, GGT and LDH were determined in serum, blood and tissue collected though cervical de capitation method. Liver homogenate was used to study the glycogen content, Nucleic acid levels, Carbohydrate metabolizing enzymes, Glycoprotein levels, and antioxidant status.

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Summary and Conclusion

Treatment with the test drug (EEHS and EELN) increased the lifespan of the tumor bearing animals up to 61.5% and 73.57% respectively. Reduced body weight, Ascites fluid volume, viable cells counts and increased non-viable cell counts were noticed. Reduction in tumor growth response clearly indicated the efficacy of the test drugs in inhibiting the proliferation of fast growing tumor cells.

The reduction in Hemoglobin and RBC counts and Elevation found in the WBC counts were effectively restored to near normalcy. These results thus suggest that the test drugs are capable of protecting the hemopoietic system.

Administration of Test drugs resulted in the restoration of altered levels of biochemical parameters such glucose, urea, uric acid, bilirubin and glycogen to near normal. Reversal of hypoglycemia, hyperbilirubinemia and hyperproteinemia depicted the role of plant drug in controlling the cellular proliferation and tissue damage.

A significant depletion of Cholesterol, Phospholipids and Triglycerides in liver and corresponding elevation in serum of cancer bearing animals were effectively restored, on treatment with EEHS and EELN. The Test drug treatment also reduced the serum levels of TG, LDL and VLDL. It is also further observed that administration of test drugs improved the HDL cholesterol level.

On treatment with test drugs Increased levels of serum hepatic marker enzymes such as AST, ALT, ALP and GGT and LDH were brought back to near normalcy and elevated levels of DNA and RNA were also reduced.

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Summary and Conclusion

Increased activity of Glycolytic enzymes such as Hexokinase, phosphogluco isomerase and decreased activity of Glucose-6- phosphatase were restored to near normalcy on test drug treatment and increased glycogen levels also noticed in liver. On treatment with EEHS and EELN the serum and liver glycoprotein levels were also reduced.

Treatment also enhanced the antioxidant status with sufficient production of antioxidant enzymes such as GPx, SOD and Catalase.

EAC bearing animals revealed loss of liver architecture such as presence of microvasiular fatty changes and hepatocytes with plehomorphic condensed nuclei. EEHS and EELN treated animals showed a cutral vein surrounded by hepatocytes and mild Fatty changes. Hepatocytes with enlarged nuclei and granular cytoplasm were also noticed.

Docking studies conducted of the ligands identified in GC-MS studies with Bcl-2 protein were also encouraging. Ligands from EELN showed more effective docking.

The data of the results obtained clearly depicted that the plant extracts possess pronounced anticancer activity at the maximum dose level and were comparable with that of standard drug (5-Fluorouracil). Among the two plant sources selected Leonotis nepetaefolia R.BR. showed better activity.
Summary and Conclusion

To conclude:
Following Botanical and Chemical Standards were determined for the selected plants:

<table>
<thead>
<tr>
<th>S.No</th>
<th><strong>Hyptis suaveolens Poit.</strong></th>
<th><strong>Leonotis nepetaefolia R.Br.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trichomes are glandular, capitate or peltate.</td>
<td>Non-glandular and glandular trichomes.</td>
</tr>
<tr>
<td>2</td>
<td>Glandular trichome have one or two celled stalk.</td>
<td>Glandular trichome have one or two celled stalk.</td>
</tr>
<tr>
<td>3</td>
<td>Stem four angled and four cornered</td>
<td>Stem four angled with four thick ridges and wide shallow furrows.</td>
</tr>
<tr>
<td>4</td>
<td>Presence of pith canal</td>
<td>Absence of pith canal</td>
</tr>
</tbody>
</table>

