**REVIEW OF LITERATURE**

Chemotherapy is an effective treatment against cancer either singly or in combination with surgery and/or radiotherapy. In chemotherapy, drugs *viz.* cisplatin, carboplatin, cyclophosphamide, doxorubicin, melphalan, mitomycin-c, gemcitabine etc. have been used for the treatment of cancer (Black & Livingston, 1990). However, 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 & Livingston, 1990; Kartalou & Essigmann, 2001). In an attempt to abate these side effects and as a better remedy against various malignancies, many plant derivatives have been used with varying degrees of success (Raja and Rao, 2000). Higher plants, a source of medicinal compounds, play a dominant role in health care of human beings (Huang *et al.*, 1992). More than 50% of modern drugs in clinical use are of natural origin (Huang *et al.*, 1992; Raja and Rao, 2000). Many natural products have also been recognized to have the ability to induce apoptosis in various tumor cells of human origin, (Taraphdar *et al.*, 2001). Drugs made from or designed from natural products are important sources for new anticancer pharmaceuticals (Cragg *et al.*, 1997). The Vinca alkaloids Vinblastine and Vincristine are derived from *Catharanthus roseus* and paclitaxel (Taxol™) was originally isolated from *Taxus brevifolia*. The efficacy of these agents led to the development of second generation drugs, including vinorelbine and docetaxel. Etoposide and teniposide are analogues of podophyllotoxin, an antitumour compound from *Podophyllum peltatum*. China’s “Tree of Joy” *Camptotheca acuminata*, is the source of camptothecan, the precursor of the clinical cancer drugs topotecan and irinotecan (Chin *et al.*, 1999).
2.1 Cytotoxic screening models

Cytotoxic screening models provide important preliminary data to select plant extracts with potential antineoplastic properties (Cardellina et al.; 1999). As part of a permanent screening programme, which considers the search for plants and natural products with anticancer properties, the plants are subjected to bioscreening assay testing for cytotoxicity. The cell viability and cellular proliferation can be detected by two methods (1) DNA synthesis (2) measurement of metabolic activity.

Common methods for determining cell viability depend upon

(1) Membrane integrity (Jackway and Shevach, 1984; Jackway, 1986; Hartwig, 1986). This is a direct unequivocal measure of the number of viable cells remaining at the end of an assay but it is not practical for large number of samples because it is time consuming and tedious. Example is trypan blue exclusion method.

(2) Incorporation of nucleotides (Trentymen et al.; 1984). Make use of incorporation of 3H thymidine, or BrdU. The most obvious disadvantage of the assay is the use of radioactive isotopes.

(3) Clonogenic assays (Selby et al.; 1983). The assays of chemosensitivity have been used extensively for both established tumour cell lines and freshly biopsied tumour tissue. Low plating efficiencies, clumping artifacts, and long assay duration are technical problems that limit the wide spread applicability of clonogenic assays on human tumor material.

