Review of Literature
Herbal medicine which involves the therapeutic use of plant products, is amongst the oldest of medical practices known to mankind. It is a central feature of many forms of folk and traditional medicine and is used in the treatment of a wide range of health disorders for which no allopathic treatments are available. Several plants used in traditional systems of medicine have been shown to modulate immune responses. Many active principles have been isolated from these plants and have been characterized (24).

Many of the compounds used as medicines in the allopathic treatment are derived directly or indirectly from the plants. While several classic plant derived drugs have lost much ground to synthetic competitors, others have gained new investigational or therapeutical status in recent years and many novel plant derived substances have entered into Western drug market (25). Our current knowledge of the therapeutic activity of plant products indicates that many useful drugs can be developed from them in the future, or that they could be used as template for further research and development of more useful compounds.

During the last two decades there has been an upsurge in the search for new plant-derived drugs containing medicinally useful alkaloids, glycosides, polyphenolics, steroids, and terpenoid derivatives. Farnsworth et al. identified 119 secondary metabolites, isolated from higher plants that were being used globally as drugs (26). Secondary metabolites isolated from medicinal plants can serve as precursors or models for the preparation of effective agents through semi-synthetic approaches or lead-based total synthesis.
Curcumin, an antioxidant present in turmeric (*Curcuma longa*), has been reported to have immunomodulatory activities. It has been found to inhibit carcinogenesis induced by chemicals in animal models and has also been reported to be an anti-inflammatory agent (27). It is found to increase, significantly, the total count of WBC and circulating antibody titre against SRBC when injected into animals. A significant increase in macrophage phagocytic activity was also observed in Curcumin treated animals (28). Amongst many cancer preventive activities of Curcumin it is known to inhibit cyclooxygenase and lipoxygenase dependent metabolism of arachidonic acid to synthesize prostaglandins and hydroxyeicosatetraenoic acids (HETEs) (29, 30).

Pretreatment with curcumin significantly inhibited IL-12 production by macrophages stimulated with either lipopolysaccharide (LPS) or heat-killed *Listeria monocytogenes* (HKL). When treated with Curcumin and macrophages reduced their ability to induce IFN-gamma secretion in Ag-primed CD4+ T cells. It was also observed that such Ag-primed CD4+ T cells under such conditions secreted significantly higher levels of IL-4. *In vivo* administration of curcumin resulted in the inhibition of IL-12 production by macrophages stimulated *in vitro* with either LPS or HKL, leading to the inhibition of secretion of Th1 cytokines (decreased IFN-gamma and increased IL-4 production) in CD4+ T cells. It was therefore concluded that curcumin may inhibit Th1 cytokine production by CD4+ T cells by suppressing IL-12 production in macrophages, and this points to a possible therapeutic use of curcumin in the immune diseases where
Th1 cell activation is the primary cause of pathological conditions (31).

Curcumin reduced the expression of inducible nitric oxide synthase (iNOS) gene in murine macrophages, _in vitro_, and in the liver, _in vivo_. _In vitro_, curcumin reduced the expression of tumor necrosis factor α (TNF-α) and interleukin-1beta (IL-1β) genes by LPS-stimulated peritoneal cells. _In vivo_, it reduced the expression of TNF-α and IL-1β genes in the liver and spleen, and reduced the expression of interferon -γ (IFN-γ) gene in the spleen and lymph nodes. Nitric oxide (NO) enhances the activity of cyclooxygenase-2 (COX-2); it was found that curcumin also reduced COX-2 gene expression in RAW 264.7 macrophage cell lines (32). Curcumin, which itself possesses antitumour activity against experimental tumours, is also reported to enhance the antitumour effect of the widely used anticancer drug like cisplatin, when used in combination against fibrosarcoma (33-35).

Ginsenoside, isolated from _Panax ginseng_ (family; Araliaceae) roots has shown strong stimulation of inducible nitric oxide synthase in a dose dependent manner. As nitric oxide plays an important role in immune function, _Panax ginseng_ treatment could modulate several aspects of host defense mechanisms, which are dependent upon stimulation of the inducible nitric oxide synthase (36). The crude extract and saponins of Indian _P. pseudoginseng_ were also found to be potent immunostimulants.

