Chapter-II

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2.1. Immunology and plants as immunomodulatory agents

The immune system which is responsible for protecting the organism against various infections, maintaining homeostasis and immune surveillance has evolved as an integrated network of interacting cells and cell products that co-ordinately recognize and respond to foreign materials (Jerne, 1973). It is a complex and highly interactive network of cells and their products with characteristic features of Antigenic-specificity, Diversity, Immunologic memory and Self/nonself recognition. The cellular constituents that constitute the frame work of immune response include mononuclear phagocytes, granulocytes, lymphocytes (T and B lymphocytes), plasma cells, mast cells, and blast cells. These cells circulate in the blood and are concentrated in lymph nodes, lymph glands, spleen and thymus. This cellular complex provides not only specifically sensitized cells and immunoglobulin antibody but also an inherent control system that limits the extent of the response (Sell, 1980). Following an activation of the immune system a spectrum of cellular and humoral events occur that comprise the non-specific and specific immune responses (Fig. 2.1.1). Moreover co-stimulatory signals are the most important signaling molecules playing important role in the cell-cell cross-talk for the delivery of the required immune response. The lymphocytes interact with the other cell types and sensitized lymphocytes for the expression and regulation of the immune response through the liberation of different lymphokines.

Fig. 2.1.1. Immune response against invading pathogens involving different cells & signal molecules.
T cells differentiate into two different subsets: CD4+ (i.e. Th or T helper cells) and CD8+ (i.e. Tc or T cytotoxic cells) T lymphocytes, which play different roles in immunomodulation. Th cells can promote proliferation, maturation, and immunologic function of other cell types, and specific cytokines secreted by Th cells are very important for the activities of B cells, macrophages, and CTLs. The Th1 cells are able to produce IL-2 and IFN-γ whereas Th2 cells can produce IL-4. Since IFN-γ is an important immunoregulatory molecule which protects against viral infections, induces the generation of T cells, activates macrophages and regulates crossly Th1 and Th2 cells (Fig. 2.1.2). Investigation of the balance of Th1 and Th2 cytokine production are helpful in understanding the outcomes of different immune responses and are clinically useful in treating immunologically deregulated states. The progress in the clinical immunology has unravelled the involvement of immune system in the pathogenesis of several disease conditions like in arthritis, cancer, chronic and recurrent viral and bacterial infections due to the altered functioning of the immune system. This has led to enormous search for development of the agents which could modulate the immune system for the treatment of these diseases.

![Fig. 2.1.2 Role of Th1 and Th2 cells in regulating immune responses against various pathogenic challenges with the secretion of specific types of chemical signals (cytokines/lymphokines).](image)
2.1.1 Immunomodulation

The term "immunomodulation" denotes a change, a strengthening or suppression of the indicators of cellular and humoral immunity and nonspecific defense factors through any pharmacological agent. Hence both immunostimulating agents and immunosuppressing agents have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world. Natural adjuvants, synthetic agents and antibody reagents are used as immunosuppressive and immunostimulative agents. It is generally agreed that the concept of immunomodulation emerged in 1796 when Jenner undertook the first "vaccination". Immunomodulation (immunotherapy) is now increasingly employed clinically in the treatment of various diseases associated with immune dysfunction. For years, various immunomodulating substances, often derived from microorganisms and plants, have been used to enhance general resistance to infectious agents. A few of the agents capable of modulating the immunological network and which have undergone significant clinical development are Levamisole, Liposaccharide, Cytokines, Interferons (IFNs), Tuftsin, BCG, Cyclosporin, Cyclophosphamide, etc.

2.1.2 Immunomodulating agents

An immunomodulator may be defined as a substance, biological or synthetic, which can stimulate, suppress or modulate any of the components of the immune system including both innate and adaptive arms of the immune responses. In clinical perspective some immunomodulators are immunostimulants or immunerestorators used for enhancing body’s resistance against infections, allergy, immune deficiency, and cancer, and some are immunosuppressants used for control of pathological immune response in autoimmune diseases, graft rejection, graft versus host disease and hypersensitive reaction. These immunomodulating agents are also referred to as immunoaugmentors or biologically response modifiers (BRM). These agents can modulate a host’s immune system by one of the several mechanisms to the disease with resultant therapeutic benefit.
2.1.3 **Plant as immunomodulatory agents**

Medicinal plants are a rich source of substances that are claimed to induce paraimmunity, the non-specific immunomodulation of granulocytes, macrophages, natural killer cells and complement function in mammalian systems. In the recent past, scientific studies on plants used in ethno medicine have led to the discovery of many valuable drugs. Several structural analogues are also in clinical use and most notable of these are vinorelbine and vindesine. The strong historic bond between plants and human health began to unwind in 1897, when Friedrich Bayer and Co. introduced synthetic acetyl salicylic acid (aspirin) to the world from willow bark was discovered independently by residents of both the New and Old worlds as a remedy for aches and fevers. A number of medicinal plants and various ‘rasayanas’ have been claimed to possess immunomodulatory activity (Fig.2.1.3).

![Diagram](image-url)

**Fig.2.1.3.** Schematic representation of the affecting of immunomodulatory agents on the adaptive immune system leading to activation of antimicrobial and antitumor pathways. Ag, antigen; PPCs, polysaccharide peptide/protein complexes; FTPs, fungal immunomodulatory proteins; APC, antigen presenting cells; TLR, toll like receptor, MHC II, major histocompatibility complex; TCR, T-cell receptor; NO, nitric oxide; IL, interleukin; IFN, interferon; LT, lymphotoxin; TH, T helper cells.
Medicinal plants and fruits produce different classes of secondary bioactive phytochemicals with immunomodulatory potential, reported to modulate different components of the immune network (Waldmann, 2003). Plant extracts or herbal preparations, display an array of immunomodulatory effects in various in vitro and in vivo studies. The ethanolic extract of medicinal plants like *Tinospora cordifolia* revealed significant improvement in cell mediated immunity and non-specific resistance in mice and immunomodulating agents of plant origin (Atal et al., 1986). Jiao et al. (1999) investigated the effect of flavonoids from *Astragalus membranaceus* stem and leaves on the cell mediated immune response in mice. The study revealed that the flavonoids obtained could enhance the proliferation of lymphocytes induced by Con A., increase the T-cell count and also regulate the T-cell subset disorders, elevate the LAK activity induced by IL-1.

Rezaeipoor et al. (1999) studied the aqueous extract of *Achillea talagonica* consisting of a mixture of alkaloids, terpenoids and flavonoids for the humoral antibody responses in BALB/c mice. Prior to immunization with sheep red blood cells, resulted in a significant dose dependent decrease in haemagglutinating antibody (HA) titre, by decreasing the dosage a significant decrease in HA titre was observed. Agarwal et al. (1999) documented the immunomodulatory activities of extracts of *Withania somnifera* L. Dunal (*Solanaceae*) namely WST and WS2 in mice for immune inflammation, active paw anaphylaxis and delayed type hypersensitivity (DTH). The results inferred that a significant increase in leucocyte count was observed in animals treated with WST. Further immunosuppression was counteracted by treatment with WS2, revealing significant increase in haemagglutination antibody response and hemolytic antibody response towards sheep RBC’s.

Barua et al. (2000) evaluated the immunomodulatory effect of bark of *Albizia lebbeck* on the humoral and cell mediated immune response. The *A. lebbeck* treated mice developed higher levels of serum antibody titre as compared to control animals. The results showed that *A. lebbeck* could be used as an immunomodulatory agent.

Latha et al. (2000) studied the immunoimodulatory and anti-tumor properties of *Psoralea corylifolea* seeds. The study revealed that the seed extract increased the efficacy of immune system in experimental mice. Also administration of the extract was found to inhibit EAC ascetic tumor growth and stimulated natural killer NK-cell
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activity, antibody dependent cellular cytotoxicity (ADCC), antibody forming cells and the antibody complement mediated cytotoxicity (ACMC) during tumour growth.

Benencia et al. (2000) investigated in vitro and in vivo immunomodulatory activities of Trichilia glabra aqueous leaf extract. The study revealed that in vitro proliferation of T and B lymphocytes was completely impaired. Besides, the extract significantly diminished both antibody and delayed hypersensitivity responses in treated mice. These results suggested that the extract caused marked immunosuppressive effect on the murine immune system.

Amirgufran et al. (2000) evaluated the immunomodulatory activity of five medicinal plants- Silybum marianum, Matricaria chamomilla, Callindulla officinalis, Cichorium intybus and Dracocephalum kotschyi. The plants were extracted with ethanol 70% and the mitogenic activity was studied on human peripheral blood lymphocytes and thymocytes. The in vitro study showed that all the extracts except Dracocephalum enhanced proliferation of lymphocytes after stimulation with allogeneic cells.

Smit et al. (2000) studied the in vitro and in vivo immunomodulatory properties of rhizomes of Picrorhiza scrophulariiflora. The diethyl extracts showed a potent inhibition activity towards the classical pathway of the complement system, the respiratory burst of polymarphonuclear leucocytes and the nitrogen induced proliferation of T-lymphocytes.

