CHAPTER 6

Study on antioxidant and anticancer activity of

Emilia sonchifolia DC.
6.1 Introduction

Plants are invaluable in the search for new drugs. There is a tremendous historical legacy in folklore uses of plant preparations in medicine (209). Scientific studies on plants used in ethno medicine led to the discovery of many valuable drugs. Some examples of plant derived drugs are taxol, camptothecin, vincristin and vinblastin.

_Emitia sonchifolia_ (L) DC (Compositae), is a herbaceous plant found in India and other countries in Asia. In India it is used in folklore medicine, against inflammation, rheumatism, cough, cuts and wounds (209). In China, the leaves were used in fever and dysentery (210). It is also used as an analgesic agent and antibiotic (211). The aqueous extract of this plant showed antimicrobial activity (212). The aerial part of the plant contains alkaloids (213) and flavanoids (212). Plants belonging to Compositae showed antioxidant, antitumor and anticarcinogenic properties (214, 215). A detailed investigation has been made to ascertain the antioxidant, anti-inflammatory antitumor and anticarcinogenic property of this plant.

6.2 Materials and methods

The plants were collected from Amala cancer hospital campus during the months of January 1997. The plants were identified by Dr. Sasi, KFRI, Peechi.

6.2.1 Preparation of crude extract from plant

The air-dried plants powdered. And the preparation of the extract was according to the procedure referred in materials and methods section (2.2.6).
6.2.3 Animals

Swiss albino mice (20-25 gm, 6 weeks old) were used throughout the study. They were housed in well-ventilated cages and given normal diet and water *ad libitum* (section 2.1.5).

6.2.4 Superoxide scavenging activity

It was determined by the nitroblue tetrasodium (NBT) reduction method of McCord and Fridovic (185) as described in the materials and method (section 2.4.1).

6.2.5 Hydroxyl radical scavenging activity

Thiobarbuturic acid reacting substances produced as a result of the degradation of deoxyribose by OH\(^+\) radical generated from Ferric/ascorbate system *in vitro* were assessed (section 2.4.2) (186).

6.2.6 Anti-inflammatory activity

Anti-inflammatory activity was studied using the carageenan induced paw oedema in mice as described in the materials and method (section 2.4.4).

Four groups of mice (6/ group) received carageenan (3mg/ kg body wt.) in saline (0.1ml) subplantarly into left hind paw. One group was kept as the control. The other two groups received 250mg and 500mg / kg b.wt. of the plant extract i.p. 1 h prior to the subplantar carageenan injection. Oedema measurements were made before and after injection of carageenan, at 1h intervals up to 4h. The percentage inhibition of oedema
was calculated vs. control group. Indomethacin (10mg/ kg. b. wt. i.p.) was used as reference compound.

6.2.7 *In vitro* cytotoxicity of the extract

Short term cytotoxicity was assessed by incubating $1 \times 10^6$ DLA, EAC and normal human lymphocytes (separated by Ficol hypaque method) in 1ml PBS with varying concentrations of the methanolic extract at $37^\circ$C for 3h. The cell viability was determined by trypan blue exclusion method (section 2.3.1).

Long term cytotoxicity of the extract to L-929 cells was assessed as described in the materials and method (section 2.3.2).

6.2.8 Effect of methanolic extract on ascites tumor in mice

A group of 18 animals were injected with EAC ($1 \times 10^6$) intraperitoneally to develop ascites. The animals were divided into three groups.

**Group A:** Kept as control received saline

**Group B:** Treatment started 24 h after tumor transplantation (100mg/kg.b.wt) orally for 10 consecutive days

**Group C:** Treatment started (100 mg/kg.b.wt) orally 11th day onwards for 10 days.

Percentage increase in life span was calculated, compared to that of control animals, as described in the materials and methods (section 2.3.5)
6.2.9 Effect of methanolic extract on solid tumor development.

A group of 18 animals were injected subcutaneously with $1 \times 10^6$ DLA cells into right hind limb for the development of solid tumor. The animals were divided into three groups as given for ascites model. The mode of administration and schedule of drugs were the same as those in the case of ascites tumor model. The diameter of the tumor was measured every 5th day. The volume of tumor mass was calculated as described in materials and method (Section 2.3.6).

6.2.10 Effect of methanolic extract on DNA synthesis

DLA cells ($2 \times 10^6$) were incubated in three ml MEM containing $2 \mu$Ci ($^3$H)-labelled thymidine. The cells were exposed to various concentrations of the test material (100-500μg) at 37°C for 4 h. The uptake of labeled thymidine was calculated from the radioactivity measured as described in material and method (2.3.4).

6.2.11 Partial purification of *E. sonchifolia* extract by XAD amberlite column

The crude methanol extract was loaded on XAD-2 amberlite column and eluted with varying ratio of water:methanol (100 H$_2$O, MeOH: H$_2$O : 20:80,40:60,60:40,80:20, v/v and 100 %MeOH)

6.2.12 Phytochemical analysis of the active fraction

Active fraction was chromatographed on silica gel G coated glass plates using the solvent system BAW (Butanol: Acetic acid: H$_2$O). This chromatogram sprayed with different spray reagents as given in the materials and methods (section: 2.2.8)
6.2.13 uv visible absorption of the active fraction

The partially purified fraction was dissolved in ethanol and absorption maxima were taken between 200-700nm.