(4) Tetrazolium assays

Due to the practical limitation of the above methods, there was a need to develop a colourimetric assay, which would have an upper hand over the earlier assay methods. In one of the earliest efforts to develop a practical in vitro drug sensitivity assay, Black and Speer (1953) utilized
tetrazolium/formazan methods to assess inhibition of succinate dehydrogenase activity by cancer chemotherapeutic drugs in slices of excised tissue. As an in situ vital staining process, this phenomenon has also been used for identifying viable colonies of mammalian cells in soft agar culture (Schaeffer and Friend, 1976) and for facilitating in vitro drug sensitivity assays with human tumour cell populations in primary culture (Alley and Lieber, 1984). Since then, tetrazolium reagents have become popular as convenient non-radioactive alternatives for determining the number of viable cells in proliferation and cytotoxicity assays. Moreover, tetrazolium assays can be semi automated with the use of 96 well microtitre plates to provide for easy and rapid analysis of large number of samples (Finlay et al.; 1984). MTA using MTTC (3- (4, 5- dimethyl thiazol – 2 – yl)-2, 5 – diphenyl tetrazolium bromide) depends upon the reduction of tetrazolium salt to an insoluble coloured formazan dye by mitochondrial succinate dehydrogenase, the intensity of which can be quantified spectrophotometrically. Comparison of these values to those of an untreated control provides a relative increase in cellular proliferative activity. Conversely in cells, those undergoing apoptosis; MTT reduction decreases, reflecting the loss of cell viability (Mosmann, 1985). Table 2.1 gives studies on cytotoxic and antiproliferative activities of certain medicinal plants.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Plant/Compound</th>
<th>Cell line used for screening</th>
<th>Method of screening</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous extract of <em>Bacopa monnieri</em></td>
<td>Sarcoma – 180</td>
<td>Tritiated thymidine in corporation</td>
<td>Elangovan et al.; 1995</td>
</tr>
<tr>
<td>3</td>
<td>Methanolic extract of <em>Centella asiatica</em></td>
<td>Murine cell lines</td>
<td>Trypan blue exclusion</td>
<td>Babu et al.; 1995</td>
</tr>
<tr>
<td>4</td>
<td>Amaryllidaceae alkaloids</td>
<td>Molt-4, HepG-2</td>
<td>MTT assay</td>
<td>Weniger et al.; 1995</td>
</tr>
<tr>
<td>5</td>
<td>Uvaol from <em>Calluna vulgaris</em></td>
<td>HGT, MCF-7</td>
<td>MTT assay</td>
<td>Es et al.; 1995</td>
</tr>
<tr>
<td>6</td>
<td>Ether extract of <em>Rhaphidophora korthalsii</em></td>
<td>P. 388, Molt 4, KB, SW 620</td>
<td>MTT assay</td>
<td>Wong and Tan, 1996</td>
</tr>
<tr>
<td>7</td>
<td>Methylene chloride fraction of <em>Picrolemma huberi</em></td>
<td>MDA – MB 231, PC3, KB</td>
<td>MTT assay</td>
<td>Rodrigues et al.; 1997</td>
</tr>
<tr>
<td>8</td>
<td>Tanshinones from <em>Salvia miltiorrhiza</em></td>
<td>A 549, SK-OV-3, SK – MEL-2, XF 498m HCT-15</td>
<td>SRB assay</td>
<td>Shi et al.; 1997</td>
</tr>
<tr>
<td>10</td>
<td>Sterol from <em>Turbinaria ornata</em></td>
<td>KB, P-388, A 549, HT-29</td>
<td>MTT assay</td>
<td>Jyh et al.; 1997</td>
</tr>
<tr>
<td>11</td>
<td>Artemesinin from <em>Artemesia annua</em> L</td>
<td>EAC (murine)</td>
<td>MTT assay</td>
<td>Aaron et al.; 1998</td>
</tr>
<tr>
<td>12</td>
<td>Diterpenes from <em>Citrus reticulatus</em></td>
<td>Human leukemic cell lines</td>
<td>Trypan blue exclusion</td>
<td>Kostas Dimas et al.; 1998</td>
</tr>
<tr>
<td>13</td>
<td>Boswellic acid from <em>Boswellia serrata</em></td>
<td>HL-60</td>
<td>Trypan blue exclusion</td>
<td>Yu et al.; 1998</td>
</tr>
<tr>
<td>No.</td>
<td>Compound/Extract</td>
<td>Source Cells/Methods</td>
<td>Assay(s)</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>-----</td>
<td>------------------------------------------</td>
<td>----------------------</td>
<td>------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>14</td>
<td>Alkaloids from <em>Brunvigia littoralis</em></td>
<td>BL-6 mouse melanoma</td>
<td>MTT assay</td>
<td>William et al.; 1998</td>
</tr>
<tr>
<td>15</td>
<td>Pennogenin glycoside from <em>Majanthemum dilatatum</em></td>
<td>Cultured human cells lines</td>
<td>MTT assay</td>
<td>Eun-Rhan Woo et al.; 1998</td>
</tr>
<tr>
<td>16</td>
<td>Corosolic acid from <em>Crataegus pinnatifolia</em></td>
<td>Cultured human cancer cell lines</td>
<td>SRB assay</td>
<td>Kyung-Seop Ahn et al.; 1998</td>
</tr>
<tr>
<td>17</td>
<td>Iscador an aqueous extract of <em>Viscum album</em></td>
<td>DLA and EAC cell lines</td>
<td>Trypan blue exclusion</td>
<td>Girja et al.; 1999</td>
</tr>
<tr>
<td>18</td>
<td>Essential oils and leaf extracts of <em>Cochlospermum tinctorium</em> and <em>C. planchonii</em></td>
<td>K 562</td>
<td>Tritiated thymidine incorporation assay</td>
<td>Francoise Benoit – Vical, 1999</td>
</tr>
<tr>
<td>19</td>
<td>Hemistepsins from <em>Hemisteptia lyrata</em></td>
<td>Cultured human cell lines</td>
<td>SRB assay</td>
<td>Dae Sik Jang et al.; 1999</td>
</tr>
<tr>
<td>19</td>
<td>Components from bark of <em>Kalopanax pictus</em></td>
<td>Murine and human cancer cell lines</td>
<td>MTT assay</td>
<td>Kyung-Tae Lee et al.; 1999</td>
</tr>
<tr>
<td>21</td>
<td>Constituents of <em>Corydalis tashiroi</em></td>
<td>Human cancer cells</td>
<td>MTT assay</td>
<td>Jih-Jung Chen et al.; 1999</td>
</tr>
<tr>
<td>22</td>
<td>Aporphine alkaloids from <em>Magnolia obovata</em></td>
<td>Human cancer cell lines</td>
<td>SRB assay</td>
<td>Huang Kyoon and Shi Yong, 1999</td>
</tr>
<tr>
<td>23</td>
<td>Natural and synthetic ginseng glycosides</td>
<td>Human cancer cell lines</td>
<td>MTT assay</td>
<td>Lyubov et al.; 1999</td>
</tr>
<tr>
<td>24</td>
<td>Parthenolide from <em>Tanacetum parthenium</em></td>
<td>Panel of human cancer cell lines</td>
<td>Clonogenic and Br du incorporation</td>
<td>Jonathan et al.; 1999</td>
</tr>
<tr>
<td>25</td>
<td>Indole alkaloids from <em>Alstonia macrophylla</em></td>
<td>Panel of human ling cancer cell line</td>
<td>SRB assay</td>
<td>Niwat et al.; 1999</td>
</tr>
<tr>
<td>26</td>
<td><em>Cinnamomum cassia</em> extract</td>
<td>Panel of human cancer cell lines</td>
<td>SRB assay</td>
<td>Chang et al.; 1999</td>
</tr>
<tr>
<td>27</td>
<td><em>Croton cajucara</em> diterpenes</td>
<td>Murine cancer cell lines</td>
<td>MTT assay</td>
<td>Noema et al.; 1999</td>
</tr>
<tr>
<td></td>
<td>Compound</td>
<td>Test System</td>
<td>Assay</td>
<td>Reference</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------</td>
<td>------------------------------------</td>
<td>----------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>28</td>
<td>Neolignans from <em>Machilus obovatifolia</em></td>
<td>Panel of human cancer cell lines</td>
<td>MTT assay</td>
<td>Ian Lih et al.; 2000</td>
</tr>
<tr>
<td>29</td>
<td>Triterpenes from <em>Physocarpus intermedius</em></td>
<td>Panel of cultured tumour cells</td>
<td>SRB assay</td>
<td>Young et al.; 2000</td>
</tr>
<tr>
<td>30</td>
<td>Methanolic extract of <em>Emilia sonchifolia</em></td>
<td>DLA, EAC, L-929</td>
<td>Trypan blue exclusion</td>
<td>Shylesh and Padikkala, 2000</td>
</tr>
<tr>
<td>31</td>
<td>Kiwifruit extracts</td>
<td>HSC-2, HGF</td>
<td>MTT assay</td>
<td>Noboru et al.; 2002</td>
</tr>
<tr>
<td>32</td>
<td><em>Tabernaemontana calcarea</em> extracts</td>
<td>Panel of cultured cancer cell lines</td>
<td>MTT assay</td>
<td>Chaturvedula et al.; 2003</td>
</tr>
<tr>
<td>33</td>
<td>Draconins from <em>Dracaena draco</em></td>
<td>HL-60</td>
<td>MTT-assay</td>
<td>Gonzalez et al.; 2003</td>
</tr>
<tr>
<td>34</td>
<td>Members of <em>Zingiberaceae</em></td>
<td>Different cancer cell lines</td>
<td>MTT assay</td>
<td>Kirana et al.; 2003</td>
</tr>
<tr>
<td>35</td>
<td>Bochmeriasin A</td>
<td>A panel of different human cancer cell lines</td>
<td>MTT assay</td>
<td>Luo et al.; 2003</td>
</tr>
<tr>
<td>36</td>
<td>Betulinic acid from <em>Shefflera rotundifolia</em></td>
<td>Murine and human cell lines</td>
<td>MTT assay</td>
<td>Braca et al.; 2004</td>
</tr>
<tr>
<td>37</td>
<td>Cytotoxic compounds from <em>Boenninghausinia japonica</em></td>
<td>HeLa, Mk-1 B/6 F10 (Murine melanoma)</td>
<td>MTT assay</td>
<td>Chaya et al.; 2004</td>
</tr>
<tr>
<td>38</td>
<td>Methylene chloride fraction of <em>Spatholobus suberectus</em></td>
<td>U 937</td>
<td>MTT assay</td>
<td>Ha et al.; 2004</td>
</tr>
<tr>
<td>39</td>
<td>Ethanol extract of turmeric</td>
<td>Fibrosarcoma</td>
<td>Tritiated thymidine incorporation</td>
<td>Chakravarty et al.; 2004</td>
</tr>
<tr>
<td>40</td>
<td>Ethanol extract of <em>Sutherlandia frutescens</em></td>
<td>Human cancer cell lines</td>
<td>Trypan blue exclusion</td>
<td>Joseph et al.; 2004</td>
</tr>
<tr>
<td>41</td>
<td>Flavonoids from <em>Scutellaria sp</em></td>
<td>Human leukemia cell lines</td>
<td>WST 8 assay</td>
<td>Maki et al.; 2004</td>
</tr>
<tr>
<td>42</td>
<td>Thai medicinal plants</td>
<td>Human cancer cell lines</td>
<td>SRB assay</td>
<td>Arunpom et al.; 2004</td>
</tr>
<tr>
<td>43</td>
<td><em>Rotula aquatica</em> extract</td>
<td>Cultured human cancer cell lines</td>
<td>MTT assay</td>
<td>Patil et al., 2004</td>
</tr>
<tr>
<td>44</td>
<td>Phytoestrogen rich Thai medicinal plants</td>
<td>MCF 7</td>
<td>Protein quantification</td>
<td>Cherdshewasart et al.; 2004</td>
</tr>
<tr>
<td>45</td>
<td>Andrographolides from <em>Andrographis paniculata</em></td>
<td>Cultured human cancer cell lines</td>
<td>SRB assay</td>
<td>Ajayakumar et al., 2004</td>
</tr>
<tr>
<td>46</td>
<td>Organic extracts of <em>Platycodon grandiflorum</em></td>
<td>Cultured cancer cell lines</td>
<td>MTT assay</td>
<td>Ji-Young et al.; 2004</td>
</tr>
<tr>
<td>47</td>
<td>Piperine from <em>Piper longum</em></td>
<td>SNU 4</td>
<td>MTT assay</td>
<td>Sunila and Kuttan, 2004</td>
</tr>
<tr>
<td>48</td>
<td>Methanolic extract of <em>Garcinia mangostana</em></td>
<td>SKBR 3</td>
<td>MTT assay</td>
<td>Prim Chanien et al.; 2004</td>
</tr>
<tr>
<td>49</td>
<td>Cranberry extracts</td>
<td>Colon cancer and oral cancer cell line</td>
<td>MTT assay</td>
<td>Sreram et al.; 2004</td>
</tr>
<tr>
<td>50</td>
<td>polyphenols from <em>Acasia nilotica</em></td>
<td>human cancer cell lines</td>
<td>ATP determination</td>
<td>Kamaljit et al.; 2005</td>
</tr>
<tr>
<td>51</td>
<td>TRIPHALA</td>
<td>Cultured human cancer cell lines</td>
<td>Tritiated thymidine incorporation</td>
<td>Swayamjot et al.; 2005</td>
</tr>
<tr>
<td>52</td>
<td><em>Oroxylum indicum</em> extract</td>
<td>Cultured human cancer cell lines</td>
<td>MTT assay</td>
<td>Leticia et al.; 2005</td>
</tr>
<tr>
<td>53</td>
<td>Extract of <em>Citrus reticulate viride</em> pericarp</td>
<td>SNU 4</td>
<td>MTT assay</td>
<td>Soon et al.; 2005</td>
</tr>
<tr>
<td>54</td>
<td>Diaryl heptanoids and gingerol from rhizome of Chinese Ginger</td>
<td>HL 60</td>
<td>MTT assay</td>
<td>Qing-Yi et al.; 2005</td>
</tr>
<tr>
<td>55</td>
<td><em>Antrodia camphorata</em> fruiting bodies</td>
<td>HepG2, PLC/PRF/5</td>
<td>MTT assay</td>
<td>Ya-Ling et al.; 2005</td>
</tr>
<tr>
<td>56</td>
<td>Methanolic extract of <em>Pereskia bleo</em></td>
<td>T 47D (breast carcinoma cell line)</td>
<td>MTT assay</td>
<td>Tan et al.; 2005</td>
</tr>
<tr>
<td>57</td>
<td>Tea root extract</td>
<td>Human cell lines</td>
<td>MTT assay</td>
<td>Papiya et al.; 2006</td>
</tr>
<tr>
<td>58</td>
<td>Caulespia racemosa</td>
<td>SHSY5Y Kelly</td>
<td>MTT assay</td>
<td>Levent et al.; 2006</td>
</tr>
<tr>
<td>59</td>
<td>Isocostunolide from <em>Inula helenium</em> roots</td>
<td>A2058, HT 29, HepG2</td>
<td>MTT assay</td>
<td>Chia-Nan et al.; 2007</td>
</tr>
</tbody>
</table>
2.2. Tumour models for testing antitumour drugs

Spontaneous and artificially produced phenotype diseases have been described in a wide range of animal species with differing potentials for modeling human diseases. Mammals would be expected to provide better disease models, but for a variety of reasons, our closest relatives, the great apes have not been very useful in providing disease models. Instead, other mammals, notably mice have been used widely as models for human diseases.

Mice are small, and can be maintained in breeding colonies comparatively cheap. They have a short life span (~2-3 years), a short generation time (~3 months) and are prolific. Because of these reasons they are preferred to other mammals as models against human diseases.