Ross et al. (2001) studied the immunomodulatory potential of an aqueous suspension of Punica granatum L. fruit rind powder (PGFRP) in rabbits with respect to the cell-mediated and humoral components of the immune system. The study revealed that PGFRP elicited an increase in antibody titer to typhoid-H antigen and also enhanced the inhibition of leucocyte migration in LMI test and induration of skin in delayed hypersensitivity test.

Ganguly et al. (2001) determined the cellular targets of Tylophora alkaloids in an in vitro system and in vivo cellular immune responses. The study revealed that alkaloid mixture was found to inhibit proliferation of splenocytes at higher concentrations and augment the same at lower concentrations. Both macrophages and T cells were found to be vulnerable to Tylophora alkaloids. The alkaloid mixture suppressed IL-2 production in Con A stimulated splenocytes at the inhibitory or higher
concentrations and enhanced production at the lower concentrations. IL-1 production by activated macrophages on the contrary was doubled in the presence of inhibitory concentrations of *Tylophora*. The results indicated that *Tylophora* alkaloids have a concentration dependent biphasic effect on Con A induced mitogenesis.

Wang *et al.* (2001) investigated the pharmacological effects of a polysaccharide from *Urtica fissa*. The polysaccharides inhibited foot pad swelling induced by carrageenan and the pinna swelling induced by xylene in rats. The results showed that polysaccharides have the significant effect of anti-inflammation and immunity enhancement.

Kiseleva *et al.* (2002) investigated the anti-inflammatory action of gamma-plant (GP) glycoprotein in case of reactive arthritis. The results showed that glycoprotein (mm. 70kD) extracted from plant *Solanum tuberosum* had anti-inflammatory action in case of reactive arthritis. The GP was found active against HIV-1. IC 50 for GP varied from 40 to 100µg/ml against different HIV-1 strains including clinical isolates and reference strains. The antiviral and immunologic activity of GP could give a new approach in therapy of HIV-infection.

Sai-Ram *et al.* (2002) studied in vitro cytoprotective and immunomodulatory properties of fruit extract of Amla on lymphocytes using chromium (Cr VI) as an immunosuppressive agent. The results showed, Amla restored the immunosuppressive effects of Cr on lymphocyte proliferation, IL-2 and gamma-IFN-γ production considerably. Amla also significantly inhibited Cr-induced free radical production and restored the antioxidant status back to control level. Interestingly Amla inhibited apoptosis and DNA fragmentation induced by Cr. Thus fruit of *Emblica officinalis* (Amla) proved to have strong antioxidant and immunomodulatory properties.

Mediratta *et al.* (2002) studied the effect of *Ocimum sanctum* seed oil (OSSO) on some immunological parameters in both non-stressed and stressed animals. After the detailed studies, the results showed that OSSO at (3ml/kg, ip) produced a significant increase in anti-sheep red blood cells (SRBC) antibody titre and a decrease in percent histamine release from peritoneal mast cells of sensitized rats, footpad thickness and percent leucocyte migration inhibition (LMI) in stressed animals. Thus, OSSO appears to modulate both humoral and cell-mediated immune responsiveness and these immunomodulatory effects may be mediated by GABA ergic pathways.
Geetha et al. (2002) reported that alcoholic extracts of leaves and fruits of seabuckthorn exhibits cytoprotective activity and enhances immune response.

Chiang et al. (2003) investigated the immunomodulatory activities of five classes of pure compounds obtained from the Plantago genus on human peripheral blood mononuclear cells (PBMC). Studies were conducted on lymphocyte transformation by BrdU immunoassay and secretion of interferon gamma (IFN $\gamma$) using an ELISA assay. The results showed that the water soluble compounds enhanced the activity of human lymphocyte proliferation and secretion of IFN $\gamma$. Among the water–insoluble compounds with the exception of luteolin, both baicalein and baicalin showed an enhancement of the human PBMC. Although oleanolic acid and ursolic acid of the triterpenoids did not significantly affect the proliferation of PBMC, but they exhibited a stimulation of IFN $\gamma$ -secretion.

Akbay et al. (2003) studied the immunomodulatory activities of compounds isolated from methanolic extract of the aerial parts of Urtica dioica L. The immunomodulatory activities were studied in vitro by chemotaxis (Boyden Migration Chamber) and intracellular killing activity (NBT reduction) tests. The results of both assays confirmed the immunostimulatory activity of the flavonoid fraction and the isolated flavonoid glycosides on neutrophils suggesting that they could possibly be useful for treating patients suffering from neutrophil function deficiency and chronic granulomatous diseases.

Manosroi et al. (2003) evaluated the in vitro immunomodulatory activities of aqueous extract, acetone extract and the Thai folklore extract of Clausena excavata Burm. on mouse immune system. The phagocytic activity of macrophages and splenocyte proliferation in the absence and presence of mitogens (lipopolysaccharide, LPS) or pokeweed mitogen, PWM) were assayed. The results showed that aqueous extract exhibited the maximum effect on both respiratory burst response and lysosomal enzyme activity more than the acetone extract and the Thai folklore extract indicating effective phagocytic activation. For splenocyte proliferation assay, the Thai folklore extract with LPS gave the maximum activity higher than that with PWM, suggesting specificity towards B cell proliferation through T cell independent pathway the same as LPS.
Auttachoat et al. (2004) studied the hematological and immunological effects of the Dok Din Daeng (DDD) (*Aeginetia indica* Roxbert) whole plant extract using water (WDDD) or ethanol (EDDD) as the solvent. The extracts were administered to female B6C3F1 mice by gavage for WDDD (10-100%) and intraperitoneally for EDDD (0.25-250 mg/kg) for 28 days. In addition to hematological evaluation, several quantitative measures and functional assays (e.g., the splenic phenotypic analysis, IgM antibody-forming cell responses, natural killer cell activity, mononuclear phagocyte system [MPS] and neutrophil activity) were employed to examine the effects of DDD extracts on the innate and humoral immunities. The results demonstrated that exposure to WDDD and EDDD produced minimal changes in the activities of B cells and natural killer cells, macrophages and neutrophils. Overall, hematological parameters were not affected by exposure to WDDD or EDDD.

Gautama et al. (2004) evaluated the immunoadjuvant potential of aqueous extract of *Asparagus racemosus* in Swiss albino mice immunised with diphtheria, tetanus, pertosis (DTP) vaccine. Administration of extract for 15 days resulted in an increase in antibody titre to *Bordetella pertussis* as compared to untreated (control) animals. Immunised animals (treated and untreated) were challenged with *B. pertussis* 18323 strain and the animals were observed for 14 days. Results indicated that the treated animals did show significant increase in antibody titres as compared to untreated animals after challenge, showing its immunoadjuvant property.

Manosroi et al. (2005) evaluated the immunomodulatory and antioxidant effect of the methanolic stem bark extract from *Pouteria cambodiana* in BALB/c mice. The extract exerted a dose-response effect in the peritoneal macrophage phagocytosis assay and also activated lysosomal enzyme activity. In the splenocyte proliferation assay, the extract without mitogen was active (EC50, 0.01 mg/ml) while the EC50 of the extract with (LPS) and (PWM) were 0.02 and 0.41 mg/ml, respectively. The extract showed low free radical scavenging activity in the DPPH radical assay being less active than ascorbic acid. The Thai folklore plant had potent immunological but no antioxidant activity and is applied in the treatment of fever and skin eruption.

Mishra et al. (2005) isolated nineteen compounds of various classes from *Desmodium gangeticum* whole plant and studied their immunomodulatory activities. The novel compound Aminoglucosyl glycerolipid exhibited in vitro antileishmanial and
immunomodulatory activities, as it enhanced nitric oxide (NO) production and provided resistance against infection established in peritoneal macrophages by the protozoan parasite *Leishmania donovani*. Another known compound, glycosphingolipid (cerebroside) also possessed significant in vitro antileishmanial and immunomodulatory activities against the same parasite. Other compounds were found to be inactive. Thus current study demonstrated the isolation and characterization of two biologically active glycolipids, which were possibly responsible for immunostimulatory activity of *Desmodium gangeticum*.

Nevin and Vijayanmal (2005) studied the immunomodulatory effect of *Aerva lanata* in Daltons lymphoma ascites bearing mice. The authors noticed that the extract exhibited toxicity towards the cell line and also showed stimulation in lymphocyte proliferation in both the in vitro as well as in vivo conditions.

Gabhe *et al.* (2006) studied the immunomodulatory activity of various extracts from the aerial roots of *Ficus benghalensis* using the in vitro polymorphonuclear leucocyte (human neutrophils) function test. The extract with maximum potency was also assessed for hypersensitivity reaction (DTH) and hemagglutination activity in rats. The results inferred that the methanol extract exhibited maximum immune potentiation effect both in in vitro as well as in vivo systems.

Shivaprasad *et al.* (2006) investigated the aqueous fruit extract of *Terminalia chebula* for its effect on cell-mediated and humoral components of the immune system in mice. Administration of *T. chebula* extract caused an increase in humoral antibody (HA) titer and delayed-type hypersensitivity (DTH) in mice. The study revealed that *T. chebula* extract produced a dose-dependent increase in both the parameters (i.e., antibody production and delayed-type hypersensitivity). It was concluded that the *T. chebula* is a promising drug with immunostimulant properties.