6.2.14 Protective effect of *Emilia sonchifolia* partially purified fraction on DMBA/ croton oil induced skin papilloma formation in mice

The anticarcinogenic effect of *Emilia sonchifolia* active fraction was evaluated in Swiss albino mice. The dorsal side of the mice shaved (2cm diameter) 2 days before the application of DMBA and croton oil. The croton oil and DMBA were applied on the shaved area as described in materials and method (section 2.4.5). The animals were treated in the following way:

**Group I**: Animals (n=10) received DMBA + croton oil served as control

**Group II**: Animals (n=10) received DMBA + croton oil + 250µg of the active fraction topically

**Group III**: Animals (n=10) received DMBA + croton oil + 500µg of the active fraction topically

**Group IV**: Animals (n=10) received DMBA + croton oil + 1000µg of the active fraction topically

**Group V**: Animals (n=10) received DMBA + croton oil + 100µg of curcumin topically and served as standard
6.3 Results

6.3.1 Antioxidant and anti-inflammatory activity of the methanolic extract of *E. sonchifolia*

Table 6.1 shows the superoxide and hydroxyl radical scavenging activity of the fresh juice and methanolic extract of *E. sonchifolia*. The IC$_{50}$ value of fresh juice was 300µg/ml in the case of hydroxyl radical generation and 426µg/ml for superoxide radical generation, whereas the IC$_{50}$ of the methanolic extract was 3µg/ml and 26µg/ml respectively. Intraperitoneal administration of the methanolic extract at doses of 250 and 500mg/kg b. wt. was found to inhibit the carrageenan-induced paw oedema (Table 6.2) with efficacy comparable to that of indomethacin (10mg/kg b.wt i.p).

6.3.2 Cytotoxic and antitumor property

In the short term assay the methanolic extract of *E. sonchifolia* was found to be cytotoxic to EAC and DLA cells, whereas human lymphocytes were unaffected (Table 6.3). The concentrations required for 50% activity were 1.5mg/ml and 1mg/ml for DLA and EAC respectively. In long term chemosensitivity assay 15µg/ml of the extract produced 50% death of L929 cells whereas 2µg/ml camptothecin produced the same result (Table 6.3).

Oral administration of methanolic extract (100mg/kg b.wt) significantly increased the life span of the tumor bearing mice when compared to the control group animals (Table 6.4).
In the solid model tumor studies, the oral administration of the extract significantly reduced the solid tumor development. On the 30th day the tumor volume of the control animal (group A) was 4.25 cm³ whereas that of the treated animals group B and C were 1.25 cm³ and 2.5 cm³ respectively (Fig 6.1).

6.3.3 Effect of methanolic extract on DNA synthesis

The Thymidine incorporation assay indicates that the extract inhibited DNA synthesis in a dose dependent manner in DL cells (Fig 6.2).

6.3.4 Antioxidant and anticarcinogenic activity of partially purified extract

The partially purified extract was found to be potent inhibitor of free radicals and DMBA induced carcinogenesis. Of the different fractions tested for its antioxidant property, fraction M-60 (60:40 MeOH: H₂O v/v) was found to be most active in inhibiting the superoxide and hydroxyl radical formation in vitro (Table 6.5). The same fraction (M-60) was also found to be a potent inhibitor of DMBA/croton oil induced skin papilloma.

In this experiment, 100% tumor incidence was observed in control (Group I) animals the onset of papilloma formation started on 4th week onwards and 100 % tumor incidence was noted on 16 week. Where as in the treated group animals (group II and III and IV) the onset of tumor incidence was found to be delayed. The inhibition of tumor incidence was dose dependent. Only 10% inhibition was observed in the lower concentration of the active fraction (250 μg). Where as 35 and 85% tumor
Fig. 6.1 Effect of methanolic extract of *E. sonchifolia* on solid tumor (DLA) in mice (mean ± SD) ◆—◆, control; ▲—▲, drug (100mg/kg, b.wt) was administered daily for 10 days starting from 24 h after tumor challenge. ■—■, drug (100 mg/kg, b.wt.) was administered daily for 10 days starting from the 11th day after tumor challenge. *P < 0.01; ** P < 0.001 (compared with control)
Fig. 6.2 Effect of methanolic extract of *E. sonchifolia* on $^3$H-thymidine incorporation by Daltons lymphoma cells. Cells were incubated for 4 h in the presence of indicated concentrations of the drug. Values are mean ± SD *$P<0.02$; **$P<0.001$ compared with control. (ii) $^3$H-thymidine incorporation is given as % of control.
Fig. 6.3 Inhibitory effect of topically administered M-60 on Croton oil induced tumor promotion.  
- 250µg drug/animal;  
- 500µg drug/animal;  
- 1000 µg/animal;  
- 1000µg curcumin/animal.
inhibition was noted in animals treated with 500 µg and 1000µg of the partially purified fraction respectively, when compared with the control animals. (Fig 6.3).