_Planthago major_ Linn. and _P asiatica_ Linn. (Plantaginaceae) are commonly used as folk medicine for treating infectious diseases
related to the respiratory, urinary and digestive tracts and various human leukemias, lymphomas and carcinomas. The hot water extract of *P asiatica* has been shown to possess significant inhibitory activity on the proliferation of lymphoma (U937) and carcinoma (bladder, bone, cervix, kidney, lung and stomach) cells and on viral infection (HSV-2 and ADV-11). *P. major* and *P. asiatica*, both exhibited dual effects on immunodulatory activity, enhancing lymphocyte proliferation and secretion of IFN-γ at low concentrations, and inhibiting this effect at high concentration, which modulates cell-mediated immunity (37-39).

Biologically active polysaccharide, acemannan isolated from gel of *Aloe vera* leaves, has been reported to have immunomodulatory activities. Dendritic cells were treated by acemannan and examined for phenotypic and functional properties. Phenotypic analysis for the expression of class II MHC molecules and co-stimulatory molecules such as B7-1, B7-2, CD40 and CD54 confirmed that acemannan could induce maturation of immature DCs. Functional maturation of immature DCs was supported by increased allogenic mixed lymphocyte reaction (MLR) and IL-12 production. The adjuvant activity of acemannan was proposed, at least in part, due to its capacity to promote differentiation of immature DCs (40). Acemannan has been reported to increase the number of spleen cell monocytes in tissue culture and enhanced the function of peritoneal macrophages as phagocytic cells against sheep red blood cells (41, 42).
TNF-α is one of the proinflammatory cytokines that is primarily secreted by activated macrophages and monocytes (43). TNF-α enhances the production of other proinflammatory cytokines by autocrine fashion, induces inflammatory mediators by synovial fibroblast-type cells and endothelial cells and activates other inflammatory cells (44, 45). Because of its pivotal role in pathogenesis of many human diseases like rheumatoid arthritis, septic shock and other inflammatory diseases, a significant effort has been focused on developing therapeutic drugs that interfere with TNF-α production. Plants are a valuable source of a vast array of lead compounds, which decreases TNF-α production from which more potent and less toxic drugs can be synthesized. Plant flavonoids are a group of such compounds. The methanolic extract of Amorpha fruticosa, which is applied traditionally to treat hypertension, hematomas and contusions in China, Japan and Korea, was reported to inhibit TNF-α production in LPS stimulated RAW264.7 cells. A flavonone has been identified from Amorpha fruticosa, which is been reported to be responsible to inhibit the production of TNF-α by RAW264.7 cells (56).

In many cases, plant components have been used as starting material for making semi-synthetic drugs; an example is taxol, (anticancerous drug) obtained from bark of Taxus baccata (47, 48). Plant based clinical research has made particularly rewarding progress in the important field of anti-cancer drugs (e.g., taxol and camptothecins). Paclitaxel (Taxol) and docetaxel (Taxotere) are the first representatives of a new class of antitumor compounds. These two
taxoids are clinically active against breast, ovarian and lung cancers (49). Taxoids are highly complex diterpenoids form natural origin. Paclitaxel is extracted from the barks of the Pacific, yew tree *Taxus brevifolia* whereas docetaxel is prepared by semisynthetically starting from 10-deacetyl-baccatin III, an inactive precursor found in the needles of the European yew tree *Taxus baccata*. These two drugs are active in various *in vitro* and *in vivo* preclinical models (cell lines, human tumor stem cells, murine grafted tumors, and human xenografts). Taxoids constitute a new class of anti-mitotic agents different from vinca-alkaloids. On the one hand, paclitaxel and docetaxel can be considered as inhibitors of the reaction of depolymerization of microtubules into tubulin; on the other hand, vinca-alkaloids inhibit reaction of polymerization of tubulin into microtubules (50).

Many important modern plant drugs, such as vinblastine and vincristine, have also been discovered by following leads from traditional medicines. Vincristine is the drug of choice for the treatment of childhood leukemia; vinblastine is a secondary drug for the treatment of Hodgkin's disease and other neoplasms (51, 52).

Certain steroids and alkaloids, which are used in drug manufacturing by the pharmaceutical industry, include steroidal sapogenins. *Digitalis* glycoside, digoxin which continues to have an important role in long-term outpatient management of heart failure (53, 54).