Shokri *et al.* (2006) tested the effects of *Zataria multiflora* on the function of innate immunity including phagocytic activity and TNF-alpha secretion in BALB/c mice. The results showed significant increase in phagocytic activity and TNF-alpha secretion by *Z. multiflora* treated group as compared to control. Thus *Z. multiflora* essence can stimulate remarkably innate immunity function and it may be used as therapeutic adjuvant alone or in combination with other immunostimulatory agents.
Sangvanich et al. (2007) studied the hemagglutinating activity of *Curcuma longa* plants. The crude proteins obtained by Mg/NP-40 extraction exhibited hemagglutinating activity against rabbit blood showing the immunomodulatory potential of the extract.

Herbert (2007) evaluated the medicinal importance of *Pelargonium* species, most notably *P. reniforme* and *P. sidoides*, by testing their extracts and isolated constituents for antibacterial activity and for their effects on nonspecific immune functions. The results showed that the samples exhibited merely moderate direct antibacterial capabilities against a spectrum of Gram-positive and Gram-negative bacteria. Various functional bioassays revealed significant immunomodulatory properties of these samples. Further ELISA confirmed the protein production of TNF-a, IL-1a and IL-12, while FACS analyses reaffirmed the cytokines IL-1a and IL-12 at the singular cell level. The current data provided convincing support for the improvement of immune functions at various levels.

Pinto et al. (2007) demonstrated the immunosuppressive effect of aqueous extract of *Echinodorus macrophyllus* in mice. The treatment with extract inhibited B cell antibody production, delayed type hypersensitivity reaction, reduced subcutaneous tissue infiltration and also inhibited NO production in J774 cells in a dose dependent manner.

Arulkumarran et al. (2007) investigated the effect of Kalpaannaruthaa (Siddha formulation of *Semecarpus anacardium* and *Phyllanthus emblica*) on cellular and humoral immune response in Albino Wister rats, immunised with SRBC. The test sample was found to enhance heamagglutination titre, delayed type hypersensitivity and phagocytic index, showing the immunostimulatory potential of extract.

Ismail et al. (2008) evaluated the anti-arthritic nature of evening primrose oil (EPO) on adjuvant-induced arthritic rats. The study revealed that oral administration of EPO exerted a significant elevation in serum IgG and IgM levels. In addition, normalization of body weight, serum IL-4 and TNF- levels were observed. In fact, oral supplementation of EPO showed homologous effects on the biochemical parameters studied as compared to the administration of diclofenac sodium with few qualitative and quantitative distinctions.
Gangwal et al. (2008) investigated the immunomodulatory effect of n-butanol soluble and ethyl acetate soluble fractions from methanolic extract of *Lagenaria siceraria* fruits in rats. Oral administration of these fractions significantly inhibited delayed type hypersensitivity reaction and also produced a dose-dependent increase in both primary and secondary antibody titres. Further the fractions also significantly increased the white blood cell and lymphocyte count.

Kim et al. (2008) evaluated the immunoregulatory effects of arctiin on the production of various kinds of cytokines in primary and cultured macrophage (with various doses of arctiin). The results showed that arctiin dose dependently decreased the expression levels of inducible NO synthase (iNOS), IL-1β, IL-6, and TNF-α gene expression and western blot analysis also supported that arctiin decreased expression levels of these cytokines. Thus arctiin has immunomodulating effects in both innate and adaptive immunity and could be a new suppressive immunomodulator.

Satpute et al. (2009) screened *Randia dumetorum* Lamk. methanolic fruit extract and its petroleum ether, chloroform, ethyl acetate and methanol fractions for immunomodulatory effects on cell mediated and humoral components of the immune system in mice. Administration of chloroform fraction at dose 100 mg/kg produced statistically significant results as evidenced by increase in humoral antibody (HA) titre and delayed type hypersensitivity (DTH) response. This fraction also enhanced the total WBC level in cyclophosphamide induced myelosuppression model. Petroleum ether fraction and methanol fraction affected only cell mediated immunity. The results therefore revealed that *R. dumetorum* holds promise as immunomodulatory agent.

Shukla et al. (2009) studied the immunomodulatory potential of ethanolic seed extract of *Caesalpinia bonduc* in rat model. The administration of extract increased the neutrophil adhesion to nylon fibres as well as dose dependent increase in antibody titre, potentiated delayed type hypersensitivity reaction and prevented myelosupression in cyclophosphamid treated rats.

Patil et al. (2009) evaluated the immunomodulatory activity of *Toxicodendron pubescens* in SRBC immunised C57/BL6 mice. The administration of plant sample at various dilutions intensified the antibody titre and delayed type hypersensitivity response in mice. It also stimulated the phagocytosis, candidacidal activity chemotaxis in human PMN cells.
Sharififar et al. (2009) evaluated the immunomodulatory potential of aqueous extract of top flowering of Achillea wilhelmsii in Swiss albino mice. The extract potentiated both the cellular as well as humoral immune immunity as was evidenced by enhanced DTH response and hemagglutination titre.

Chen et al. (2009) proved immunosuppressive activity of homoisoflavonoids isolated from the methanolic extract of leaves of Agave sisalana. The authors showed that homoisoflavones (±)-3,9-dihydroeucomin, dihydrobonducellin and 5,7-dihydroxy-3-(4-hydroxybenzyl)-4-chromanone suppressed the production of IL-2 and IFN-Y and thus showing immunosuppressive effects.

Sudha et al. (2010) investigated the immunomodulatory potential of methanolic extract of Moringa oleifera (MEMO) in experimental animal models of cellular and humoral immunity. Different immunological parameters revealed that MEMO stimulated both cellular and humoral immune response. However, low dose of MEMO was found to be more effective than the high dose. It was concluded that the test extract possessed promising immunostimulant properties.

Dhasarathan et al. (2010) studied the ethanolic extracts of Punica granatum L., Annona squamosa L. and Cucumis melo Blanco for their effect on cell mediated and humoral components of the immune system in mice. The results showed that the fruit extracts of Punica granatum, Annona Squamosa and Cucumis melo significantly stimulated both CMI and humoral immunity, as evidenced by the enhancement of T cell, B cell count, antibody titre, delayed type hypersensitivity and IgG concentration.

Patel and Asdaq (2010) assessed the immunomodulatory potential of methanolic extract of Aegle marmelos fruit (FEAM) in experimental model of immunity. FEAM produced significant increase in adhesion of neutrophils and an increase in phagocytic index in carbon clearance assay. Both high and low doses of FEAM significantly prevented the mortality induced by bovine Pasteurella multocida in mice. Treatment of animals with FEAM significantly increased the circulating antibody titre in indirect haemagglutination test. Thus FEAM possessed potential for augmenting immune activity by cellular and humoral mediated mechanisms.

Dashputre and Naikwade (2010) studied the immunomodulatory activity of aqueous and ethanol extracts of leaves of Abutilon indicum Linn. in albino mice. A. indicum showed a significant increase in both primary and secondary HA titre in
treated group and potentiated the DTH reaction by facilitating the footpad thickness response to SRBCs in sensitized mice. Also *A. indicum* evoked a significant increase in percentage of neutrophil adhesion to nylon fibers and phagocytic activity. The aqueous and ethanolic extracts of *A. indicum* leaves may be beneficial in the treatment of impaired immunity.

Yue *et al.* (2010) investigated the immunomodulatory activities of the polar fractions of *Curcuma longa* (CL) hot water extract using human peripheral blood mononuclear cells (PBMC). The results showed that the high polarity fraction of the hot water extract exhibited stimulatory effects on PBMC proliferation as shown by (methyl-3H)-thymidine incorporation assay. Thus crude extracts of *Curcuma longa* could be used as an adjuvant supplement.

Azadmehr *et al.* (2011) evaluated the in vivo immunomodulatory and in vitro anti-cancer effects of *Scrophularia megalantha* extract. The study revealed that in vitro exposures of the Jurkat cells to *S. megalantha* extract significantly suppressed their growth in a dose-dependent manner. Moreover, the production of specific antibody to SRBC antigen in immunized mice significantly increased by different concentrations of *S. megalantha* extract. Thus *S. megalantha* extract could be used to develop an immunomodulator and anticancer agent.

Siveen and Kuttan (2011) studied the in vivo immunomodulatory and antitumor activity of ethanolic extract of whole plant of *Aerva lanata*. Intraperitoneal administration of five doses of the extract was found to enhance the total WBC count and bone marrow cellularity. *Aerva* treatment also showed enhanced proliferation of splenocytes, thymocytes and bone marrow cells both in the presence and absence of specific mitogens in vitro and in vivo. The extract was 100% cytotoxic to Dalton's lymphoma ascites (DLA) and Ehrlich ascites carcinoma (EAC) cells. It was also found to be cytotoxic toward L929 and HELA cells. Thus *Aerva lanata* possessed promising immunomodulatory and antitumor properties.

Govinda and Asdaq (2011) investigated the immunomodulatory potential of methanol extract of *Aegle marmelos* in an experimental animal model of cellular and humoral immunity. Results showed that methanol extract of *Aegle marmelos* possessed immunomodulatory potential by stimulating cellular and humoral immune mechanisms. However, low dose of methanol extract of *Aegle marmelos* was more effective for
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augmenting cellular immunity, whereas, high dose was more inclined towards humoral immunity.