The evaluation of cancer cytotoxic agents necessitates screening of a great number of chemicals. Animal models especially murine cancer models have played an important role in drug evaluation. The use of murine leukemia in drug screening had its beginning in the 19th century (Griswold and Harrison, 1991). Although spontaneous tumours in animals were used as models, it was the ability to transplant tumours that made development possible to tumour systems, which could be used both for large scale screening and detailed evaluation of anticancer agents. Further more, the development in the 1920’s of inbred strains of mice allowed the successful transplantation of numerous tumour systems (Zubrod, 1979). Soon anticancer drug screening programmes were initiated, one of which was the Memorial – Sloan – Kettering programme that used the mouse sarcoma 180 (SA-180) as its screening models. Since additional drugs exhibited some anticancer activity and the supply of new agents exceeded the screening capacity, a national drug development programme was started by NCI (National Cancer Institute) as directed by the US Congress. In 1955, the Cancer Chemotherapy National Service Centre (CCNSC) was
created. The initial CCNSC screen consisted of three mouse tumours L1210 leukemia, SA-180, and mammary adenocarcinoma 755 (Goldin et al.; 1966). Over the years, the primary screen changed from the original three tumours to L1210 plus two arbitrarily selected tumours to L1210 plus Walker 256 carcinosarcoma to L1210 plus p388 leukemia to L1210 plus p385 B16 melanoma or Lewis lung tumor (Carter, 1974). In 1976, a major change occurred in the NCI primary screening. The new screening consists of a panel of colon, breast, and lung tumour models (murine and human); however, drugs were initially screened in p388 leukemia. The low number of drugs with marked activity against human solid tumours led to a radical change in the screening programme that had used murine leukemia models. In the mid-1980’s NCI developed a new screening based on the use of established human tumour cell lines in vitro (Alley et al.; 1988). These two screening were to be conducted in parallel.

2.3 Primary screening of plant compounds for anticancer activity in mice tumour models

William and Maria (1964) observed that Colchicine, vinblastine and vincristine inhibits phenol extractable RNA production in the EAC ascitic tumour bearing mice, which is a strong evidence of reduction in tumour proliferation of ascitic tumours in mice. Funoran is an algal polysaccharide from Gloiopeltis tenax, which significantly inhibits the growth of EAC ascitic tumour and solid tumours, Meth A fibrosarcoma and sarcoma-180 tumours (Ren et al.; 1995). Babu et al. (1995) reported that oral administration of Centella asiatica extracts retards DLA and EAC solid and ascites tumours and increases life span of tumour bearing mice. Yasuyuki et al (1995) reported that antitumour effect of adriamycin increases 2.5 times by a combination chemotherapy involving caffeine in EAC cells. He also reported that thianine a component of green tea leaves increases 2.1 times the antitumour potential of adriamycin in EAC tumour cells
Sobatum is the active ingredient from *Solanum trilobatum*, which significantly reduces peritoneal tumours induced by DLA and EAC tumour cell lines (Mohanan and Devi, 1996). Further, they also reported that sobatum reduces solid tumour growth induced by DLA and EAC tumour cells (Mohanan and Devi, 1997). Many of the medicinal plants from Northeast of Brazil shows tumour reducing property in animal models with tumours induced by Walker carcinosarcoma 256, Yoshida sarcoma and EAC (Moraes et al.; 1997). *Ixora coccinea* flowers have been found to inhibit ascitic tumours produced by DLA and EAC cell lines and increased the life span of tumour bearing animals (Latha and Panikkar, 1998).

*Lindera megaphylla* roots yielded a compound namely d-dicentrine, which was found to be inhibitory to tumour incidence of leukemia cell line K562 in SCID mice (severe combined immunodeficiency) (Huang et al.; 1998). Echitamine chloride an indole alkaloid extracted from the bark of *Alstonia boonei* Willd showed highly promising anticancer activity against fibrosarcoma developed by S-180 cell line (Saraswathy et al.; 1998). Methanolic extract of *Emelia sonchifolia* is able to reduce EAC ascitic and solid tumours and increased the life span of these tumour-bearing mice (Shylesh and Padikkala, 1999). Green tea flavonoids have a synergistic effect on doxorubicin induced antitumour activity on EAC tumour bearing mice (Yasuyuki et al.; 2000). Methanolic extract of *Cassia fistula* seed has been remarkable in reducing EAC ascitic tumours and increasing lifespan of the animals bearing the tumour, in addition to the normalization of haematological parameters (Gupta et al.; 2000). *Picrorhiza kurroa* extract has been able to reduce DLA ascites tumour and solid tumour development in mice (Joy et al.; 2000). It is also interesting to note that the ethanolic extract of chicory is found to be having tumour reducing properties in EAC tumour models (Hazra et al.; 2002). Similarly, *Barringtonia racemosa* Roxb seed extract has tumour-reducing properties in DLA solid and ascites tumour bearing mice (Jose et al.; 2002). Another
interesting study was that ripe banana diet has tumour-reducing property on EAC induced malignant ascites in mice (Guha et al.; 2003). Ramnath et al (2002) reported that Abrin a galactose specific lectin from *Abras precatorius* is effective in reducing solid tumour mass and increases lifespan of DLA and EAC tumour bearing mice. TLC purified petroleum ether extract of *Aerva lanata* has tumour-reducing effect in DLA solid tumours in mice (Nevin and Vijayamma, 2003). Ethyl acetate and methanol extracts of *Phellinus rimosus* show *in vitro* cytotoxicity against DLA and EAC cell lines. Ethyl acetate, methanol and aqueous extracts of the plant are highly effective in reducing solid tumour induced by DLA cell line in mice and ethyl acetate fraction prevented EAC induced ascites tumour development (Ajith and Janardhanan, 2003). Avermectins are a group of flavonoids, which possesses a pronounced antitumour action against ascitic tumour developed by EAC, p388 lympholeukaemia and solid EAC and 755 carcinoma in mice (Victor et al.; 2004). The ethanolic extract of *Withania somnifera* has antitumour effect against ascites tumour induced by DLA (Christina et al.; 2004). Rajkapoer et al (2004) found that oral administration of *Indigofera aspalathoides* increases the survival time and normal peritoneal cell count in EAC tumour bearing animals. Methanolic extract of *Bauhinia racemosa* is found to reduce solid tumour volume in EAC tumour bearing animals. It also increases the life span and corrects the haematological parameters and hepatic antioxidant enzymes (Gupta et al.; 2004). Soamsam an herbal remedy of traditional Korean medicine possesses antitumour activity in mouse colon adenocarcinoma cell line CT-26 in murine model (Sung et al.; 2004). Similarly Sunila and Kuttan (2004) reported that alcoholic extract of *Piper longum* and piperine can inhibit DLA solid tumours in mice. Similarly, aqueous extract of *Curcuma zedoaria* Rosae has been found to possess ability to reduce metastasis of B16 melanoma C57 B2/6 mice (Woon-Gyo et al.; 2005). Somepalli et al.;
(2005) reported that curcumin analogues have promising tumour-reducing activity on Dalton’s lymphoma ascites tumour.

2.4 Oxidants, anti-oxidants and carcinogenesis

Chemical compounds capable of generating potential toxic oxygen species or free radicals are referred to as pro-oxidants. On the other hand compounds and reactions disposing off these species, scavenging, suppressing their formation or opposing their action are called antioxidants. In normal cells, there is an appropriate pro-oxidant; anti-oxidant balance. Antioxidants act as disease preventing agents because any imbalance between reactive oxygen species and activity of antioxidant defense results in oxidative stress.

Free radical is any species capable of independent existence that contains one or more unpaired electrons. Free radicals have very short half life and high reactivity and damaging activity towards macromolecules like proteins, DNA, and lipids. These species may be either oxygen derived or nitrogen derived.

Reactive oxygen species (ROS), which include superoxide anions ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ('OH) are highly toxic to cells. Generation of ROS overtakes the anti-oxidant defense of the cells, oxidative damage of the cellular macromolecules (lipids, proteins and DNA) occurs leading finally to various pathological conditions. The degenerative diseases induced by ROS include hypercholesterolemia, atherosclerosis, carcinogenesis, cataractogenesis, insulin dependant diabetic mellitus and ischemic reperfusion cardiac injury.

ROS are controlled at physiological concentrations by cellular anti-oxidant defenses such as superoxide dismutase (SOD), catalase, glutathione reductase (GR), reduced glutathione (GSH), etc. These
defense exist to intercept or scavenge free radicals. Certain other natural defense systems exist in the cell.

### 2.4.1 Oxidants

1) **Superoxide**

Superoxide anion is the first reduction product of oxygen. In aqueous solution, at neutral or slightly acidic pH superoxide is a relatively non-reactive species and dismutates to $\text{H}_2\text{O}_2$. This reaction either occurs spontaneously or is catalyzed by intracellular enzyme SOD.

It has been proposed that $\text{O}_2^*$ owing to its unreactivity can diffuse through a long way from its site. At low pH in the cell, it becomes protonated ($\text{HO}_2^*$) and, hence reactive. The most important source of superoxide is oxidative enzymes, among which xanthine oxidase and NADPH /NADH oxidase is the most effective sources. Superoxide anion itself directly affects the activity of catalase and peroxidase. It directly affects some intracellular enzymes and changes their activities. It is also involved in the oxidation of epinephrine. It may also capable of initiating the peroxidation of unsaturated lipids. Photochemical or enzymatic generation of superoxide anion results in the chromosome breakage, rearrangement, and sister chromatid exchange. Thus, superoxide anion may be one of the possible factors for increased risk of carcinogenesis.