Secondary natural products often have highly complex structures with many chiral centers, which may determine biological activity; such complex compounds cannot be synthesized economically. A good
example of a secondary metabolite having a high degree of structural complexity is the naturally occurring plant insecticide azadirachtin. The effect of azadirachtin, a triterpenoid derived from *Azadirachta indica* on humoral immune response was shown in the freshwater teleost, *O. mossambicus*. Bovine serum albumin (BSA) and sheep erythrocytes (SRBC) were used as antigens to evoke immune response. The immune responses in fish as measured by quantifying antibodies produced and counting the peripheral blood leucocytes were found to be significantly enhanced by azadirachtin in a dose dependent manner. An inverse relationship was observed between the dose of azadirachtin and the degree of immunostimulation. Timing of azadirachtin administration in relation to immunization revealed that the maximum enhancement of antibody response was observed when the stimulant was given two days prior to immunization (55, 56).

The extracts of fruit of *Emblica officinalis* (Amla) have been reported to have strong anti-oxidant, immunomodulating and cytoprotective properties against chromium (VI) induced oxidative damage in murine macrophages. It enhanced free radical production and decreased reduced glutathione (GSH) levels and glutathione peroxidase activity in macrophages. Amla inhibited chromium induced immunosuppression and significantly restored both phagocytosis and IFN-γ production by macrophages (57,58). Pyrogallol, has been identified as the common compound present both in the unfractionated and n-butanol fractions of *Phyllanthus emblica* extracts. Anti-proliferative effects of pyrogallol were, therefore, determined on human tumor cell lines, including human
erythromyeloid cell line K562, B-lymphoid cell line Raji, T-lymphoid cell line Jurkat and erythroleukemic cell line HEL. It was found to be the most active in inhibiting \textit{in vitro} cell proliferation (59). The extracts from leaves \textit{P. emblica} showed inhibitory activity of against human polymorphonuclear leukocyte (PMN) and platelet functions was studied (60). The immunomodulatory effects of \textit{P. emblica} on immune profile of tumor bearing mice were determined. When administered orally, \textit{P. emblica} fruit powder was found to enhance NK cell activity and antibody dependent cellular cytotoxicity in syngeneic Balb/c mice bearing Dalton's lymphoma ascites tumor (61, 62).

\textit{Tinospora cordifolia} is widely used in traditional system of medicines. It is known for its immunomodulatory, anti-hepatotoxic, anti-stress and anti-oxidant properties. The active principles of \textit{Tinospora cordifolia}, Syringin and cordiol inhibited the \textit{in vitro} immunohemolysis of antibody-coated sheep erythrocytes by guinea pig serum. The reduced immunohaemolysis was found to be due to inhibition of the C3-convertase of the classical complement pathway. However, higher concentrations of the active principle showed inhibitory effects. The compounds also significantly increased the level of IgG antibodies in serum. Humoral as well as cell-mediated immunity were enhanced dose-dependently. Macrophage activation was reported by cordioside, cordiofolioside A and cordiol and this activation was more pronounced with increasing incubation times (63). It has been used in combination with other plant products to prepare a number of drug preparations used in traditional system of medicine. \textit{Tinospora cordifolia} has been reported to have
radioprotective effect in Swiss albino mice, thereby enhancing the survival of mice against a sub-lethal dose of gamma radiation (64, 65). Effect of *Tinospora cordifolia* extract on modulation of hepatoprotective and immunostimulatory functions in carbon tetrachloride (CCl₄) intoxicated mature rats has been reported. CCl₄ administration has been found to cause immunosuppressive effects of phagocytic capacity, chemotactic migration and cell adhesiveness of rat peritoneal macrophages. However, treatment with *T. cordifolia* extract (100 mg/kg body weight for 15 days) in CCl₄ intoxicated rats was found to protect the liver and also delete the immunosuppressive effect of CCl₄. A significant increase in the functional capacities of rat peritoneal macrophages was observed following *T. cordifolia* treatment (66, 67). The water and ethanol extracts of stems of *Tinospora cordifolia* and *T. sinensis* were shown to alleviate immunosuppression caused by cyclophosphamide (68). An arabinogalactan of mean molecular weight 2.2 x 10⁶ has been isolated from the dried stems of *Tinospora cordifolia* as examined by methylation analysis, partial hydrolysis and carboxyl reduction. Purified polysaccharide showed polyclonal mitogenic activity against B-cells, while their proliferation did not require macrophages (69, 70). Exposure of HeLa cells to the extracts of *Tinospora cordifolia* (methanol, aqueous and methylene chloride) resulted in significant increase in cell killing in a dose dependent manner when compared to non-drug-treated controls. It killed the cells very effectively *in vitro* and deserves attention as an anti-neoplastic agent (71).
Podophyllin, an ethanolic extract of *Podophyllum peltatum* L., is a good source of the aryltetralin-type lignan, podophyllotoxin. The latter compound, as well as its congeners and derivatives were shown to exhibit pronounced biological activity, mainly as strong anti-viral agents and as anti-neoplastic drugs. The podophyllotoxin derivatives etoposide, etopophos (etoposide phosphate), and teniposide are thus successfully utilized in the treatment of a variety of malignant conditions (72). Etoposide, a semi synthetic antineoplastic agent derived from the mayapple (*Podophyllum peltatum*), is reported to be useful in the chemotherapeutic treatment of refractory testicular carcinomas, small cell lung carcinomas, non-lymphocytic leukemias, and non-Hodgkin's lymphomas (73, 74). *Withania somnifera* (Ashwagandha) has been used in traditional system of medical practice for several drug preparations. The extracts of *Withania somnifera* were found to enhance the proliferation of lymphocytes, bone marrow cells and thymocytes in response to mitogens. Both PHA and Con A along with *Withania* extract treated splenocytes, bone marrow cells and thymocytes could proliferate at a much greater rate than the untreated cell. *Withania* extract along with the mitogen LPS (10 μg/ml) would respond to splenocytes proliferation six times more than the normal. Natural killer cell activity (NK) was found to be enhanced significantly in both the normal and the tumor bearing group. Antibody dependent cellular cytotoxicity (ADCC) was found to be enhanced in the *Withania* extract treated group. An early Antibody dependent complement mediated cytotoxicity (ACC) was also observed in the *Withania* treated group (75,76).
The effect of a methanolic extract (1-256 μg/ml) from the roots of *Withania somnifera* on nitric oxide (NO) production in J774 macrophages was found to have produced a significant and concentration-dependent increase in NO production, an effect which was abolished by N(G)nitr-o-L-arginine methyl ester (L-NAME, 3-300 μM), a non-selective inhibitor of NO synthase (NOS), dexamethasone (10 μM), an inhibitor of protein synthesis and N (alpha-p)-tosyl-L-lysine chloromethyl ketone (TLCK, 0.01-10 μM), an inhibitor of nuclear factor-kappaB (NF-κB) activation. Moreover, western blot analysis showed that *Withania somnifera* extract increased, in a concentration-dependent fashion, inducible NOS protein expression. It was concluded that *Withania somnifera* extract may induce the synthesis of inducible NOS expression, possibly by acting at transcriptional level. The increased NO production by macrophages could account, at least in part, for the immunostimulant properties of *Withania somnifera* (77).