More and Pai (2011) evaluated the effect of *Tinospora cordifolia*, as an immunomodulator for activation of macrophages. The direct drug treatment to J774A cells showed activation as assessed by biochemical assays. Enhanced secretion of lysozyme by macrophage cell line J774A on treatment with *Tinospora cordifolia* and lipopolysacharide was observed, suggesting activated state of macrophages. The enhanced inhibitory effects of *T. cordifolia* (direct effect) and *T. cordifolia* treated cell supernatant (indirect effect) on the bacteria (*E. coli*) indicated the susceptibility of bacteria. The results proved experimental basis for immunomodulation by biological response modifier (BRM).

Zhu *et al.* (2012) evaluated anti-inflammatory and immunomodulatory effects of IGPS and its mechanism on UAN rats. The histopathology of renal tissues in UAN rats were assessed for conventional morphological evaluation. The results showed that IGPS exerted a protective effect against renal injury in UAN rats and possessed anti-inflammatory and immunomodulatory effects by inactivating NF-κBp65 pathway transmembrane signal transduction, down regulating the expression of MCP-1 and α-SMA to modulate pro-inflammatory mediator production in nephropathy tissue to improve renal fibrosis in UAN rats.

Santander *et al.* (2012) evaluated the immunomodulatory activity of aqueous and organic fractions from *P. alliacea* using human monocyte-derived dendritic cells. The phenotype, cytokine secretion and gene expression were estimated after treatment with the plant fractions. The study revealed that *P. alliacea* aqueous fraction induced morphological changes and co-stimulatory expression of CD86, indicating partial DC maturation. In addition, pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-10, IL-12p70, and TNF-α were secreted. The fraction also increased NF-κB gene expression while down-regulating TGFβ gene expression. It is important to state that the organic fraction by itself does not showed any immunomodulatory activity.

Zhao *et al.* (2012) studied the effects of a polysaccharide-protein complex from *Scolopendra subspinipes mutilans* L. Koch (SPPC) on the tumor growth and immune function in sarcoma S180 and hepatoma H22 bearing mice. The results showed that SPPC significantly inhibited the growth of S180 transplanted in mice and prolonged the
survival time of H22-bearing mice. In S180-bearing mice, it promoted specific and nonspecific immune response as evidenced by enhancing the activities of natural killer (NK) cells, cytotoxic T lymphocytes (CTL) and the ratio of Th1/Th2 cytokines. Furthermore, SPPC significantly inhibited mRNA expression and production of the immunosuppressive cytokines (IL-10 and TGF-β) in tumor-associated macrophages (TAMs). Taken together, results indicated that SPPC could act as an anti-tumor agent with immunomodulatory activity.

Banji et al. (2012) evaluated the immunomodulatory effects of alcoholic and hydro-alcoholic extract of leaves of *Moringa olifera* on various immune paradigms using SRBC as an antigen. The study revealed that hydro-alcoholic extract of *M. olifera* substantially enhanced cellular immune response, humoral immune response, neutrophil index and phagocytic activity at lower dose while as ethanolic extract was efficient immune regulator at higher dose. Thus *M. olifera* has a significant role to play as an immune stimulator.

Sharma et al. (2012) isolated immunomodulatory active compounds from *Tinospora cordifolia*. The immunomodulatory activity of different extracts, fractions and isolated compounds was investigated in human neutrophil cells using the PMN phagocytic function studies, NBT, NO and chemiluminescence assay. The results indicated that ethyl acetate, water fractions and hot water extract exhibited significant immunomodulatory activity with an increase in percentage of phagocytosis. Chromatographic purification of these fractions led to the isolation of a mixture of two compounds 2, 3 isolated for the first time from natural source and five known compounds. Thus seven immunomodulatory active compounds belonging to different classes isolated and characterised, indicating that the immunomodulatory activity of *T. cordifolia* may be attributed to the synergistic effect of group of compounds.

Daoudi et al. (2013) evaluated the immunomodulatory activity of protein extracts (PEs) of 14 Moroccan medicinal plants. All prepared extracts of plant samples were tested using MTT assay on the splenocytes with or without stimulation by concanavalin-A (Con-A), a mitogenic agent used as positive control. The results of the study indicated different activity spectra. Three groups of activities were observed. Therefore present study revealed an interesting immunomodulating action of certain PEs, as their traditional use.
2.2. Cancer and anti-cancer drug discovery from plants

Cancer is the general name for over 100 medical conditions involving uncontrolled and dangerous cell growth, a neoplastic condition resulting from genetic changes that control the proliferation, maturation, metastatic behaviour and senescence of cells. These errors may arise due to certain causes including mutation, chemical carcinogens, ionizing radiation, infection, hormonal imbalances, immune system dysfunction, heredity, environmental exposures, diet and exercise. Other cancer-promoting genetic abnormalities may randomly occur through errors in DNA replication, or are inherited, and thus present in all cells from birth. The heritability of cancers is usually affected by complex interactions between carcinogens and the host’s genome (Anand et al., 2008). Genetic abnormalities found in cancer typically affect two general classes of genes viz. Cancer-promoting proto-oncogenes and tumor suppressor genes. Most cancers can be treated and some cured, depending on the specific type, location, and stage (MacDiarmid et al., 2009) (Fig. 2.2.1).

Despite the progress made in targeted therapy drugs, cancer is still the point of concern for the world. According to a recent report by the World Health Organization, there are now more than 10 million cases of cancer per year worldwide. In 2010 cancer caused about 13% of all human deaths worldwide (7.9 million) and it is projected to be 12 million deaths per year by 2030. Hence at present, there is an urgent need for developing new approaches and drugs to prevent as well as cure this devastating disease (Coseri, 2009; Karikas, 2011). Considering that many chemotherapeutic agents against tumor cells without sparing normal cells remain a major obstacle and development of multidrug resistance further limits chemotherapy in cancer. Within the scientific community, interest in natural compounds is increasing and now a days newer drug are being prepared by using the natural basic skeleton of an isolated component that targets the unique makeup mechanism of cancer cells.
2.2.1 Hallmarks of cancer

The hallmarks of cancer comprise of six biological capabilities acquired during the multistep development of human cancers. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Conceptual progress in the last decade has added two emerging hallmarks of potential generality to this list i.e. reprogramming of energy metabolism and evading immune destruction (Douglas and Robert, 2011) (Fig. 2.2.2.). Cancer results from a multistage, multi-mechanism carcinogenesis process that involves mutagenic cell death and epigenetic mechanisms, during the three distinguishable but closely allied stages: initiation, promotion, and progression. Since reducing the initiation phase to a zero level is impossible, the most effective intervention would be at the promotion phase to eliminate premalignant cells before they become malignant (Trosko, 2005).

Recent developments in cancer treatment have led to greatly improved survival and quality of life for cancer patients in the past three decades. The most important therapies employed for the treatment of cancer are: surgery, radiation
therapy, cancer vaccines, hormonal therapy, gene therapy, immunotherapy, chemotherapy and herbal therapy.

![Diagram of cancer hallmarks]

**Fig. 2.2.2. Hallmarks of the cancer.**

### 2.2.2. Cancer chemo prevention with dietary phytochemicals

The field of cancer chemoprevention began in 1966, when Lee Wattenberg demonstrated that compounds associated with fruits and vegetables (indoles and isothiocyanates) could prevent cancer development in an animal model. Many mechanisms have been shown to account for the anticarcinogenic actions of dietary constituents, but attention has recently been focused on intracellular-signalling cascades as common molecular targets for various chemopreventive phytochemicals. The concept of delaying or preventing this transformation remains a viable and attainable goal for the future (Brenner and Gescher, 2005; Manson *et al.*, 2000). Natural products consist of a wide variety of biologically active phytochemicals including phenolics, flavonoids, carotenoids and alkaloids which have been shown to suppress early and late stages of carcinogenesis. The American National Cancer Institute has identified about 35 plant-based foods containing 1,000 different phytochemicals that possess cancer-preventive properties. The most exciting findings have been achieved with antioxidant and their precursors, which are found in dark, leafy green vegetables and coloured fruits (Karikas, 2010). It seems that most dietetic products with anticancer activity act as strong antioxidants and modify the activity of one or more protein kinases involved in cell cycle control. Kinases such as protein kinase A, protein kinase B, protein kinase C,
JNK-1, CDK-2, and CDK-4 are either activated or deactivated by these antioxidants, as shown in Fig. 2.2.3. Phytochemicals in fruit and vegetables help to metabolize drugs, toxins, carcinogens and mutagens by neutralizing free radicals, inhibiting carcinogen activating enzymes and inducing carcinogen inactivating enzymes (Young-Joon, 2003; Anne et al., 2004; George, 2011).

Fig. 2.2.3. Effect of dietary phytochemicals on Cancer cells.

2.2.3. Plants as anticancer agents

An alternative solution to Chemo. and Radiotherapy embodied with many side effects is the use of medicinal plant preparations to arrest the insidious nature of the cancer disease. Throughout history, natural products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy and biochemistry (Karikas, 2010). This is especially obvious in the case of antitumor drugs, as exemplified by paclitaxol, taxol, vincristine vinblastine, teniposide etc.