2) **Hydrogen peroxide**

It is the most stable ROM. This is to say that it is the least reactive and the most readily detected. Hydrogen peroxide may be generated directly by divalent reduction of oxygen or indirectly by univalent reduction of superoxide. Experiment with antioxidant enzymes show that $\text{H}_2\text{O}_2$ rather than superoxide is the more essential species to induce cell injury (Rao et al., 1996).
Fanton Reaction
\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \text{OH} \]

Haber –Weiss reaction
\[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \cdot \text{OH}^- + \text{OH} \]

Hydrogen peroxide stimulated the proliferation of smooth muscle cells. It is involved in the initiation and promotion of carcinogenesis. \( \text{H}_2\text{O}_2 \) induces DNA breaks in intact cells and in purified DNA.

3) Hydroxyl radical (\( \cdot \text{OH} \))

It is highly reactive. It is short lived (Ray and Hussain, 2002). Due to its short life span it reacts with an organic substrate at the sites or near the sites of its formation. Its life span at 37\(^\circ\) C is \( 10^{-9} \) sec. Due to its short lifetime it is very difficult to investigate the \( \cdot \text{OH} \) by conventional methods. As a result of interaction of hydroxyl radical with DNA, formation of many types of oxidized nucleosides has been reported. Hydroxyl radical is known to induce conformational changes in DNA including strand breaks, base modifications, damage to tumor suppressor gene and enhanced expression of protooncogenes. Hydroxyl radical is responsible for DNA damage, high frequency of sister chromatid exchange, (SCEs) and lipid peroxidation (LPO).

4) Malondialdehyde

Malondialdehyde is the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (Vaca et al.; 1998). MDA, a secondary product for LPO, is used as an indicator of tissue damage by series of chain reactions.

5) Nitric oxide

Nitric oxide is an inorganic free radical gas; containing odd number of electrons and can form a covalent bond with other molecules by sharing
a pair of electrons. It is synthesized by a family of isoenzymes called nitric oxide synthase, located in various tissues, and plays an active role in free radical and tumor biology (Felly, 1998).

These free radical causes oxidative stress in the body. Oxidative stress can be implicated in the pathology of many disease conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing. (Marx, 1987)

2.4.2 Enzymatic antioxidants

The first line defense against superoxide radical and peroxide radical mediated injury is antioxidant enzymes like superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase. SOD, CAT, GSH etc are antioxidant enzymes that protect the cells from oxygen and is essential for aerobic life process. However, cells under aerobic conditions are threatened with the insult of ROMs that are effectively taken care of by the powerful antioxidant system in the human body. Aerobic life is characterized as continuous production of oxidants balanced by equivalent synthesis of antioxidants (Rice and Diplock, 1993). Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals inhibiting lipid peroxidation and many other mechanisms and thus prevent diseases (Youdin and Joseph, 2001).

1) Superoxide dismutase (SOD)

Superoxide dismutase is family of metalloenzymes that convert superoxide radicals to hydrogen peroxide. It is the most important enzyme because it is found in all aerobic organisms. It protects the cells from damage caused by superoxide radical. SOD acts as an anti carcinogen, and inhibits the initiation and promotion/ transformation stages in carcinogenesis. SOD activity increased significantly in Hodgkin’s disease, leukemia and in breast cancer SOD is the most important enzyme because
it is found virtually in all aerobic organisms. SOD is considered to be a stress protein, which is synthesized in response to oxidative stress (Mc Cord; 1993).

During oxidative stress, cell responds to ROMs with SOD. Accumulation of superoxide anions and peroxide radical results in the production of hydroxyl radical by a metal catalysed reaction. SOD is reported to inhibit hydroxyl radical production (Fridovich, 1995).

2) Catalase

Catalase is an enzyme, which is present in most cells, and catalyses the decomposition of $H_2O_2$ to water and oxygen. Catalase is a haeme containing protein. The mechanism of action is,

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

CAT is found to reduce SCE levels resulting from treatment with $H_2O_2$. Both GPx and CAT inactivate the environmental mutagens. In breast cancer, plasma catalase activity decreases because of higher ROMs production especially superoxide radical.

3) Glutathione peroxidase

Glutathione peroxidase is a well-known first line of defense against oxidative stress, which in turn requires glutathione as a co-factor. GPx catalyses the oxidation of GSH to GSSG at the expense of $H_2O_2$.

2.4.3 Antioxidant vitamins

Antioxidant vitamins have a number of biological activities such as immune stimulation, inhibition of nitrosamine formation and alteration of metabolic activation of carcinogenesis. Vitamin A, Vitamin E and Vitamin C have antioxidant activity.
2.4.4 Oxidants and carcinogenesis

DNA damage leads to cancer. Any agent that is capable of reacting with DNA and chemically modifying it could be carcinogenic. Exposure of organisms to ionizing radiations leads to the development of cancer. It appears that radiation induced carcinogenesis involves both initiation and promotion.

Compounds with antioxidant property can inhibit tumour initiation, promotion and progression. Reactive Oxygen Species involves in the initiation and promotion of multistage carcinogenesis. The ROS can act as initiator and/ or promoter of multi stage carcinogenesis. The ROS acting as initiator and/ or promoter cause DNA damage, activate carcinogens, alter the cellular antioxidant defense system and phase two metabolizing enzymes. Hydroxyl radical, attack upon DNA and generates a whole series of modified purine and pyrimidine bases. Attack of OH upon deoxyribose yields a multiplicity of products. So Reactive Oxygen Metabolites (ROMs) such as superoxide anion, hydrogen peroxide, hydroxyl radical, malondialdehyde and nitric oxide are directly or indirectly involved in the multistage process of carcinogenesis.

Photochemical or enzymatic generation of superoxide anion resulted in an increase in chromosome breakage, rearrangement and sister chromatid changes (SCEs). So this anion may be one of the possible factors for increased risk of carcinogenesis. Recent studies found higher generation of superoxide radical in breast cancer patients (Ray and Hussain, 2002).

Certain estrogen metabolites such as catacholestrogens are involved in carcinogenesis, where hydrogen peroxide plays an important role. The formation of catacholestrogens induces DNA strand breaks has been associated with the utilization of superoxide anion and the generation
of hydrogen peroxide. Hydrogen peroxide is believed to be involved in initiation, and promotion of carcinogenesis (Pryor, 1986).

Lipid is very susceptible to hydroxyl radical attack and initiate lipid peroxidation. Hydroxyl radical is the most reactive among ROMs, reacting with a wide range of macromolecules at a high rate constant. This radical known to induce conformational changes in DNA including strand break, base modifications, damage to tumour suppressor gene and enhanced expression of proto-oncogenesis (Cerutti et al; 1994). Hydroxyl radical is responsible for DNA damage, high frequency of sister chromatid exchanges and lipid peroxidation (Chessman and Slater, 1993).

Malondialdehyde is a mutagenic and genotoxic agent that may contribute to the development of cancer. Lipid hydroperoxides may directly induce DNA chain breaking and lipid peroxide and alkoxy radical may cause base oxidation in DNA.

Nitric oxide and derivatives produced in inflamed tissues contribute to carcinogenesis. It is believed that nitric oxide plays a dual role in cancer. This radical plays an active role in free radical and tumour biology. Moreover, it may have a role in carcinogenesis by inducing DNA strand breaks (Yoshie and Okuda, 1998).

Epidemiological studies have suggested that high endogenous level of oxidative adducts and deficiencies in antioxidant levels are likely to be important risk factors for cancer. SOD can act as anticarcinogenesis agent and inhibitor at initiation and promotion stages in carcinogenesis. During oxidative stress, cell responds to ROMs with SOD.

Glutathione peroxidase scavenges the hydrogen peroxide. GPx catalyses the oxidation of GSH to GSSG. This oxidation reaction occurs at the expense of \( \text{H}_2\text{O}_2 \). This enzyme and catalase were found to be important in the inactivation of many environmental mutagens. Catalase
also prevents the chromosomal aberration caused by hypoxanthine or xanthine oxidase (Iwata et al.; 1984).

### 2.4.5 Antioxidants from plants

Many plant extracts have antioxidant activities. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases. In this respect flavonoids and other polyphenolic compounds have gained the greatest attention (Sundararajan et al.; 2006). Diterpenoids from *Rosmarinus officinalis* inhibited the superoxide anion production. These phenolic diterpenes were shown to be effective to protect biological systems against stress (Haraguchi et al.; 1995). Products of lipid peroxidation may cause DNA damage. LPO may also play an indirect role in the conversion of procarcinogens (O Brien, 1994). In higher animals lipid peroxidation is known to cause destabilization and disintegration of the cell membrane leading to liver injury, atherosclerosis, and kidney damage and ageing and susceptibility to cancer (Osawa et al.; 1990) Dihydrochalcones from *Myrica gale* inhibited the lipid peroxidation (Mathiesen et al.; 1995).