*Allium sativum* (Garlic) extract has been shown to have immunomodulatory and anti-inflammatory properties. Garlic powder extracts (GPE) and garlic metabolites modulated lipopolysaccharide (LPS) induced cytokine levels in human whole blood cell culture. These cytokines also reduced the expression of nuclear factor kappaB (NF-κB) activity in human cells. Pretreatment with GPE reduced the ability of LPS-induced production of proinflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor-α (TNF-α), whereas the expression of the anti-inflammatory cytokine like IL-10 was not affected. The garlic metabolite diallydisulfide also significantly
reduced IL-1β and TNF-α expression. Interestingly, exposure of human embryonic kidney cell line (HEK293) to GPE-treated PBMC supernatants reduced NF-κB activity compared to control cells exposed to supernatants from untreated PBMC, as measured by a NF-κB-driven luciferase reporter gene assay. (78).

Several compounds isolated from *Allium sativum* modulate leukocyte proliferation and cytokine production. Ajoene, a major compound containing sulfur in oil-macerated garlic products, has shown anti-platelet and anti-tumor activity in the skin of mice (79, 80). Treatment of human promyeloacticleukemic cell line such as HL-60 with the experimental antileukemic drug ajoene has been found to induce the activation of the mitogen-activated protein kinases (MAPKs) c-Jun NH (2)-terminal kinase (JNK), p38 and extracellular signal-regulated kinases (ERK)1/2, as well as the survival kinase Akt. Blockade of ERK1/2, but not Akt pathways lead to promoting sensitization of cells against ajoene-mediated apoptosis. This supported the view that inhibition of ERK1/2 is a valuable strategy to increase the sensitivity of promyeloleukemic cells towards ajoene (81). Ajoene has also been shown to inhibit proliferation and induce apoptosis of human CD34-negative leukemia cells including HL-60, U937, HEL and OCIM-I. More significantly, ajoene was been shown to induce 30% apoptosis in myeloblasts from a chronic myeloid leukaemia patient. Acute myeloid leukaemia (AML) is a heterogeneous malignant disease in which disease progression at the level of CD34-positive cells has a major impact on resistance to chemotherapy and relapse (82). Anti-proliferative activity of ajoene has been demonstrated against a panel
of human tumor cell lines and normal marsupial kidney cells (PtK2) (83, 84). Epidemiological as well as experimental data have also supported a role for constituents of allium vegetables, such as garlic and onion, in the prevention of gastrointestinal cancer (85).