Natural products or related substances or extracts of folk medicine accounted for 30% of the top 35 worldwide natural product-based drugs sold in recent years (Butler, 2004). It is, for instance, only upon the addition of the Vinca alkaloid vincristine or oncovin isolated from Catharanthus roseus, (Johnson et al. 1963) to mechlorethamine, prednisone, and procarbazine (the MOPP regimen) that the first cures in a human cancer (Hodgkin's disease) were achieved (Devita et al., 1970). Homoharringtonine is an alkaloid isolated from the Chinese tree Cephalotaxus harringtonia and has shown
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efficacy against various leukemias (Kantarjian et al., 1996). 4-Ipomeanol is a
pneumotoxic furan derivative isolated from the sweet potato (Ipomoea batatas,
Convolvulaceae) that has been under clinical evaluation as a lung-cancer-specific
antineoplastic agent (Rowinsky et al., 1993). Turmeric has been shown to possess
variety of pharmacological properties such as anti-inflammatory, anti-carcinogenic and
anti-oxidant. Turmeric also activates the lymphocytes and induces apoptosis of tumor
cells (Yasmin et al., 1998).

Kavimani et al. (1999) evaluated the antitumour activity of the methanolic
extract of Glinus lotoides (MGL) against Dalton's ascitic lymphoma (DAL) in Swiss
albino mice. A significant enhancement of mean survival time of tumor bearing mice
and inhibition of tumor cell growth in treated animals was observed with respect to the
control group; MGL also was able to reverse the changes in the haematological
parameters, protein and packed cellular volume consequent to tumour inoculation.

Prakash et al. (2002) investigated chemopreventive effect of hydroalcoholic root
extract of Withania somnifera (WSRE) on 7, 12-dimethylbenz[a]anthracene (DMBA)-
induced skin cancer in Swiss albino mice. The results of the study showed a significant
decrease in incidence and average number of skin lesions in mice compared with
DMBA alone. Also a significant impairment was noticed in the levels of reduced
 glutathione, malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase,
and glutathione S-transferase in skin lesions of DMBA-treated control mice compared
with vehicle-treated mice. The above findings were supported by histopathological
studies and thus study showed that WSRE possesses potential chemopreventive activity
in this experimental model of cancer.

Ruffa et al. (2002) evaluated cytotoxic effect of Argentine medicinal plant
extracts on human hepatocellular carcinoma cell line under in vitro test system.
The methanolic extracts were prepared from Achyrocline satureioides (DC.) Lam,
Aristolochia macroura Gómez, Lithraea molleoides (Vell.) Engl. and Schinus molle L.
The results inferred that the almost all sample extracts showed cytotoxic activity against
a human hepatocellular carcinoma cell line, Hep G2, but Schinus molle L. was the most
active (IC50=50+/-7 microg/ml).

Gururaj et al. (2002) investigated effect of curcumin on the growth of Ehrlich
ascites tumour (in-vivo), endothelial cells (in-vitro) and regulation of tumour
angiogenesis and their receptor gene expression. The study showed that curcumin, when injected intraperitoneally (i.p) into mice, effectively decreased the formation of ascites fluid in EAT bearing mice in vivo. The reduction in number of EAT cells and human umbelical vein endothelial cells (HUVECs) in vitro by curcumin was attributed to induction of apoptosis as evidenced by FACS analysis. However, curcumin had no effect on the growth of NIH3T3 cells. Curcumin proved to be a potent angioinhibitory compound, as demonstrated by inhibition of angiogenesis in two in vivo angiogenesis assay viz. peritoneal angiogenesis and chorioallantoic membrane assay. The angioinhibitory effect of curcumin in vivo was corroborated by results on down-regulation of the expression of proangiogenic genes, in EAT, NIH3T3, and endothelial cells. Because of its non-toxic nature, curcumin could be developed chemopreventive agent.

Bhattacharyya et al. (2003) delineated the apoptogenic effect of black tea extract in Ehrlich's ascites carcinoma (EAC)-bearing Swiss albino mice. Black tea administration to EAC-bearing mice caused a significant decrease in the tumor cell count in a dose-dependent manner. Flowcytometric analysis showed an increase in the number of cells in the sub-G (0)/G (1) population signifying tumor cell apoptosis by black tea. These results were further confirmed by nuclear staining that demonstrated distinct morphological features of apoptosis. The data also showed an increase in the expression of pro-apoptotic protein p53 in EAC. Interestingly, anti-apoptotic protein Bcl-2 was down regulated resulting in decrease in Bcl-2/Bax ratio. All these observations together signify that black tea-induced apoptogenic signals overrode the growth-arresting message of p21, thereby leading the tumor cells towards death.

Furusawa et al. (2003) studied antitumor potential of a polysaccharide-rich substance from the fruit juice of Morinda citrifolia (Noni) on sarcoma-180 ascites in mice. The antitumor activity of polysaccharide-rich substance Noni produced a cure rate of 25%-45% in allogeneic mice and its activity was completely abolished by the concomitant administration of specific inhibitors of macrophages, T cells or natural killer cells. Noni showed synergistic effects when combined with a broad spectrum of chemotherapeutic drugs. Noni also demonstrated beneficial effects when combined with the Th1 cytokine, interferon gamma, but its activity was abolished when combined with
Th2 cytokines, interleukin-4 or interleukin-10, thereby suggesting that Noni-ppt induces a Th1 dominant immune status in vivo.

Gupta et al. (2004a) investigated the antitumor and antioxidant status of methanol extract of Bauhinia racemosa stem bark against the Ehrlich ascetic carcinoma (EAC) in mice. The administration of extract for 14 days showed decrease in tumor volume, packed cell volume and viable cell count, and increase in the mean survival time thereby increasing lifespan of EAC tumor bearing mice. Further treatment with this extract decreased lipid peroxidation and increased the levels of glutathione, superoxide dismutase and catalase and augmenting antioxidant defense system in EAC bearing mice.

Gupta et al. (2004b) studied the effect of methanol extract of Caesalpinia bonducella leaves (MECB) for antitumor activity against Ehrlich ascetic carcinoma (EAC) bearing mice. MECB caused significant decrease in tumor volume, packed cell volume, and viable cell count; and it prolonged the life span of EAC-tumor bearing mice. Hematological profile converted to more or less normal levels in extract-treated mice. MECB significantly decreased the levels of lipid peroxidation and effectively increased the levels of GSH, SOD, and CAT. Thus MECB exhibited significant antitumor and antioxidant activity in EAC-bearing mice.

Nguyen et al. (2004) evaluated the cell viability and mechanistic study of natural product quercetin against A549 lung carcinoma cells. The study showed that treatment of A549 cells with quercetin resulted in a dose-dependent reduction in cell viability and DNA synthesis. Quercetin also induced the cleavage of caspase-3, caspase-7 and PARP (poly ADP-ribose polymerase). The results suggested that in addition to inactivation of Akt-1 and alteration in the expression of the Bcl-2 family of proteins, activation of MEK-ERK is required for quercetin-induced apoptosis in A549 lung carcinoma cells.

Yuan et al. (2004) investigated the effects of tanshinone II-A (an alcohol-extracted product from roots of Salvia miltiorrhiza) Bange on inducing growth inhibition and apoptosis in human hepatocellular carcinoma (HCC) cells. The growth and proliferation of SMMC-7721 cells were found to supress in a dose and time-dependent manner. The morphology of cellular growth inhibition and characteristics of apoptosis were observed under light and transmission electron microscopes. The cells were arrested in G0G1 phase and expression of apoptosis related genes bcl-2 and c-myc were down-regulated and fas, bax, p53 up-regulated.
López et al. (2005) investigated the anticancer potential of dichloromethane extract of leaves of *Lithraea molleoides* and its compounds against human hepatocellular carcinoma cell line. The study revealed that dichloromethane leaf extract showed a profound cytotoxicity on human hepatocellular carcinoma cell line. Bioactive guided fractionation of this extract led to the isolation of a new bioactive 5-alkyl resorcinol: 1,3-dihydroxy-5-((tridec-4',7'-dienyl) benzene. This novel compound showed cytotoxic activity on 3 human tumoral cell lines: hepatocellular carcinoma cell line-Hep G2, mucoepidermoid pulmonary carcinoma cell line-H292 and mammary gland adenocarcinoma cell line -MCF7. Thus leaves of *Lithraea molleoides* contain promising anticancer molecules.

Deep et al. (2005) evaluated the chemopreventive potential of Triphala (a popular formulation of the Ayurvedic system of medicine) in tumor bearing mice. The results showed that Triphala in diet significantly reduced the benzo(a)pyrene [B(a)P] induced forestomach papillomagenesis in mice. It was observed that Triphala was more effective in reducing tumor incidences compared to its individual constituents. Triphala also significantly increased the antioxidant status of animals which might have contributed to the chemoprevention. The results inferred that the concomitant use of multiple agents seemed to have a high degree of chemoprevention potential.

Padmavathi et al. (2005) studied the effect of dietary administration of *Withania somnifera* roots on hepatic phase I, phase II and antioxidant enzymes as well as in attenuating carcinogen- induced forestomach and skin tumorigenesis in the Swiss albino mice. The results showed that roots of *W. somnifera* inhibited phase I, and activated phase II and antioxidant enzymes in the liver. In a long-term tumorigenesis study, *Withania somnifera* roots inhibited benzo(a)pyrene-induced forestomach papillomagenesis, and skin papillomagenesis, showing inhibition in tumor incidence and multiplicity.