Diterpenoids from *Podocarpus nagi* is effective to protect biological system and functions against various oxidative stresses (Haraguchi et al.; 1997). Free radical scavengers can protect against the genotoxicity induced by chemical carcinogens by decreasing oxidative damage. In rats treated with \( \text{KB}_2\text{O}_2 \) which is a Kidney specific carcinogen, it is found that treatments with melatonin, resveratrol, butylated hydroxyl toluene, α– phenyl–N-tert-butyl nitrone (PNB) and vitamin E (all known antioxidants), reduce the oxidative damage (Susana and Gustavo, 1999). *Cassia fistula L.* has antioxidant and anticancer activity (Gupta et al.; 2000). Stem bark of *Cassia fistula L.* had more antioxidant activity in terms of reducing power, inhibition of peroxidation and radical scavenging activity as proven *in vitro*
Chapter 2

studies (Sidhuraju et al., 2002). Genistein is a common precursor in the biosynthesis of antimicrobial phytoalexins and phytoanticipins in legumes, and an important neutraceutical molecule found in soybean seeds. Genistein is a phytoestrogen with a wide variety of pharmacological effects in animal cells, including tyrosine kinase inhibition, and a range of potential health beneficial effects, like chemoprevention of breast and prostate cancer (Richard and Ferreira, 2002). Resveratrol an antioxidant from grape has antimitostatic, and antiproliferative effect on B16 melanoma cells (B16M) (Miguel et al.; 2002). Caffeine is the natural ingredient present in coffee, tea, and cocoa. Development of EAC cell decreased the activities of hepatic CAT and SOD and increased lipid peroxidation. But chronic ingestion of caffeine increases the activities of the hepatic enzymes catalase and, SOD and along with decreases lipid peroxidation in mice. So caffeine is an antioxidant and may act as an anti carcinogen (Mukhopadhyay et al.; 2003). Citral, isolated from the methanol extract of lemon grass (Cymbopogon citrates) induced GST in the mouse skin and prevented the mouse skin cancer, showing its antioxidant nature (Yoshimasa et al.; 2003). Zerumbone, a sesquiterpene compound occurring in tropical ginger Zingiber zerumbet Smith induces GST, GPx, and neutralizes the lipid peroxidation in hepatocytes. The chemopreventive action of zerumbone against, skin and colon cancer might be due to this antioxidant activity (Yoshimasa et al.; 2004). Auricularia auricula is an edible mushroom showing significant inhibition of lipid peroxidation and potent hydroxyl radical scavenging activity and it have shown nitric acid synthase activation (Acharya et al., 2004). Curcumin enhance the activity of SOD and CAT and prevent oxidative damage (Padmaja and Raju, 2004).

Acetone extract of butter fruit or Avocado (Persea amaricana) containing carotenoids and tocopherols, which are known dietary antioxidants, inhibits the growth of androgen dependent (LNCaP) and
androgen independent (PC-3) prostate cancer cell lines. In common with other colourful fruits and vegetables, the avocado contains numerous bioactive carotenoids. They may contribute to the significant cancer risk reduction, associated with a diet of fruits and vegetables (Qing-Yi et al.; 2005).

Brahma Rasayana a polyherbal preparation rich in phenolics reduced the palpable tumours, delayed the tumour occurrence and lowered tumour volumes in MAT-LyLu (prostrate cancer) cell line inoculated rats (Thangapazham et al.; 2006). Similarly, melatonin an antioxidant has reported to have antitumour activity in murine lung adenoma model (Vesnushkin et al.; 2006). In addition, ascorbic acid was able to inhibit and redifferentiate cultured malignant human gastric cells (Sun et al., 2006). It is also reported that the intake of fruits, vegetables, vitamins A, C, E, and carotenoids reduce the risk of renal cell cancer in human subjects (Lee et al.; 2006). Phenolic compound rich plants like Punica granatum (Kaur et al., 2006), Cytisus scoparius (Sundararajan et al.; 2006), Diospyros malabarica (Mondal et al.; 2006), and Aspalathus linearis (Joubert et al.; 2005) show strong antioxidant activities.

Similarly, vitamin E and epigallo catechin gallate are antioxidants commonly consumed by cancer patients as dietary supplements. Mice bearing MCa – IV solid tumours (a breast carcinoma), when fed with vitamin E and epigallo catechin gallate, tumour cell apoptosis was increased (Lawenda et al.; 2007). This proves the antitumour activity of antioxidants. Precancerous gastric lesions were reduced in subjects receiving fruits and antioxidants vitamins through diet in Venezuela (Plummer et al.; 2007). Similarly consuming diets containing fruits and vegetables mainly antioxidant rich tomatoes and broccoli, reduced experimental murine tumours (Canene – Adams et al., 2007).
Many antioxidants are reported to have inhibitory actions against in vitro invasion, growth and metastasis of murine colon 26-L5 carcinoma cells (Ogasawara et al., 2007).

Dietary antioxidants are gaining immense importance by virtue of their critical role in disease prevention. Vegetables, fruits, spices, cereals, vegetable oils, tea and red wines constitute rich sources of antioxidants. Regular intake of these in diet can protect the body against most of the disease and the disorders. It has been estimated that 30-40% of all kinds of cancer can be prevented with a healthy lifestyle and dietary measures. A low use of fibres, the intake of red meat and an imbalance of Omega-3 and Omega-6 fats may contribute to increase the risk of cancer. On the other hand, the consumption of lots of fruit and vegetables may lower the risk of cancer. Protective elements in a cancer-preventive diet include selenium, folic acid, vitamin B12, vitamin D, chlorophyll and antioxidants such as carotenoids (alpha-carotene, beta-carotene, lycopene, lutein, cryptoxanthin). Ascorbic acid has limited benefits if taken orally, but is effective through intravenous injection. A supplementary use of oral digestive enzymes and probiotics is also an anticancer dietary measure. A diet drawn up according to the proposed guidelines could decrease the incidence of breast, colon-rectal, prostate and bronchogenic cancer (Divisi et al.; 2006).

2.5 Chemical Carcinogenesis an overview

A genetic basis for human carcinogenesis has been established through biochemical and molecular analyses of the disease. Many types of human cancer have been caused by occupational exposure while others have been attributed to environmental exposure to chemical and/or viral agents. Chemical carcinogenesis is a multistage process that begins with exposure, usually to complex mixtures of chemicals that are found in the human environment. Carcinogenesis can be divided conceptually into four
Review of Literature

steps, tumour initiation, tumour promotion, malignant conversion and tumour progression. Tumour initiation results from irreversible genetic damage, i.e. a chemical carcinogen cause a mutation by modification of the molecular structure of DNA. Tumour promotion comprises the selective clonal expansion of initiated cells. Clonal expansion of initiated cells produces a larger population of cells that are at risk of further genetic changes and malignant conversion. Tumour promoters generally are non mutagenic, are not carcinogenic alone, and often are able to mediate their biologic effects without metabolic activation. There are chemicals capable of both tumour initiation and promotion. They are called complete carcinogens. Examples are benzo@pyrene, and 4 amino bisphenyl. Malignant conversion is the transformation of a preneoplastic cell into one that expresses the malignant phenotypes by further genetic changes. Tumour progression comprises the expression of the malignant phenotype and the tendency of already malignant cells to acquire more aggressive characteristics in time.

2.6 Chemical Carcinogenesis models

Numerous models exist for chemical carcinogenesis. These models facilitate the study of the mechanism of chemical carcinogenesis and also help to study antimutagenic properties of both synthetic and natural substances.

(a) Mouse skin tumour promotion model:

In this model, a single sub-threshold dose of a carcinogen such as 7, 12 dimethyl Benz (a) anthracene (DMBA) is administered followed by the repetitive application of a tumour promoter such as 12-0-tetra decanoyl phorbol-13-acetate (TPA). Benign squamous papilomas generally develop within 10 weeks and virtually all papilomas contain Ha-ras mutations. A small percentage of these eventually progress to malignant squamous cell
carcinomas (Quintanilla et al.; 1986). Croton oil isolated from *Croton tiglium* seeds has been used widely as a tumour promoter in murine skin carcinogenesis and its main constituent is TPA. It acts by activating protein kinases. The same mechanism is found to operate in other rodent tissues, including bladder, colon, esophagus, liver, lung, mammary gland, stomach and trachea.

(b) **Hepatocellular carcinoma model**

In this model hepatoma is induced by single *i. p.* injection of *N*-nitrosodiethylamine and promoted by administration of Phenobarbitol orally. In another model HCC is induced by single intraperitoneal dose of aflatoxin B₁.

(c) **Mouse fore-stomach papillomagenesis model**

Papillomas are developed by feeding Benzyl (a) pyrene prepared in peanut oil. Being a complete carcinogen, no promoter is required here.

(d) **Mouse fibrosarcoma model**

Fibrosarcoma are solid tumours, characterized by the malignant proliferation of fibroblasts, arising in the external soft tissues as a gray white firm tabulate mass with a good circumscription (Barnes, 1985). They have a low metastatic rate, but are characteristically recurrent and radio resistant with a poor prognostic index (Fletcher and McKee, 1985). The fibrosarcoma is developed in mice by administering a single dose of 20- methyl chlolanthrene subcutaneously on the dorsal side.