**TEST FOR IDENTITY, PURITY AND STRENGTH:**

<table>
<thead>
<tr>
<th>S.No</th>
<th><strong>Name of the Standard</strong></th>
<th>Hyptis suaveolens Poit.</th>
<th>Leonotis nepetaefolia R.Br.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Foreign Matter (Not more than)</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>2</td>
<td>Total Ash (Not More than)</td>
<td>5.1%</td>
<td>4.7%</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash (Not More than)</td>
<td>1.9%</td>
<td>2.9%</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble Ash (Not More than)</td>
<td>3.2%</td>
<td>1.7%</td>
</tr>
<tr>
<td></td>
<td><strong>Solubility</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Alcohol (Not less than)</td>
<td>8.9%</td>
<td>14.3%</td>
</tr>
<tr>
<td>8</td>
<td>Water (Not less than)</td>
<td>13.8%</td>
<td>21.2%</td>
</tr>
<tr>
<td></td>
<td><strong>Extractive Values</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Hexane (Not less than)</td>
<td>5.1%</td>
<td>5.6%</td>
</tr>
<tr>
<td>10</td>
<td>Chloroform (Not less than)</td>
<td>5.9%</td>
<td>6.25%</td>
</tr>
<tr>
<td>11</td>
<td>Ethyl acetate (Not less than)</td>
<td>4.1%</td>
<td>4.9%</td>
</tr>
</tbody>
</table>

**BIOCHEMICAL EVALUATION OF TWO TRADITIONAL DRUG SOURCES AGAINST EHRLICH ASCITES CARCINOMA**

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Important Chemical Constituents:

Alkaloids, Flavones, Sterols and Terpenes

TLC Profiles:

TLC Profiles of the ethanol extract of the HS (Fig 8) on Silica gel “G” plate using Toluene : Ethyl acetae : Formic acid (5:5:0.5) as mobile phase revealed 3 spots in UV 254nm at Rf 0.30(green), Rf 0.55 (Green) and Rf 0.62(Green), 8 spots in UV 336nm at Rf 0.11(Blue) Rf 0.30(Blue), Rf 0.49 (Pink), Rf 053 (Blue), Rf 0.55(Pink), Rf 0.60(Pink), Rf 0.74(Pink), Rf 0.85(Pink), With Vanillin-Sulphuric acid as spraying reagent revealed 9 spots at Rf 0.11(Grey), Rf 0.17 (Grey), Rf 0.36(Grey), Rf 0.47 (Grey), Rf 0.53 (Grey), Rf 0.55 (Violet), Rf 0.60 (Grey), Rf 0.74 (Violet), Rf 0.83(Grey).

TLC Profiles of the ethanol extract of the LN (Fig 35) on Silica gel “G” plate using Toluene : Ethyl acetae : Formic acid (5:5:0.5) as mobile phase revealed 3 spots in UV 254nm at Rf 0.53(green), Rf 0.60 (Green) and Rf 0.85(Green), 4 spots in UV 336nm at Rf 0.53 (Pink) Rf 0.60(Pink), Rf 0.75 (Pink), Rf 0.85 (Pink), With Vanillin-Sulphuric acid as spraying reagent revealed 8 spots at Rf 0.11(Grey), Rf 0.43 (Grey), Rf 0.45(Grey), Rf 0.55 (Grey), Rf 0.64 (Grey), Rf 0.68 (Grey), Rf 0.72 (Grey), Rf 0.81(Dark Green).

Thus botanically and chemically standardized plants were used to prepare water and ethanolic extract and subjected to anticancer screening against Ehrlich Ascites Carcinoma cell lines and derived mechanism of action is enumerated below.
Proposed Mechanism of Action:

Plant extracts under study showed potent cytotoxic activity against Ehrlich Ascites Carcinoma cell lines which is determined through following scientific evidences.

- Increased Life span of Tumor bearing animals
- Inhibition of Ascites fluid volume and viable cell counts
- Normalises the Hematological and Biochemical profiles
- Decreased Lipolysis
- Blocking of nucleic acid synthesis
- Prevention of cell damage and necrosis
- Regularization of Carbohydrate metabolism
- Cytostabilisations
- Suppression of malignancy and metastases
- Restored Antioxidant status
- Restoration of Hepatic Architecture to near normalcy

Activation of Apoptotic Pathway in EAC cells which is confirmed through

- Membrane disturbances
- Mitochondrial disarray and
- DNA Fragmentation

Of EAC cells.

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