Xia et al. (2005) investigated mechanism of cytotoxic effect of the boswellic acid acetate (isolated from *Boswellia carterri* Birdw) on six human myeloid leukemia cell lines (NB4, SKNO-1, K562, U937, ML-1, and HL-60 cells). The morphologic and DNA fragmentation assays indicated that the cytotoxic effect of boswellic acid acetate was mediated by induction of p53 independent apoptosis. Boswellic acid acetate also induced Bid cleavage and decreased mitochondrial membrane potential without
production of hydrogen peroxide. These results suggested that boswellic acid acetate induced myeloid leukemia cell apoptosis through activation of caspase-8 by inducing expression of DR4 and DR5.

Nakatani et al. (2005) studied the effects of uvaretin, isouvaretin and diuvaretin isolated from Uvaria in human promyelocytic leukaemia cell line. Hoechst-33258 staining showed the presence of apoptotic population after treatment with isolated components. The components also exhibited DNA fragmentation and induction of caspase-3. The cytotoxicity of uvaretin and diuvaretin was stronger than isouvaretin.

Senthilnathan et al. (2006) studied the chemotherapeutic effect of a combination of paclitaxel with W. somnifera against lung cancer in mice model. The serum, lung and liver were investigated biochemically for various enzymes and other markers of cancer. Administration of paclitaxel, with W. somnifera extended its chemotherapeutic effect through modulating protein-bound carbohydrate levels and marker enzymes, as they are indicators of cancer. The results showed that combination of paclitaxel with W. somnifera could effectively treat the benzo(a)pyrene-induced lung cancer in mice by offering protection from reactive oxygen species damage and also by suppressing cell proliferation.

Meena et al. (2006) investigated the chemopreventive activity of Acacia nilotica (L.) Willd. ex Delile gum, flower and leaf aqueous extracts, on 7,12-dimethylbenz(a)anthracene (DMBA) induced skin papillomagenesis in male Swiss albino mice. The study revealed a significant reduction in the values of tumor burden, tumor incidence and cumulative number of papillomas observed in mice treated with the A. nilotica gum, flower and leaf extracts as compared with the control group. The chemopreventive and antimutagenic activity of the leaf extract of Acacia nilotica was most significant followed by the flower extract and then by gum.

Barbini et al. (2006) studied the mechanism of cytotoxicity of a new bioactive compound 5-alkyl resorcinol [1, 3-dihydroxy-5- (tridec-4’, 7’-dienyl) benzene] isolated from Lithraea molleoides leaves on human hepatocarcinoma cell lines (HepG2 and Hep3B). The cell lines were treated with inhibitory concentrations, 50% of the compound, for 24 h and induction of apoptosis was detected in treated cells by analysis of DNA fragmentation, DNA content, and acridine orange and propidium iodide staining. The results showed that after 24 h of 5-alkyl resorcinol treatment, both cell
lines showed the typical morphological alterations of apoptosis; DNA fragmentation, appearance of a subG0 population and condensed and fragmented nuclei. Thus compound exerted its cytotoxic effect in both hepatocellular cell lines through apoptotic cell death.

Wang et al. (2006) studied in vitro and in vivo, anticancer activity of Litchi fruit pericarp LFP extracts on human breast cancer and elucidated its mechanism of action. LFP extract demonstrated a dose- and time-dependent inhibitory effect on cell growth and it significantly inhibited colony formation and BrdU incorporation of human breast cancer cells. A 40.70% tumor mass volume reduction and significant increase of casepase-3 protein expression were observed in in-vivo experiment. The results of the study suggested that LFP extract might have potential anticancer activity on both ER positive and negative breast cancers, which could be attributed, in part, to its DNA damage effect, proliferating inhibition and apoptosis induction of cancer cells.

Kumarappan and Mandal (2007) studied polyphenolic extract (PPE) of the leaves of Ichnocarpus frutescens for in vivo antitumor activity using murine Ehrlich ascites carcinoma (EAC) model and in vitro cytotoxicity in U-937 monocytoid leukemia and K-562 erythroleukemia cell lines. The results of in vivo study showed a significant decrease in tumor volume, viable tumor cell count and a significant increase of life span in the PPE treated group compared to untreated one. PPE effectively inhibited in vitro proliferation of U-937 and K-562 cell lines and also exhibited pronounced radical scavenging activity.

Faried et al. (2007) isolated the natural antioxidant gallic acid (GA) from fruits of an Indonesian medicinal plant Phaleria macrocarpa (Scheff) Boerl and studied its anticancer potential. GA demonstrated a significant inhibition of cell proliferation in a series of cancer cell lines and induced apoptosis in esophageal cancer cells (TE-2) but not in non-cancerous cells (CHEK-1). Observation of the molecular mechanism of apoptosis showed that GA up-regulated the pro-apoptosis protein, Bax, and induced caspase-cascade activity in cancer cells. On the other hand, GA down-regulated anti-apoptosis proteins such as Bcl-2 and Xiap.

Ozaslan et al. (2007) investigated the antitumor activity of Plantago major L. extract in Ehrlich ascites tumor (EAT) bearing Balb/C mice. The results showed minimal weight gain was recorded in treatment Groups as compared to the negative
control group. Pathological studies showed that *P. major* L. extract has an inhibitory effect on EAT in a dose dependent manner.

Zhao et al. (2007) studied the in vitro anticancer and immunomodulatory activities of flavonoids extracted from litchi (*Litchi chinensis* Sonn.) pericarp. The litchi pericarp extract was subjected to partition by hexane, ethyl acetate and water. Epicatechin, proanthocyanidin B2 and proanthocyanidin B4 were isolated from ethyl acetate fraction. Epicatechin and proanthocyanidin B2 had lower cytotoxicities to human breast cancer cell MCF-7 and human embryonic lung fibroblast than reference standard paclitaxel. The immunomodulatory activities of isolated components and the ethyl acetate fraction were examined using proliferation of mouse splenocytes and results showed all these samples had much higher stimulatory effects on splenocyte proliferation than that of the reference, rutin.

Garg et al. (2008) studied the effect of dietary curcumin (turmeric) on benzo(a)pyrene induced tumor. The results highlighted that curcumin mediated the activation of Nr12, leading to increased detoxification of B(a)P. The study also showed that curcumin mediated the decrease in B(a) P- induced phase I enzyme and concomitantly induced phase II enzymes.

Cochrane et al. (2008) investigated the anticancer effects of alcoholic extracts prepared from leaves, pulp and seeds of *Annona glabra* (pond apple), on human leukemia cell lines. The results inferred that seed extract was more potent than leaf and pulp extracts. Treatment of CEM and CEM/VELB cells with seed extract induced apoptosis and necrosis in both sensitive and resistant leukemia cells in a concentration dependent manner and also up-regulated the expression of cyclin kinase inhibitor (WAF1/p21) contributing to the arrest of cells at the G0/G1 phase of the cell cycle. Thus present study supported the traditional use of *Annona glabra* (pond apple).

Muthuraman et al. (2008) evaluated the antitumour and antioxidant potential of the ethanol extract of *Tragia plukenetii* (ETP) in Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. The ethanolic extract effectively enhanced the life span and decreased the tumor volume, and viable cell count. The ETP brought back the altered levels of the hematological and antioxidant parameters in a dose dependent manner in EAC bearing mice.
Mothana et al. (2009) investigated 26 medicinal plants belonging to 17 families collected from Yemen (Soqotra Island) for their in vitro anticancer and antioxidant activities. Evaluation for in vitro anticancer activity was done against three human cancer cell lines (A-427, 5637 and MCF-7) by using an established microtiter plate assay based on cellular staining while as antioxidant activity was investigated by measuring the scavenging activity of DPPH radical. The results proved that notable cancer cell growth inhibition was observed for extracts from *Ballochia atrovirgata*, *Eureiandra balfourii* and *Hypoestes pubescens*. In addition, the methanolic extracts of few plant samples showed good antioxidant potential at low concentrations.

Manoharan et al. (2009) documented the chemopreventive potential of curcumin and piperine against 7,12-dimethylbenz[a]anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Oral administration of curcumin and piperine to DMBA-painted hamsters on alternate days to DMBA painting for 14 weeks completely prevented the formation of oral carcinoma. Also, curcumin and piperine restored the status of lipid peroxidation, antioxidants and detoxifying agents in DMBA-painted hamsters.

Arung et al. (2009) evaluated anticancer properties of diethyl ether extract of wood from Sukun (*Artocarpus altilis*) in human breast cancer cells (T47D). The treatment of cells with the extract resulted in significant decrease in cell viability as measured by MTT assay. The nuclear morphology showed the presence of apoptotic bodies in treated cells, whereas, the cell cycle analysis revealed that number of cells in sub-G1 phase rose with increasing extract concentration. Thus Sukun wood inferred anticancer potential.