Cytokines involved in inflammatory bowel disease (IBD) direct a predominantly cell-mediated T-helper-1 (Th1) immune response. The nonspecific anti-inflammatory treatment being used in the management of patients with IBD has not changed much since the 1970s. Recently therapeutic effects of garlic have been studied in the treatment of patients with IBD. Whole blood cell and PBMCs were stimulated in the presence of various concentrations of garlic extract and its effect was determined on leukocyte cytokine production in vitro. Monocyte interleukin (IL)-12 production was inhibited significantly in the presence of low concentrations of garlic extract. Monocyte IL-10 production was increased significantly and tumor necrosis factor-alpha (TNF-α), IL-1alpha, IL-6, IL-8, produced by monocyte and interferon-gamma (IFN-γ), IL-2, and TNF-α produced by T-cells decreased significantly in the presence of high concentrations of garlic extract. Twenty to fifty percent of the immunomodulatory activity of garlic extract on cytokine production was found to be acid labile. The inhibitory activity of methylprednisolone, a commonly used anti-inflammatory drug, when used with garlic on leukocyte cytokine production was found to be additive. Therefore, it has been concluded that by inhibiting Th1 and inflammatory cytokines, but upregulating IL-10 production, treatment with garlic extract may help to resolve inflammation associated with IBD (86).
Of the many beneficial actions of garlic, inhibition of the growth of cancer is perhaps the most remarkable. Garlic extract has been reported to inhibit rat sarcoma cell migration, a critical feature of tumor cell metastasis. It has been thus envisioned that if tumor cell metastasis could be attenuated, if not completely stopped, it would be possible to stabilize the tumor in the local area for surgical removal. Unlike other method of treatment of cancer, garlic extracts may play a role in fighting cancer without significant side effects (87). In the transplanted carcinoma cell model, it has been found that aged garlic extract (AGE) significantly inhibited the growth of Sarcoma-180 (allogenic) and LL/2 lung carcinoma (syngenic) cells transplanted into mice. Concomitantly, increases in natural killer cells (NK) and killer activities of spleen cells were observed in Sarcoma-180-bearing mice administered with AGE. This study has suggested that AGE could be a promising candidate as an immune modifier, which maintains the homeostasis of immune functions and can significantly reduce the risk of cancer (88, 89). The low molecular weight organo-sulfer compounds and protein F4 found in garlic have been shown to inhibit growth of tumors in animals and modulate activity of diverse chemical carcinogens. The sulfur compounds of Garlic are reported to stimulate immunity, including macrophage activity, natural killer cells, and LAK cells, and to increase the production of IL-2, TNF, and IFN-γ.

These cytokines are associated with the beneficial anti-tumor response mediated by Th1 cells, which is characteristic of effective cancer immunotherapies. Garlic extract was been reported to stimulate the
proliferation of macrophages and lymphocytes and has been found to protect against the suppression of immunity by chemotherapy and ultraviolet radiation (90, 91). It has also been reported to enhance cytotoxicity of human peripheral blood lymphocytes against both NK cell sensitive (K562) and resistant (M14) cell lines. It was found that garlic may augment function of macrophages (oxidative burst) and T-lymphocytes (92). It has also been shown that garlic extract protects animals from ultraviolet induced suppression of contact hypersensitivity (93).