Phytochemical analysis of active fraction (M-60) showed the presence of flavonoids as the major chemical constituent. TLC plates sprayed with lead acetate showed yellow fluorescent spots under uv. The \( \lambda_{\text{max}} \) obtained at 245 nm.

6.4 Discussion

The present study reveals that fresh juice and methanolic extract of \( E. \) sonchifolia possess antioxidant and anti-inflammatory activity. Our results indicate that both fresh juice and methanol extract of \( E. \) sonchifolia leaves inhibit hydroxyl radical and superoxide radical generation \textit{in vitro}. The inhibition by methanolic extract is comparable to that of curcumin, a well known antioxidant. The role of oxygen-derived free radicals, such as hydroxyl and superoxide radicals in the inflammatory process is well known (216). It is assumed that most of the antioxidants possess anti-inflammatory effect (217).

The methanolic extract of \( E. \) sonchifolia also shows cytotoxic and antitumor properties. Interestingly, it is non-toxic to normal human lymphocytes (NHL). The cytotoxicity is more pronounced in long term exposure of fibroblast (L929) to the extract. Moreover, oral administration of this extract significantly (\( P<0.001 \)) increases the life span of EAC tumor bearing mice, and decreases the rate of solid tumor development
(P<0.001). Thymidine incorporation assay reveals that the drug inhibits DNA synthesis in a concentration dependent manner.

Carcinogenesis involves 3 distinct stages, initiation, promotion and progression (218). The mouse skin multistage model is an ideal system to study the carcinogenic and anticarcinogenic properties of the drug. In the present study the active fraction of the extract inhibit the free radical formation in vitro and significantly reduce the incidence of papilloma formation in mice in a dose dependent manner. Phytochemical analysis of the partially purified fraction shows the presence of flavanoids as the predominant compounds. Presence of these compounds may contribute to these properties.
Table 7.1 Effect of *E. sonchifolia* extract on in vitro free radical (superoxide, hydroxyl radical) generation

<table>
<thead>
<tr>
<th>Tested material</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Superoxide</td>
<td>Hydroxyl radical</td>
<td></td>
</tr>
<tr>
<td>Fresh juice</td>
<td>426.86</td>
<td>282.92</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>3.08</td>
<td>28.21</td>
<td></td>
</tr>
<tr>
<td>Curcumin</td>
<td>6.14</td>
<td>2.69</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.2 Effect of the methanolic extract of *E. sonchifolia* leaves on carageenan induced mice paw oedema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg.ip)</th>
<th>Inhibition (%) at time h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+1</td>
</tr>
<tr>
<td><em>E. sonchifolia</em></td>
<td>250</td>
<td>50.45±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.47±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>63.34±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± SD n=6. *P<0.02; bP<0.01; cP<0.001 vs. control, students-t test
Table 6.3 *In vitro* cytotoxicity of the methanol extract of *Emilia sonchifolia* to various cell lines

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Concentration required for 50% cytotoxicity mg/ml</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>Methanol extract</td>
<td>Camptothecin</td>
<td></td>
</tr>
<tr>
<td>DLA</td>
<td>1.5</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>EAC</td>
<td>1.1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>NT</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>L-929</td>
<td>0.015</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

NHL: Normal human lymphocytes  
NT: Non-toxic  
ND: Not determined  
DLA: Dalton’s lymphoma ascites  
EAC: Ehrlich Ascites Carcinoma

Table 6.4 Effect of methanolic extract of *E. sonchifolia* (100mg/ kg b.wt) on Ehrlich ascites tumor in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean survival time</th>
<th>Increase in life span %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19.66 ± 0.94</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>67.16 ± 4.5</td>
<td>241*</td>
</tr>
<tr>
<td>C</td>
<td>45.00 ± 5.0</td>
<td>125*</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six animals in each group  
*P < 0.001; (A) control; (B) simultaneous treatment; (C) drug treatment started ten days after tumor challenge (details are given in materials and methods)
Table 6.5 Effect of *E. sorchifoila* active fraction on *in vitro* free radical (superoxide, hydroxyl) generation

<table>
<thead>
<tr>
<th>Fractions</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; µg/ml</th>
<th>Superoxide</th>
<th>Hydroxyl radical</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-80</td>
<td>6.0</td>
<td></td>
<td>10.0</td>
</tr>
<tr>
<td>M-60</td>
<td>0.5</td>
<td></td>
<td>7.0</td>
</tr>
<tr>
<td>M-40</td>
<td>8.0</td>
<td></td>
<td>17.0</td>
</tr>
<tr>
<td>M-20</td>
<td>No activity</td>
<td></td>
<td>No activity</td>
</tr>
<tr>
<td>M-10</td>
<td>No activity</td>
<td></td>
<td>No activity</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>No activity</td>
<td></td>
<td>No activity</td>
</tr>
</tbody>
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