Magesh et al. (2009) tested the antitumor effects of crocetin against lung cancer-bearing Swiss albino mice. The results indicated that crocetin was capable of inhibiting tumour by inhibiting proliferating cells, glycoprotein and polyamine synthesis. The level of lipid peroxidation (LPO) and marker enzymes markedly increased in carcinogen administered animals, which was brought back to near normal by crocetin treatment. The activities of the enzymic antioxidants and glutathione metabolizing enzymes were decreased in B(a)p induced animals and increased upon drug treatment. Crocetin profoundly reverted back the pathological changes observed in cancerous animals.
Aguero et al. (2010) studied the antitumor effect of Phenoxodiol (synthetic analog of Genistein) against prostate cancer cell lines using various mechanistic assays. The results showed that Phenoxodiol treatment promoted a marked inhibition of proliferation and loss of colony formation in LNCaP cells in a dose- and time-dependent manner. Similar effects were also observed in the metastatic prostate cell lines PC3 and DU145. Oral administration of Phenoxodiol induced a considerable growth inhibition of malignant tumors generated by inoculation of LNCaP cells into Balb/c nu/nu athymic mice. The data demonstrated that Phenoxodiol promotes apoptosis, as determined by PARP-1 degradation, via mitochondrial depolarization and G1/S cell-cycle arrest thereby confirming that it is active against androgen-dependent and independent prostate cancer cells.

López-Lázaro et al. (2010) evaluated the effect of dietary flavonoids quercetin, apigenin, fisetin and myricetin on DNA topoisomerases (topo I and topo II) in K562 human leukemia cells using the cell-based TARDIS assay. The study revealed that the flavonoids quercetin and apigenin induced moderate levels of topo.II-DNA complexes and did not induce topo.I-DNA complexes in these cells. Fisetin induced neither topo.I-nor topo. II-DNA complexes, but behaved as a catalytic inhibitor of both enzymes. Myricetin induced high levels of topo-DNA complexes with both enzymes. The results supported the idea that specific concentrations of some dietary flavonoids may produce topoisomerase-mediated chemotherapeutic effects in vivo.

Svejda et al. (2010) studied the effects of novel plant extracts from *Trailliaedoxa gracilis* in SI-NET cell line KRJ-I and in the KRJ-I transplanted mice. The proliferation and viability were analyzed using cell counting and WST-1 cell proliferation assay. Apoptosis was determined by DAPI staining and electron microscopy, and quantified by luminescence assays for caspases. The results revealed that extracts of *Trailliaedoxa gracilis* showed a dose-dependent reduction of proliferation and induction of apoptosis in the KRJ-I cells. Tumor growth inhibition was also observed in heterotransplanted SCID mice. The in vitro and in vivo outcomes suggested a potential clinical effect of *Trailliaedoxa gracilis* in SI-NETs.

Talib and Mahasneh (2010) screened forty four extracts from sixteen plants for their *in vitro* antiproliferative activity against Hep-2, MCF-7, and Vero cell lines. The results showed that 20 of these extracts demonstrated significant antiproliferative
activity against one or more of the cell lines. Methanol fractions of *Ononis hirta* (aerial parts) and *Inula viscosa* (flowers) were the most active fractions against MCF-7 cells and less toxic against other cell lines. TLC analysis showed presence of flavonoids and terpenoids in active plant samples.

Yasukawa et al. (2010) investigated the in vivo anti-tumor activity of methanolic flower extract of artichoke (*Cynara cardunculus*) in 7,12-dimethylbenz[a]anthracene induced mice model. The study showed that methanolic flower extract of artichoke exhibited remarkable antitumor activity in an in vivo two-stage carcinogenesis test. Further from the active fraction of the methanol extract, four triterpene alcohols and their corresponding acetates were isolated and identified. These compounds were evaluated for their inhibitory effects on TPA-induced inflammation (1 μg/ear) in mice and results showed marked anti-inflammatory effects, with a 50% inhibitory dose of 0.50–0.91 μmol/ear.

Mishra et al. (2011) investigated in vitro anticancer potential of seed extract of *Ziziphus mauritiana* against different cell lines (HL-60, Molt-4, HeLa, and normal cell line HGF) by MTT assay as well as in vivo against Ehrich ascites carcinoma bearing Swiss albino mice. The extract was found to markedly inhibit the proliferation of HL-60 cells in a dose-dependent manner. The cell cycle analysis revealed a prominent increase in sub G0 population and Agarose gel electrophoresis confirmed DNA fragmentation in HL-60 cells after 3 h incubation with extract. Further treatment of Ehrlich ascites carcinoma bearing Swiss albino mice with varied doses of plant extract significantly reduced tumor volume and viable tumor cell count and improved mean survival time, tumor inhibition, and per cent life span.

Sharma et al. (2011) evaluated in vitro cytotoxicity of methanolic extract of *Glochidion zeylanicum* (Gaertn) A. Juss. roots using human cancer cell lines HepG2, HT-29 and PC-3 for its effects on cell viability, growth inhibition and cell morphology. The results showed decreased cell viability and increased growth inhibition as determined by XTT-assay and also altered cell morphology by DAPI staining technique after treatment with the extract. Thus data demonstrated that methanolic extract of roots of *G. zeylanicum* has a potential cytotoxicity activity on HepG2, HT29 and PC3 cells, but the effect was more significant on PC3 cell line.
Saha et al. (2011) investigated the antitumor activity of methanol extract of *Cucurbita maxima* Duchesne aerial parts (MECM) on Ehrlich Ascites Carcinoma (EAC) model in mice. The effect of drug response was made by the study of tumor growth response including increase in life span, study of haematological parameters, biochemical estimations, antioxidant assay of liver tissue and in vitro cytotoxicity. Experimental results revealed that *C. maxima* possessed significant anticancer activity and antioxidant properties.

Motaal and Shakerb (2011) studied different parts of the fruit of *Punica granatum* L. cultivated in Egypt for their anticancer and antioxidant properties. The peel extract showed the highest antioxidant activity compared to the other two extracts, as well as, a pronounced anticancer activity against MCF-7 human breast cancer cells and HCT-116 colon cancer cells. The study indicated that peel extract of Egyptian pomegranate could be used in the preparation of dietary supplements.

Viral et al. (2011) evaluated the phytochemical and anticancer activity of *Adiantum venustum* Don against Ehrlich Ascites Carcinoma in animal model. The study indicated that ethanolic extract of *A. venustum* possessed significant anticancer activity and also reduces elevated level of lipid peroxidation due to the presence of terpenoids and flavonoids.

Abiodun et al. (2011) evaluated ten Nigerian medicinal plants for their phytochemical and anticancer studies using established standard procedures. The phytochemical study revealed the presence of different classes of molecules such as alkaloids, saponins, tannins and flavonoids. *Anona muricata*, *Andrographis paniculata* and *Garcinia kola* were active against the lung cancer cell lines at various concentrations. The study has shown the anticancer activity of some of the plant samples and thus proved a strong validation for the use of the extracts of the plant for the treatment of cancer.

Liu et al. (2011) studied the cytotoxicity and anti-innovassive effect of plant-derived agent berberine against HepG2 cells. The study demonstrated that berberine exhibited significant cytotoxicity in HepG2 cells mainly through upregulation of reactive oxygen species (ROS) production but was ineffective on normal Chang liver cells. Berberine exerted anti-invasive effect on HepG2 cells through suppression of matrix metalloproteinase-9 (MMP-9) expression. Moreover, berberine significantly
inhibited the activity of PI3K-AKT and ERK pathways. Enhancement of ROS production by berberine had no influence on its suppressive effects on the activity of PI3K-AKT and ERK pathways, as well as MMP-9 expression and HepG2 cell invasion. The results suggested that berberine could be a potential alternative against invasive hepatoma cells through PI3K AKT and ERK pathways-dependent downregulation of MMP-9 expression.

Aboul-Enein et al. (2012) tested ethanol and aqueous extracts of many Egyptian spices and herbs for their in vitro anticancer potential against Ehrlich ascites Carcinoma Cells (EACC) and HepG2 cells. Results showed that both of ethanol and water extracts of some plants possessed high cytotoxic and antioxidant activities and inhibited the cell growth of cancer cells.

Saroja et al. (2012) evaluated antitumor activity of a flavonoid fraction of Terminalia catappa (TcFf) against Ehrlich Ascites Carcinoma (EAC) in mice. After administration of the last dose followed by 18h fasting, mice were sacrificed for observation of antitumor activity for each treatment period. The activity of enzymic and non enzymic antioxidants in the liver homogenate of control and experimental mice were also determined. Administration of TcFf significantly altered the antioxidants level and MDA to normal level. The result suggested that flavonoid fraction of Terminala catappa exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defence system in EAC bearing mice.

Pradhan et al. (2012) investigated the anticancer activity of Limonia acidissima Linn. (Rutaceae) against human breast cancer cells- SKBR3 and MDA MB435. The results of the extract of Limonia acidissima showed that fraction 3 of the ethanol extract had anticancer activity against SKBR3 and MDA-MB435 human breast cancer cells. In MDA-MB435 cells, cell cycle analysis showed that fraction 3 induced the accumulation of cells in G2/M phase, but no significant change in cell cycle was detected in SKBR3 cells.

Ravichandran et al. (2012) studied Phyllanthus maderaspatensis L. methanol extract for phytochemical analysis and in vitro cytotoxicity. Preliminary phytochemical analysis of the P. maderaspatensis methanol extract revealed the presence of tannins, triterpenoids, flavonoids, proteins and carbohydrates. Trypan blue method was employed to assess the cytotoxic potential of the plant extract. The results of in vitro...
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cytotoxicity study depicted that the methanol extract of *P. maderaspatensis* possessed good cytotoxic potentials at higher concentration (93.62% of inhibition observed in 1000 μg/ml). The activity might be due to the presence of secondary metabolites such as flavone and phyllanthin present in this extract.