Methanol extracts and aqueous suspensions of Ocimum sanctum (Tulsi) leaves have been reported for their immunoregulatory effect to antigenic challenge by Salmonella typhosa and sheep erythrocytes (94). Ocimum sanctum seed oil and linolenic acid are found to possess significant anti-inflammatory activity against prostaglandin, leukotriene and arachidonic acid-induced paw edema. Ocimum oil has also been found to have the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonic metabolism, which may be responsible for its anti-inflammatory activity (95). Ocimun oil has been found to inhibit enhancement of the vascular/capillary permeability and leucocyte migration following inflammatory stimulus. The lipoxygenase inhibiting, histamine antagonistic and antisecretory effects of the oil could probably contribute towards anti-ulcer activity and it has been considered to be a drug of natural origin, which possesses both antiinflammatory and anti-ulcer activity (96-99).
Boerhaavia diffusa, has been used in Indian traditional system of medicine. Ethanolic extracts of B. diffusa roots were reported to inhibit human NK cell cytotoxicity in vitro, production of NO in mouse macrophage cells and production of IL-2 and TNF-α by human PBMCs (100). The ethanolic extract of Boerhaavia diffusa, also inhibited T cell mitogen phytohemagglutinin and concanavalin A-stimulated proliferation of human peripheral blood mononuclear cells (PBMC). It also inhibited purified protein derivative (PPD) stimulated proliferation of PBMC and mixed lymphocyte cultures in human. In addition, B. diffusa extract inhibited the growth of several cell lines of mouse and human origin, such as mouse macrophage cells (RAW 264.7), human macrophage cells (U937), human monocytic cells (THP-1), mouse fibroblast cells (L929), human embryonic kidney cells (HEK293), mouse liver cells (BNLCL.2), African green monkey kidney cells (COS-1), mouse lymphoma cells (EL-4), human erythroleukemic cells (K562), and human T cells (Jurkat) (101-105).

Echinacea purpurea is a widely used herbal medicine, reported to have immunomodulatory properties. It has been reported to activate macrophages, polymorphonuclear leukocytes and natural killer cells (106-108). Three components isolated and purified from Echinacea purpurea are cichoric acid, polysaccharides and alkylamides. The rats were gavaged orally two times a day for 4 days with three different concentrations of each of Echinacea components. Among the components, alkylamides at the dose level of 12 μ/kg body weight/day significantly increased the phagocytic activity as well as phagocytic
index of the alveolar macrophages. The alveolar macrophages obtained from this group of rats also produced significantly more TNF-α and NO after an in vitro stimulation with LPS than any other active component or the control. None of the components at any concentration had any effect on the release of TNF-α, IFN-γ and IL-2 by the splenocytes. These results suggest that the alkylamides are one of the active constituents of *E. purpurea* plant. At a dose level of approximately 12µg/kg body weight/day, they effectively stimulate alveolar macrophage function in healthy rats (109-111). Treatment of PBMC with *Echinacea* extract overnight resulted in the activation of CD69 expression and increase in mean fluorescence intensity in both the CD16+ and CD16+CD56+ NK subsets. However, the frequency of CD16+ cells was decreased as well as the mean fluorescence intensity was down regulated. NK cell mediated cytotoxicity was augmented 100% in a short time (4-h) assay at the concentration of 0.1 µg/ml of *Echinacea* extract. Examination at the single cell level revealed augmentation of the frequency of CD56+ NK-target conjugates and a plateau was reached after 30-60 min of incubation. Likewise, the frequency of CD56+ killer cells in the conjugates was also significantly increased by *Echinacea* extract. There was recruitment of non-conjugated CD56+ cells into CD16+ NK-target conjugates and activation of the NK-target non-killer conjugates into killer cells. *Echinacea* extracts have been reported to be potent activators of NK cell mediated cytotoxicity. *Echinacea* augments the frequency of NK cell target conjugates and activates the programming for lysis of NK cells (112, 113). Complement modulation, either
inhibition or stimulation, is an interesting target for drug development. Arabinogalactan-protein (AGP), a new arabinogalactan-protein type II isolated from pressed juice of the aerial parts of *Echinacea purpurea*, was clearly identified as a stimulator of both the classical and alternative pathway of complement activation. Selective removal of the arabinose side chains of AGP resulted in considerably reduced activity. Therefore, the three-dimensional structure of the polysaccharide, i.e., a backbone branched by side chains, is supposed to be important for the interactions with the complement system (114, 115). Polysaccharides isolated from large-scale plant cell cultures of *Echinacea purpurea*, have been shown to activate human and murine phagocytes. Polysaccharide, fractions were found to be effective in activating peritoneal macrophages isolated from animals after administration of cyclophosphamide (CP) or cyclosporin A (CsA). Macrophages treated with polysaccharides have been shown to exhibit increased production of TNF-α and enhanced cytotoxicity against tumor cell targets such as WEHI 164 and against the intracellular parasite *Leishmania enrietti*. After a CP-mediated reduction of leukocytes in the peripheral blood, the polysaccharides have been shown to induce an earlier influx of neutrophil granulocytes as compared to PBS-treated controls. Polysaccharides, treatment of mice, immunosuppressed with CP or CsA, restored their resistance against lethal infections with the predominantly macrophage-dependent pathogen *Listeria monocytogenes* and predominantly granulocyte-dependent pathogen *Candida albicans* (116-119). The apoptotic process can be modulated by various stimuli, including hormones, cytokines, growth factors, and some chemotherapeutic agents.
Echinacea purpurea and Hypericum perforatum are able to regulate the process of apoptosis in vivo and to define the role of the Fas-Ag and Bcl-2 signal transduction cascade. The splenic lymphocytes from mice treated with E. purpurea and H. perforatum have been shown to be significantly more resistant to apoptosis than those from mice treated only with the vehicle. In addition, mice treated with the natural substances showed a decrease in expression of Fas-Ag and an increase of Bcl-2 expression (120).