2.7 **Chemopreventive properties of medicinal plants**

Inhibitors of chemical carcinogens and antimutagens can have a profound effect on the expression of experimental tumours (Waltenberg, 1985). Such inhibitors are often seen in the plant kingdom. Curcumin the yellow pigment obtained from *Curcuma longa* (turmeric) is found to be
antimutagenic in two stage skin chemical carcinogenesis model in mice using DMBA as initiator and croton oil as promoter (Soudamini and Kuttan, 1989). Similarly topical applications of *Ixora javanica* flower extract, and *Ixora coccinea* flower extract inhibits the growth and delayed the onset of papilloma formation in mice in the same model as above (Nair *et al*.; 1991, Latha and Panikkar, 2000). Crude ethanolic extract of the leaves of *Juniperus chinensis* inhibits and delays the onset of tumours in the two stage murine skin carcinogens in using DMBA as initiator and TPA as promoter (Ali *et al*.; 1996). Lantadene, the triterpenes isolated from *Lantana camara* inhibits mouse skin papillomas induced by DMBA and promoted by TPA and mouse hepatic tumour induced by N-nitroso diethylamine and phenobarbitol (Akira *et al*.; 1997). The flavonoid-containing drug obtained from *Semecarpus anacardium* improves the antioxidant defense system in the aflatoxin B₁ induced hepatocellular carcinoma conditions. It was also observed that the anticancer property of *Semecarpus anacardium* extract might be explained by its strong antioxidant capacity and capability to induce the in vivo antioxidant system (Premalatha and Sachdanandam, 1999). The active fraction obtained from *Psoralea corylifolia* seeds on topical application inhibits the growth and delayed the onset of papilloma formation in mice initiated with DMBA and promoted using croton oil (Latha and Panikkar, 1999). It was also reported that the oral administration of *Picrorhiza kurroa* extract reduces the tumour, and tumour related deaths by 20-methylcholanthrene (20MC) induced sarcoma in mice (Joy *et al*.; 2000). Tenuzonic acid a mycotoxin isolated from *Alternaria alternata* inhibits and delays the papilloma formation on DMBA induced murine skin carcinogenesis model (Mary *et al*.; 2002). By feeding rats with a mixed diet containing guar gum and cellulose, a reduction in the incidence of colorectal tumours induced by 1, 2-dimethyl-hydrazine is observed (Okazaki *et al*.; 2002). Topical application of *Boerhaavia diffusa* Linn. Extract reduces the growth and delays the onset of papilloma formation
in DMBA induced skin papilloma genesis model (Rupjyothi et al.; 2002). The ethanolic fraction of *Hemidesmus indicus* applied topically reduces and delays the tumour formation in 2-stage skin chemical carcinogenesis in mice by DMBA and TPA (Sultana et al., 2002). Bitter melon (Momordica charantia Linn.) fruit extract was tested against 3, 4 benzo (a) pyrene induced forestomach papilomagenesis in Swiss albino mice. A significant decrease in tumour burden is observed by the treatment (Deep et al., 2004). The ethanolic extract of *Becopa monnieri* promotes the antioxidant status, reduces lipid peroxidation, and markers of tumour progression like lactic dehydrogenase, creatine kinase, alanin transaminase, aspartate transamise, and sialic acid in the rats bearing 20-MC induced fibrosarcoma (Rohini et al.; 2004). Depletion of DNA synthesis, ornithine decarboxylase activity and xanthine oxidase activity in mice skin is observed by topical application of acetone extracts of *Salix caprea* on two stage murine skin carcinogenesis by DMBA and TPA. This supports the anticarcinogenic potential of the extract (Sultana and Saleem, 2004). Hydroalcoholic extract (80% ethanol, 20% water) of Neem leaves (*Azadirachta indica*) reduces the papilloma formation by benzo (a) pyrene (forestomach) and DMBA (skin), on oral and topical treatments respectively (Dasgupta et al.; 2005). The plasma and liver glycolytic enzymes like hexokinase, phosphoglucoisomerase, and aldolase were significantly increased in N-nitrosodiethylamine induced hepatocellular carcinoma bearing animals, while gluconeogenic enzyme glucose-6-phosphate was decreased. By oral administration of *Terminalia arjuna* these enzymes returns to normal. This modulation of enzymes constitutes the depletion of energy metabolism leading to inhibition of cancer growth (Sivalokanathan, 2005).

Vanadium a dietary micronutrient was reported to possess antimutagenic activities against rat models of mammary, colon and hepato
carcinogenesis. This was found to be especially useful in preventing hepatocarcinoma developed by diethyl nitrosamine (Tridib et al.; 2007).

2.8 Apoptosis, the programmed cell death:

Apoptosis or programmed cell death is a normal occurrence in which an orchestrated sequence of events leading to the death of a cell (Karp, 2005). Death by apoptosis is a neat orderly process characterized by the overall shrinkage in volume of the cell and its nucleus, the loss of adhesion to neighbouring cells, the formation of blebs at the cell surface, the dissection of the chromatin into small fragments and the rapid engulfment of the corpse by phagocytosis.

John Kerr, Andrew Wyllie and Currie of the University of Aberdeen, described for the first time the coordinated events that occurred during the programmed death of a wide range of cells, coined the term apoptosis in 1972. Insight into the molecular basis of apoptosis was first revealed in studies on the nematode worm *C. elegans*, whose cells can be followed with absolute precision during embryonic development. Of the 1090 cells produced during the development of this worm, 131 cells are normally destined to die by apoptosis. In 1986, Robert Horvitz and his colleagues at the Massachusetts Institute of Technology discovered that worms carrying a mutation in the CED-3 gene proceed through development without losing any of their cells to apoptosis. This finding suggested that the product of the CED-3 gene played a crucial role in the process of apoptosis in this organism. The identification of the CED-3 gene in nematodes led to the discovery of a homologous family of proteins in mammals, which are now called caspases. Caspases are a distinctive group of cystine proteases that are activated at an early stage of apoptosis and are responsible for triggering most if not all of the changes observed during cell death. Caspases accomplish this feat by clearing a select group of essential proteins. Among the target of caspases are the following.
• More than a dozen protein kinases, including focal adhesion kinase (FAK) PKB, PKC and Raf1. Inactivation of FAK for example is presumed to disrupt cell adhesion, leading to detachment of the apoptotic cell from its neighbours.

• Lamins, which makeup the inner lining of the nuclear envelope. Cleavage of lamins leads to the disassembly of the nuclear lamina and shrinkage of the nucleus.

• Proteins of the cytoskeleton, such as those of intermediate filaments, actin, tubulin and gelsolin. Cleavage and consequent inactivation of these proteins leads to changes in cell shape.

• An endonuclease called caspase activated DNase (CAD), which is activated following caspase cleavage of an inhibitory protein. Once activated, CAD translocates from the cytoplasm into the nucleus where it attacks DNA.

Recent studies have focused on the events that lead to the activation of a cell’s suicide programme. Apoptosis can be triggered by both internal stimuli such as abnormalities in the DNA, and external stimuli, such as the removal of growth factors from the medium. Studies indicate that external stimuli activate apoptosis by a signaling pathway, called the extrinsic pathway, which is distinct from that of utilized by internal stimuli, which is called the intrinsic pathway.

2.8.1 The extrinsic pathway of apoptosis

The steps in the extrinsic pathway are illustrated in Fig. 2:1. It is seen that, the stimulus for apoptosis is carried by an extra cellular messenger protein called tumour necrosis factor (TNF), which was named for its ability to kill tumour cells. TNF is produced by certain cells of the immune system in response to adverse conditions, such as exposure to
ionizing radiations, elevated temperature, viral infection, or toxic chemical agents such as those used in cancer chemotherapy. Like any other types of first messengers TNF evokes its response by binding to a transmembrane receptor, TNFR1. TNFR1 is a member of a family of related “death receptors” that mediate apoptosis. The available evidence suggests that the TNF receptor is present in the plasma membrane as a preassembled trimer. The cytoplasmic domain of each TNF receptor subunit contains a segment of about 70 amino acids called “death domain” that mediates protein – protein interactions. Binding of TNF to the trimeric receptors produces a change in conformation of the receptor’s death domain, which leads to the recruitment of a number of proteins.

The last protein to join the complex that assembles at the inner surface of plasma membrane is two pro-caspase 8 molecules. These proteins are called ‘pro-caspases’ because each of them is a precursor of a caspase, which contains an extra portion that must be removed by proteolytic process. The synthesis of caspases as proenzymes protects the cell from accidental proteolytic damage. Unlike most proenzymes, procaspases exhibit a low level of proteolytic activity. Consequently when two or more procaspases are held in close association with one another, they are capable of clearing one another’s polypeptide chain and converting the other molecule to a fully active caspase. The final mature enzyme contains four polypeptide chains, derived from two procaspase precursors. Activation of caspase -8 is similar in principle to the activation of effectors by a hormone or growth factor. Caspase -8 is described as an initiator caspase because it initiates apoptosis by clearing and activating downstream or executioner, caspases that carry out the controlled self-destruction of the cell.
Fig 2:1 The Extrinsic Pathway of Apoptosis

Plasma membrane

Death domains

TNF

TNFR1

FADD

TRADD

Pro-caspase-8

Initiator caspase-8

Executioner procaspases

Executioner caspase

APOPTOSIS
2.8.2 The intrinsic pathway of apoptosis:

Internal stimuli, such as irreparable genetic damage, extremely high concentrations of cytosolic Ca$^{2+}$ or severe oxidative stress, and lack of survival signals, trigger apoptosis by the intrinsic pathway as illustrated in Fig 2:1. Members of the Bcl-2 family of proteins regulate activation of intrinsic pathway. Bcl-2 family members can be subdivided into two groups, proapoptotic members that promote apoptosis (e.g. Bad and Bax) and antiapoptotic members that protect cells from apoptosis (e.g. Bcl-x$_L$, Bc1-w, and Bcl-2).