Guo *et al.* (2013) studied the anti-cancer activity of licorice total flavonoids (LTFs) from *Glycyrrhiza inflata* Licorice, on cervical carcinoma cells. After determination of LTFs content by high-performance liquid chromatographic (HPLC), the finger print map was characterized by Electro Spray Mass Spectrometry. SiHa cervical carcinoma cells were treated with LTFs, and cell viability and cellular apoptosis were determined by both MTT method and flow cytometry in the dose range of 0 to 500 μg/ml LTFs. The results showed that cell viability dropped gradually with the LTFs treatment, the lowest level by MTT detection was 12%; in the dose range of 0 to 1000 μg/ml LTFs, the ratio of cellular apoptosis induced by LTFs increased gradually, the highest level by flow cytometry detection was up to 78%. It can be concluded that LTFs had relatively high anti-cancer activity on cervical carcinoma cells including the inhibition of cell growth and viability by induction of cellular apoptosis.

Sankara Aditya *et al.* (2013) evaluated the anticancer properties of three plants *Rubia cordifolia*, *Plumbago zeylanica*, *Calophyllum inophyllum* respectively by standard MTT assay against MCF-7 and HT-29 cell lines. The authors found that *Rubia cordifolia* and *Plumbago zeylanica* showed nearly 50 % MCF-7 cell line inhibition at 200μg/ml tested dose, whereas, these plant species did not display much anticancer activities against HT-29 colon cancer cell line.
2.3. Plant profile: *Prunus cerasus* L.

| Kingdom:  | *Plantae* |
| Subkingdom: | *Viridaeplantae* |
| Class: | *Spermatopsida* |
| Order: | *Rosales* |
| Family: | *Rosaceae* |
| Genus: | *Prunus* |
| Species: | *cerasus* |

**Common Names:** Sour Cherry, Morello Cherry, Pie Cherry, Tart Cherry  
**Parts used:** Fruit, leaf, fruit stalk, bark, kernel.  
**Distribution:** Native to Europe, Southwest-Asia; Kashmir in India.

### 2.3.1 Botanical description

There are 270 varieties of sour cherries, a handful of which are of commercial importance. The sour cherry is medium sized tree (Shrub) with more spreading habit, smaller than the erect sweet cherry (*Prunus avium*) and is more tolerant of extremes in temperature. The trees may reach 12 meters in height, with a trunk diameter of 30 to 45 cm. The bark is greyish-brown, flowers are white to pale pink, and leaves are ovate with serrated edging. Sour cherry fruits can grow to 20 mm in length and 18 mm in width. They are cordate drupes, with colour ranging from light to dark red. This fruit envelops a light brown seed (Fig. 2.3.1).

### 2.3.2 Traditional and ethnomedicinal uses

Cherry has been used as a medicinal plant for a long time in Asia. Red cherry fruits are used in a traditional herbal remedy for various diseases such as heart failure, beriberi, dropsy, mastitis, cystitis and urine retention. The stalk from sour cherry has been used medicinally as an astringent. Bark and stem of this cherry tree are used for detoxification and relaxation. The bark of sour cherry is astringent, bitter and an infusion of this bark has been used in the treatment of fevers, coughs and colds. The seed (pit) is nervine and an edible drying oil obtained from these seeds is also used in cosmetics. An infusion of sour cherry leaves is given for convulsions in children. A
green dye can be obtained from the leaves which can be used as a natural colouring agent (Ponnuswamy and Devairrakam, 2011). Heartwood is used in skin eruptions, erysipelas, obstinate skin diseases, haemorrhagic diseases. As a tonic for promoting conception.

2.3.3 Chemical constituents

The leaves, fruits and bark give flavone glycosides. The bark contains 5-7% tannin. The kernel contains a considerable proportion of hydrocyanic acid. The leaves contain a nitrile glycoside amygdalin. Prunus cerasus fruits produces various kinds of polyphenolics that include cyanidin derivatives (mostly cyanidin 3-glucosylrutinoside, cyanidin 3-rutinoside, cyanidin sophoroside), peonidin 3-glucoside; kaempferol, quercetin, and isorhamnetin and their derivatives, chlorogenic acid, gallic acid, p-coumeric acid, diadzein and rutin, ellagic acid as well as the alkaloid, melatonin.

2.3.4 Prunus cerasus L. as medicinal fruit

The ethnic use of Prunus cerasus L. as medicinal plant has been re-exploited for the modern medicinal system. A study published in the American Journal of Clinical Nutrition found that tart cherries ranked 14 in the top 50 foods for highest antioxidant content per serving size surpassing well-known leaders such as red wine, prunes, dark chocolate and orange juice (Halvorsen et al., 2006). Epidemiological research studies confirmed that sour cherries or the natural bioactive compounds found in these cherries, reduce inflammation and ease the pain of arthritis and gout, offer protection against cardiovascular disease and certain cancers, reduce the risk of diabetes and insulin resistance syndrome, aid in the treatment and possible prevention of memory loss and weight management.

Sour cherries are one of the richest sources of powerful antioxidants called anthocyanins—which provide the distinctive red color and may hold the key to the benefits locked inside (Wang et al., 1999). Studies suggest that these disease-fighting pigments possess antioxidant, anti-inflammatory, anti-aging and anti-carcinogenic properties (Blando, 2004). Sour cherries are rich sources of other phenolic compounds, such as gallic acid, p-coumaric acid, kaempferol, all of which are potent antioxidants.
These active compounds are being called "Mother Nature's all-natural chemotherapy agents" (Gao and Mazza, 1995).

Halliwell and Gutteridge (1989) investigated that addition of *Prunus cerasus* antioxidants is one of the popular methods to increase the shelf life of food products which is thought to be associated with lipid per-oxidation. The authors found that dietary antioxidants may be effective against the peroxidative damage in the living systems.

Haibo et al. (1999) isolated three novel antioxidant compounds as (1):2-hydroxy-3-(a-ydroxyphenyl) propanoic acid, (2):1-(3,4-dihydroxycinnamoyl)-cyclopenta-2,5-diol(3) and 1-(3,4-dihydroxycinnamoyl) cyclopenta-2,3-diol(4), determined by their spectral data from EtOAc of *Prunus cerasus*.

Navindra et al. (2001) evaluated the in vivo and in vitro efficacy of bioactive anthocyanins present in *Prunus cerasus* fruit to prevent inflammation and colon cancer. The authors isolated and characterized Protocatechuic acid as the predominant degradation product of these anthocyanins using in vitro Cell- culture studies.

Young et al. (2003) studied the potential of anthocyanins to inhibit intestinal tumor development in Apc\(^{M1}\) mice and growth of human colon cancer cell lines. Mice consuming the cherry diet, anthocyanins, or cyanidin had significantly fewer and smaller cecal adenomas than mice consuming the control diet or sulindac Colonic tumor numbers and volumes were not significantly influenced by treatment. Authors found that anthocyanins and cyanidin also reduced cell growth of human colon cancer cell lines HT 29 and HCT 116. These results suggested that tart cherry anthocyanins and cyanidin may reduce the risk of colon cancer.

Federica et al. (2004) revealed that *Prunus cerasus* acquire new interest, due to the fact that it can be considered as a” functional food” because of its high content of antioxidant compounds mainly Anthocyanins and their possible use as chemotherapeutics. The authors also studied the induction of anthocyanin biosynthesis in the sour cherry callus cell cultures. The pigments produced under in vitro conditions were different with respect to anthocyanins found in vivo in the fruits.

Daeok et al. (2005) demonstrated that Sour cherry (*Prunus cerasus*) fruit phenolics protected neuronal cells from cell-damaging oxidative stress in a dose dependent manner mainly due to presence of anthocyanins.
Haidari et al. (2009) investigated the effect of *Prunus cerasus* (sour cherry) juice on serum uric acid levels, hepatic xanthine oxidoreductase activity and two non-invasive biomarkers of oxidative stress. The data showed that Sour cherry juice treatment did not cause any significant reduction in serum uric acid levels in normal rats, but significantly reduced the serum uric acid levels of hyperuricemic rats in a time-dependent manner. *Prunus cerasus* juice also inhibited hepatic xanthine oxidase/dehydrogenase activity. These features of *Prunus cerasus* make it an attractive candidate for prophylactic treatment of hyperuricaemia, particularly if it is to be taken on a long-term basis.

Kirakosyan et al. (2010) evaluated the antioxidant capacities of various kinds of phytochemicals found in sour cherry fruit using TEAC antioxidant assay and also determined how these constituents interact in terms of expression of their antioxidant action, by isobolographic analysis. The results showed that Kaempferol, quercetin, isorhamnetin 3-rutinoside, cyanidin 3-rutinoside, and melatonin showed significant antioxidant capacities; of these, kaempferol proved to be the most active. Using different dose ratios for the selected polyphenol constituents, the authors found that three types of interactions may occur: synergistic, additive, and negative. Thus, not all polyphenols in sour cherry fruits are equally effective in alleviating oxidative stress. Those which are most effective are likely to be acting synergistically.

![Fig. 2.3.1. *Prunus cerasus* L. (Sour cherry) plant and its fruits.](image-url)