Immunomodulatory activity of aqueous extract of Trigonella foenum graecum L. (fenugreek), a widely used dietary herb in several Ayurvedic and Unani drugs, has been found to elicit a significant increase in phagocytic activity of macrophages (121). Protodioscin (PD), purified from Trigonella foenum graecum L. and identified by Mass, and 1H- and C\(^{13}\)-NMR. PD has been reported to display strong growth inhibitory effect against HL-60 cells, but weak growth inhibitory effect on KATO III cells. The fragmentation by PD of DNA to oligonucleosomal-sized fragments, that is a characteristic of apoptosis, has been observed to be both concentration- and time-dependent in the HL-60 cells. It has been suggested that growth inhibition of HL-60 cells by PD is due to induction of apoptosis by this compound in HL-60 cells (122).

Vanadate and Trigonella foenum graecum administration to diabetic animals showed an encouraging anti-oxidant property and they could be valuable candidates for the treatment of the reversal of and complications of diabetes (123). The modulatory effects of fenugreek
seeds on circulatory lipid peroxidation (LPO) and its anti-oxidant role during 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in male Wistar rats have been reported and it has been shown that enhanced LPO in the circulation of tumor bearing animals was accompanied by a significant decrease in the levels of ascorbic acid, vitamin E, reduced glutathione, glutathione peroxidase, glutathione S-transferase, superoxide dismutase and catalase. Inclusion of fenugreek in the diet has been shown to significantly decrease LPO with simultaneous enhancement of circulating antioxidants. It has been reported that fenugreek exerts its chemopreventive effect by decreasing circulatory LPO and enhancing antioxidant levels (124). The aqueous extract and a soluble gel fraction derived from its seeds have shown significant ulcer protective effects. The fenugreek seeds have also been found to have prevented the rise in lipid peroxidation induced by ethanol, presumably by enhancing antioxidant potential of the gastric mucosa, thereby lowering mucosal injury (125).

*Nyctanthes arbor tristis* Linn. (Harsingar) is widely used as a decoction in the Ayurvedic system of medicine for treatment of sciatica and arthritis. *Nyctanthes arbor tristis* (NAT) was shown to have inhibitory effects on the acute inflammatory oedema produced by different phlogistic agents, viz. carrageenan, formalin, histamine, 5-hydroxytryptamine and hyaluronidase in the hind paw of rats. NAT has also been found to inhibit the inflammation induced by immunological means, viz. Freund's adjuvant arthritis and tuberculin reaction induced by PPD. Thus, anti-inflammatory activity in the leaves of Harsingar has been shown in support of its use in various inflammatory conditions by the followers of the Ayurvedic system of
medicine (126,127). *Nyctanthes arbor-tristis* L. has recently been reported to possess strong stimulation of antigen specific and non-specific immunity. Increases in humoral and delayed type hypersensitivity (DTH) responses to sheep red blood cells (SRBC) and in the macrophage migration index (MMI), have been demonstrated in mice fed with 50% ethanolic extract of seeds, flowers and leaves of this plant. Maximum activity has been found in the seeds in which the active principle(s) appear to be mainly associated with lipids. However, in flowers and leaves, the major activity has been found in the aqueous fraction of the 50% ethanol extract (130). Oral administration of water soluble fraction of the ethanol extract of NAT on the TNF-α level in plasma of arthritic and soluble protein A (SpA)-treated Balb/c mice has shown a consistent decrease. A similar depletion of TNF-α level in the plasma of SpA-treated mice has been observed. The extract was also shown to reduce plasma IFN-γ level while the plasma IgM and IgG levels were not found to be affected (129).