In the intrinsic pathway, stressful stimuli activate certain proapoptotic members of the Bcl -2 family, such as Bax which translocates from the cytosol to the outer mitochondrial membrane. The attachment of Bax to the outer mitochondrial membrane increases the permeability of the membrane and promotes the release of certain mitochondrial proteins, most notable Cytochrome –C which resides in the intermembrane space. Release of proapoptotic mitochondrial proteins may be the crucial event that commits the cell to apoptosis. Once in the cytosol, Cytochrome C forms part of a multiprotein complex called the apoptosome that includes several molecules of procaspase -9. Like caspase -8, which is activated by the receptor mediated pathway caspase -9 is an initiator caspase that activates downstream executioner caspases in intrinsic pathway, which bring about apoptosis. The external receptor mediated and internal mitochondria mediated pathways ultimately converge by activating the same executioner caspases which cleave the same cellular targets.

As the cells execute the apoptotic program they lose contact with their neighbours and start to shrink. Finally, the cell disintegrates into a condensed, membrane enclosed apoptotic body in less than an hour. During apoptosis, a phospholipid scramblase moves phosphatidylserine that is usually present within the inner leaflet of plasma membrane to outer leaflet, where they are recognized as an ‘eat me’ signal by specialized macrophages.
Fig 2:2 The Intrinsic Pathway of Apoptosis

- Internal cellular damage
- Activation of proapoptotic Bcl-2 family members (e.g., Bad or Bax)
- Cytochrome c
  - Cytoplasmic factors (e.g., Apaf-1)
  - Procaspase-9
- Activated initiator caspase-9 complex (Apoptosome)
- Executioner procaspases
- Executioner caspase
Apoptotic cell death thus occurs without spilling cellular content into the extra cellular environment, without causing any inflammatory response.

Apoptosis has an important role in maintaining the normal homeostasis of the multi cellular organisms. During embryonic development neurons grow out of the central nervous system to innervate organs that are present in the periphery of the body. Usually many more neurons grow out than that is needed for normal innervations. Those neurons that reach their destination receive a signal from the target tissue that allows them to survive. Those neurons that fail to find their way to the target tissue do not receive the survival signal and ultimately eliminated by apoptosis. T Lymphocytes are cells of the immune system that recognize and kill abnormal or pathogen infected target cells. During embryonic development, T Lymphocytes are produced that possess receptors capable of binding tightly to proteins present on the surface of normal cells within the body. T Lymphocytes that have this dangerous capability are eliminated by apoptosis. Apoptosis is also involved in the elimination of cells that have sustained irreparable genetic damage. This is of importance because damage to the genetic blueprint can result in unregulated cell division and the development of cancer. The antiapoptotic Bcl -2 gene, when mutated, causes unregulated cell division and cancer. Finally apoptosis appears to be involved in neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and Huntington’s disease. Elimination of essential neurons during the progress of these diseases gives rise to loss of memory or decrease in motor coordination. These examples show that apoptosis is important in maintaining homeostasis in multicellular organisms and failure to regulate apoptosis can result in serious damage to the organism.
2.9 Apoptosis and cancer chemotherapy

The success of chemotherapy in the treatment of some malignant diseases has been dramatic. Before 1980, the five-year disease free survival in childhood acute lymphoblastic leukemia was 39%, but by the end of the 1990’s, this had increased to 63%. However, in common epithelial malignancies of adult life carcinoma of the breast, colon and lung, the impact of chemotherapy has been disappointing. One reason for the burgeoning interest in apoptosis research was the expectation that a greater understanding of the way in which, both cells normal and malignant, die after drug induced damage, might lead to improvements in chemotherapy. Two areas of fundamental importance were the identification of novel agents, informed by a mechanistic understanding of the process of drug-induced apoptosis and the modulation of cellular resistance to conventional agents, which derive from knowledge of the mechanisms that allow cancer cells to evade apoptosis after drug induced damage (Makin and Dive, 2001).

Abrogation of survival signaling to enhance the proapoptotic ability of conventional chemotherapeutic agents is a powerful strategy towards overcoming drug resistance. An alternative approach is to identify molecular abnormalities in tumour cells that decrease intrinsic sensitivity to apoptosis and to target these with novel agents. Other than patient survival, the most important endpoint in cancer therapy is tumour regression. To achieve this endpoint, most of the non-invasive therapeutic modalities, with the exception of anti-angiogenic approaches rely on the direct blocking of malignant cell division and / or enhancing cell death. Therefore, a crucial component of pre-clinical oncology drug development is the study and monitoring of cell death in tumour and normal tissues (Kamb and Lassota 2004). A review of almost 2000 studies shows that large majority of 39 putative cancer chemopreventive agents induces
spontaneous apoptosis (Francesco et al.; 2005). It was found that lectins isolated from *Viscum album* L induce apoptosis in cancer cell lines (Bussing, 1996). The bis-benzylisoquinoline alkaloids, tetrandrine and berbamine isolated from Chinese medical herbs known to possess antitumour activities, had concentration dependently inhibited the proliferation of human leukemic HL-60 cells. These causes shrinkage of the cell with the formation of apoptotic bodies, which showed clear evidence of DNA fragmentation (Ying et al.; 1997). Cleistanthin B isolated from the tropical plant *Cleistanthus collinus* possesses remarkable antitumour properties. This compound was also able to inhibit DNA synthesis in Chinese hamster ovary cells and induces apoptosis in cervical carcinoma (SiHa) cells. SiHa cells exposed to cleistanthin B shrank rounded up, had condensed chromatin, and fragmented nuclei, indicative of programmed cell death (Pradeepkumar et al.; 1998). Epigallocatechin gallate isolated from *Camellia sinensis* inhibits the growth of human lung cancer cell line, DC-9-dose dependent by inducing apoptosis induction (Hirota, 1999). Cycloprodigiosin hydrochloride, an H⁺/Cl⁻ symporter is found to induce apoptosis in human breast cancer cell lines KPL-1.T-47D, MCF7, MKL-F and MDA-MB-231 (Yamamoto et al.; 2000). Prodigiosin a red pigment produced by various bacteria including *Serratia marcescens* which is found to be apoptotic in cultured human colon cancer cell lines namely DLD-1 and SW-620. Prodigiosin induces apoptosis in these cell lines which is confirmed by DNA laddering pattern, condensed nuclei, and apoptotic bodies identified by fluorescent microscopy (Beatriz and Ricardo, 2001). Mitochondria play a crucial role in the induction and execution of apoptosis. Ionidamine a mitochondrial targeting drug is found to promote apoptosis in human hepatic carcinoma HepG-2 (Li et al.; 2002). Wogonin and fisetin are widely distributed plant flavonoids and these are found to be apoptotic in cultured human hepatocellular carcinoma cells SK-HEP-1.
Both these flavonoids shows dose dependent cytotoxic effects on SK-HEP-1 cells accompanied by DNA fragmentation, cellular swelling and the appearance of apoptotic bodies (Yen et al.; 2002). *Glycorrhiza glabra* and *Olenandria diffusa* and two commercially available combinations of Chinese Herbal medicines SPES and PC-SPES show cytotoxicity towards lung cancer cell lines H69 and H69 VP cell lines. These drugs are able to induce apoptosis in these cell lines and are able to elevate the expression of a number of genes involved in the apoptotic cascade (David et al.; 2002). Lup-28-al-20 (29)-en-3-one a lupane triterpene is found to be apoptotic against cultured human leukemia cell lines (Keishi et al.; 2003).

Background Capsiate, the non-pungent ester isoester of capsaicin and its dihydro derivative are the major capsaicinoids of sweet peppers. These capsiates induces apoptosis in Jurkat cells that preceded by an increase in the production of reactive oxygen species and by a subsequent loss of mitochondrial transmembrane potential (Antonio et al.; 2003). Taiwanin, a lignan isolated from *Taiwania cryptomerioides* showed apoptotic response against MCF7 cell lines, evidenced by chromatin condensation and apoptotic bodies (Shang et al.; 2003). Amooranin is a triterpene acid isolated from the stem bark of a tropical tree (*Amoora rohituka*) grown wild in India. Amooranin when added to a culture of human breast carcinoma cell line MCF7 and multidrug resistant carcinoma MCF7/TH induces apoptosis characterized by DNA ladder formation and caspase-8 activation (Thangaiyan et al.; 2003). An orally active Amazonian plant extract (BIRM) induces apoptosis in cultured metastatic hormone refractory prostrate cancer and activated cell death associated caspases (Devendra et al.; 2003). The aqueous ethanol extract prepared from *Coriolus versicolor* is shown to produce apoptosis in cultured cancer cells namely B-cell lymphoma (Raji) and two human promylocytic leukemia (HL-60, NB-4) cell lines. The dose of the extract is critical in the proliferation of the cancer cell lines and shows increased rate of nuclear fragmentation. This is
suggestive of the fact that an apoptosis dependent pathway of cytotoxicity of the extract (Lau et al.; 2004). Garlic (*Allium sativum*) has been used for centuries for treating various ailments and its consumption is said to reduce the risk of cancer and its extracts and components effectively block experimentally induced tumour. Allicin a major component of garlic inhibits murine and human cancers. It induces the formation of apoptotic bodies, nuclear condensation and a typical DNA ladder in cancer cells. Further more, it activates caspases -3, 8 and 9 and induces the cleavage of poly (ADP-ribose) polymerase, indicating its apoptosis inducing potential (Suby et al.; 2004).