Andrographolide, an active component of *Andrographis paniculata*, has been reported to inhibit inflammatory responses by rat neutrophils. Prevention of ROS production through, modulation of PKC-dependent pathway has confirmed at least in part the ability of andrographolide to down-regulate Mac-1 expression, which is essential for neutrophil adhesion and transmigration (130). Incubation of macrophages activated by BCG with the methanol extract of *A. paniculata* has been found to reduce LPS stimulated NO production in them. The diterpene lactones, andrographolide and
neoandrographolide have been isolated as active components from the extract. These compounds have been reported to be responsible for suppression of NO production (131-133).

Apocynin (4'-hydroxy-3'-methoxy-acetophenone or acetovanillone), a non-toxic compound isolated from the medicinal plant *Picrorhiza kurroa*, has been found to selectively inhibit production of reactive oxygen species by activated human neutrophils. Apocynin has proved to be effective in the experimental treatment of several inflammatory diseases such as arthritis, colitis and atherosclerosis. These features have suggested that apocynin could be a prototype of a novel series of non-steroidal anti-inflammatory drugs (NSAIDs) (134, 135).

Chemoprevention of chemically induced tumors by Picroliv, a glycoside mixture purified from *Picrorhiza kurroa*, has been shown by using 20-methylcholanthrene (20-MC)-induced sarcoma model and 7, 12-dimethylbenz[a]anthracene (DMBA)-induced papilloma in BALB/c mice. Picroliv has also been reported to exhibit anti-tumour-promoting activity on mouse skin using DMBA as an initiator and croton oil as a promoter (136-138). Picroliv has also been reported to inhibit passive cutaneous anaphylaxis in mice and rats and protected mast cells from degranulation (139).

*Centella asiatica* is a medicinal plant widely used in China and India for wound healing purposes. The activity of asiaticoside, isolated from *Centella asiatica*, has been reported to exhibit significant wound healing activity in normal as well as delayed healing models. It has been found to promote angiogenesis in the chick chorioallantoic membrane model. (140) Titrated Extract from *Centella asiatica*
(TECA) is a drug which has been used for many years in Europe for rectifying wound healing defects. It is a reconstituted mixture of 3 triterpenes namely asiatic acid, madecassic acid and asiaticoside which are extracted from the plant. Asiatic acid and asiaticoside were the most active out of the 3 triterpenes. Asiaticoside exerted a preferential stimulation of collagen synthesis and was active at low doses and was found to be the only component responsible for the same. In addition to collagen synthesis, the 3 components were also able to stimulate glycosaminoglycan synthesis. TECA and all three terpenes increased the intracellular free proline pool independent of its effect on collagen synthesis.

Since antioxidants have been reported to play a significant role in the wound healing process, and asiaticoside has been shown to affect the levels of certain antioxidants, its role in the wound healing process was studied. Application of asiaticoside (0.2%, topical) twice daily for 7 days to excision-type cutaneous wounds in rats led to increased level of enzymatic and non-enzymatic antioxidants, namely superoxide dismutase (35%), catalase (67%), glutathione peroxidase (49%), vitamin E (77%) and ascorbic acid (36%) in newly formed tissues. It also resulted in a several fold decrease in lipid peroxide levels (69%) as measured by their reactivity with thiobarbituric acid.

The crude extract (CE) of Centella asiatica as well as its partially purified fractions (AF) has been reported to have anti-tumour activity. AF dose dependently inhibited the proliferation of the transformed cell lines more significantly than the CE. It also significantly
suppressed the multiplication of mouse lung fibroblast cells (L-929). Oral administration of the extracts (CE and AF) retarded the development of solid and ascites tumours and increased the life span of these tumour bearing mice (148). Fresh juice of C. asiatica leaves (CAJ) have been reported to have anti-ulcerogenic activity against gastric ulcers in rats. The drug, when given orally in doses of 200 and 600 mg/kg body weight, twice daily for 5 days. It showed significantly inhibited gastric lesions formation (58% to 82% reduction) and decreased mucosal myeloperoxidase (MPO) activity (149, 150).

The extract was also tested for its radioprotective properties. A sublethal dose of Co 60 gamma radiation, i.e. 8 Gy was selected for the purpose. Animals, whole bodies were irradiated with Co 60 gamma radiation externally, with and without administration of drug extract. The dose that was found to be most effective against radiation was 100 mg/kg body weight. This dose increased the survival time of the mice significantly. Body weight loss of the animals in the drug treated group was significantly less in comparison with the animals that was given radiation only (151).