*Poncirus trifoliata* (PTF) fruits have been used for the treatment of various cancers among Korean Oriental Medical doctors. This fruit extract causes cytotoxicity, cell shrinkage, cell membrane blebbing, apoptotic body and DNA fragmentation. PTF induces apoptosis, which is accompanied by the activation of Caspase -3 and the specific proteolytic cleavage of poly (ADP-ribose) polymerase in human leukemia HL-60 cells. But PTF did not show cytotoxicity in normal peripheral blood mononuclear cells (Jin –Mu et al.; 2004). Colorectal cancer is the second most frequent diagnosed cancer in the US, causing significant morbidity and mortality in human beings. Curcumin a natural plant product possesses such chemopreventivity that targets multiple signaling pathways in the prevention of colon cancer. Curcumin is found to induce degradation of cell to cell adhesion molecules which cause apoptosis (Satya Narayan, 2004). Crude methanolic extract from the pericarp of *Garcinia mangostana* shows dose dependent inhibition of cell proliferation of SKBR -3 (human breast carcinoma) cell lines. Apoptotic changes are observed as nuclear fragmentation and morphological changes (Primchanien et al.; 2004). *Cimicifuga racemosa* extracts shows antiproliferative activity against MCF7 and MDA-MB 231 breast cancer cells. The mode of cell death is identified as apoptosis by microscopic examination and by the presence of activated
caspases (Hostanska et al.; 2004). Ethyl acetate fraction of *Anoectochilus formosanus* Hayata is shown to possess antitumour activity. The antitumour activity of this plant is found to be due to the induction of apoptosis as evidenced by cell morphological changes, early redistribution of plasma membrane phosphatidylserine cleavage of procaspases and poly (ADP-ribose) polymerase, and release of mitochondrial Cytochrome C (Lie-Fen et al.; 2004). A novel compound polyphyllin D induces DNA fragmentation and externalization of phosphatidylserine indicative of apoptosis in hepatocellular carcinoma cell line HepG-2. The compound was isolated from the Chinese medicinal plant *Paris polyphylla*. The compound induces the mitochondrial apoptotic pathway as evidenced by depolarization of mitochondrial transmembrane potential, generation of $H_2O_2$ and release of Cytochrome C and apoptosis inducing factor in dose and time dependent manner in treated cells (Jenny et al.; 2005). The flavonoid fraction isolated from *Rhus verniciflua* shows antiproliferative and apoptotic effect on human osteosarcoma (HOS) cells. It induces the cleavage of poly (ADP-ribose) polymerase (PARP), activated caspase 8 and Bax, inhibited Bel-2 expression and releases Cytochrome –C, promoting apoptosis in treated cells (Hyon-Seok et al.; 2005). Lanostanoid triterpenes isolated from *Ganoderma amboinense* induces cell apoptosis in human hepatoma cell line HUH-7 and inhibited topoisomerases (Chyi-Hann et al.; 2005). β- Elemene is a novel anticancer drug, which was extracted from the ginger plants. β-elemene was shown to trigger apoptosis in non-small-cell lung cancer cell lines (NSCLC) by inducing caspase –3, 7, and 9 activities, decreasing Bcl-2 expression, causes release of Cytochrome-C and increases the levels of cleaved caspase -9 and poly (ADP-ribose) polymerase in NSCLC cells (Wang et al.; 2005). Mijatovic et al (2005) investigated the effect of aloe emodin an herbal anthraquinone derivative on rat C6 glioma cell line and they found that the compound induces cell cycle block and caspase dependent apoptosis.
Denbinobin has been reported to exhibit antitumour and anti-inflammatory activity. In human myelogenous K562 leukemia cells denbinobin displays anticancer effects in K562 cells through increase in the levels of tubulin polymerization and deregulation of Bcr-Abl signaling and thereby prompted the cells to undergo apoptosis (Yu-Chun et al.; 2005). “Semecarpus Lehyam” induces apoptosis in MCF7 cell lines (human breast carcinoma) (Srinivasan et al.; 2005). Erhart et al (2005) reported that flavone the core structure of the flavone subgroup inhibits the proliferation and induces apoptosis in HCT 116 colon cancer cells. It induces the activation of caspases 2,3,8,9 and 10 and decreased mitochondrial antiapoptotic Bcl2 protein expression.

Pancreatistatin a naturally isolated compound from spider Lilly is found to induce apoptosis selectively in cancer cells (Mc Lachlan et al.; 2005). Citri Reticulate viride Pericarpium (CR) promotes cytotoxicity and apoptosis in human colon cancer cells (SNU-4) by up regulating Bax and down regulating Bcl-2 and also activated caspase -3 (Soon et al.; 2005). Components of Chinese ginger (Zingiber officinale) are found to have significant cytotoxicity against HL-60 cells and cytotoxicity was due to apoptosis (Qing – Yi et al.; 2005). Triphala an Indian herbal drug has cytotoxic and apoptotic effect in Shinogi 115, and MCF-7 breast cancer cell lines and PC-3 and Du-145 prostate cancer cell lines (Swayamjot et al.; 2005). Baizhu (Atractylodes macrocephala koidz) induces apoptosis in human leukemia cell lines Jurkat T cells, leukemia U937, and HL -60 cells (Huey-Lan et al.; 2005). Evening primrose extract induces apoptosis and inhibits DNA synthesis in Ehrlich ascites tumour cells (Arimura et al.; 2005). Dihydror帝idine isolated from Toddalia asiatica Lam induces apoptosis in human lung adenocarcinoma (A549) cells by regulating cell cycle related genes (Hironori et al.; 2006). Linum persicum and Euphorbia cheiradenia induce apoptosis in K562 and Jurkal cell lines (Zahra et al.; 2006).
Treatment with ethyl acetate extract of *Antrodia camphorata* fruiting bodies induces cytotoxicity and apoptosis in HepG2 and PLC/PRF/5 cell lines, in a p53 independent manner. This extract also initiates mitochondrial apoptotic pathway through regulation of Bcl-2 family of proteins, release of Cytochrome C and activation of Caspase-9 in both the cell lines (Ya-Ling et al.; 2005). Methanolic extract of *Pereskia bleo* (Kunth) DC. induces DNA apoptosis in breast carcinoma cell line T47-D. This compound induces DNA fragmentation, chromatin margination and apoptotic bodies. It also increases mRNAs of c-myc and caspase-3, indicative of the activation of these pathways (Tan et al.; 2005). Papiya et al.; (2006) reported that Tea root extract has remarkable apoptogenic effect in human cell lines and cell lines from leukemic patients.

Similarly extract of *A. cinnamomea* fruiting bodies had remarkable apoptogenic effect on Hep 3B liver cancer cell line. This extract triggered apoptosis induction, induces Ca\(^{2+}\)/ calpain pathway, disrupted mitochondrial function and amplified the apoptotic signaling by cross-link between the calpain / Bid / Bax / Ca\(^{2+}\) / mitochondrial apoptotic pathways (Po-Lin et al.; 2006). *Caulerpa racemosa* (Forskal) is a green marine alga which spreads from tropical to warm water regions, as a biological pollutant. This produces an important secondary metabolite known as Caulerpenyne (CPN). CPN was found to be apoptogenic in two neuroblastoma cell lines namely Kelly and SHSY5Y (Levent et al.; 2006). Similarly, isocostunolide a sesquiterpene lactone isolated from the roots of *Inula helenium*, induces apoptosis in 3 human cancer cell lines, A2028, HT-29, and Hep G-2. This compound markedly depolarizes mitochondrial membrane facilitating Cytochrome-C release into cytosol, meaning thereby that apoptosis is medicated through a mitochondrial dependent pathway (Chia-Nan et al.; 2007).
2.10 About the plant

*Cassia fistula* L. belonging to the family of Caesalpinaceae is widely distributed throughout India and is commonly called Sarakonnai in Tamil, Kanikonna in Malayalam. The English common name of the plant is Indian Laburnum. This is a tree of 6-9m height with straight trunk, smooth bark, which is pale grey when young and dark brown when old, branches spreading and slender, leaves 23-40cm long, main rachis pubescent stipules minute, leaflets 4-8 pairs, ovale or ovale-oblong and acute. Plant is widely distributed in India, Sri Lanka, Malaysia and China. All the parts of the tree are medicinally important in Indian traditional systems of medicine.

![Plate 2.1: Cassia fistula L. in full bloom](image)

**Plate 2.1: Cassia fistula L. in full bloom**

**Medicinal properties:**

The leaves lessen inflammation. The flowers are purgative. The fruit has a sweetish bad taste. It is, antipyretic, purgative, abortifacient,
demulcent, reduces inflammation and heat of the body. The fruit is useful in chest complaints, throat troubles, liver complaints, diseases of the eye, rheumatism. Seeds are emetic. The root is generally given as a tonic and febrifuge. It has been found to act as a strong purgative. In Cambodia the bark and wood is used to treat dysentery. Every part of the plant is prescribed in combination with other drugs for the treatment of snakebite (Charaka, Sushruta, Yoga Ratnakara) and scorpion sting (Charaka, Sushruta, Yoga Ratnakara). Besides this, the plant has been shown to possess several medicinal values such as hypoglycemic (Bhakta et al.; 1997) hepatoprotective (Bhakta et al.; 1999), antibacterial (Perumal Samy et al.; 1998) hypocholesterolaemic (El Saadany et al.; 1991) and antidiabetic (Esposit Avella et al.; 1991) in experimental animals. The methanolic extract of pods of the plant has shown remarkable antitumour activity against EAC cell lines in mice (Gupta et al.; 2000). The bark of the plant has remarkable antioxidant properties also (Sidduraju et al.; 2002). In addition the chemical constituents of different parts of the plant have also been reported by various authors (Murty et al.; 1966, Misra et al.; 1995). However, information on the anticancer properties of the stem bark of the plant is not available.