CHAPTER II

REVIEW OF LITERATURE

Medicinal plants have been widely used as a source of folk medicine in these systems to support health and to sustain body’s resistance against harmful diseases (Datta et al. 1999). In recent years, there has been an increase in the clinical use of native drug formulation. Herbal sources are effectively used in field of medicine for a variety of pathophysiological states (Darshaan and Dorewamy 1998). They are unlimited plant sources in nature which are being used for anti-tumour properties or to induce immunity in the standard manner of traditional medicine. Natural products have very lesser side effects especially in anti-tumour therapy as the synthetic compounds or chemotherapeutic drugs show potent anti-tumour property with many side effects (Capeli et al. 2000). Recent research using a transgenic mice model of melanoma has shown that the antiproliferative effects of well known kampo medicine were mediated through an enhanced specific-antigen antitumor T-lymphocyte cytotoxic response (Patwardan and Gautam 2005; Plaeger 2003).

A lot of proteins have been purified from seeds from different plants, for example phytohaemagglutinin (PHA), concanavalin A and various fungal immunostimulant proteins was isolated from *G. lucidium*, *F. velutipes* and *V. volvacea* have been shown to provoke a cascade of events leading to proliferation of cell, activation and that nontoxic doses of galactoside specific mistletoe lectin thus increasing the production of lymphokines (Hsu. et al. 1997). Although present researches have revealed immunomodulatory potential of plant medicines (Bueth et al. 1993), non-specific and specific aspects of immunity have been also evaluated (Ray et al. 1996; Sen et al. 1990). The plants were considered as natural biological factories, not only for compounds present in plants like proteins, carbohydrates and lipids that were utilize as food sources by human but also for a complex compounds, such as secondary metabolites etc. that yield physiological effects (Patwardan and Gautam 2005). The compounds which are responsible for beneficial effects are usually derived metabolites present in plant. These medicinal plants provide an extensive range of natural products mainly secondary metabolites with immunomodulating potential (Patwardhan and Gautam 2005).
Many plants have been reported to have immunomodulatory activity (Atal et al. 1986). According to Ayurveda, they toughen tissues of the body, promote intelligence, prevent aging, and prevent infections and syndromes (Ataal et al. 1986; Patwardan et al. 1990; Puri et al. 1994; Ziaudin et al. 1996). From ancient times, natural remedy has been a basis of treatments and at present receiving consideration as source of synergistic combinations.

Immunomodulators become popular in the natural healthiness manufacturing as people realized the significance of human immune system in the preservation of health (Ramasundharam et al. 2005). It is quite extensive to facilitate the immune system ensuing in the body defense to help itself. An immunomodulator is a compound that helps to maintain the immune system and its immune response (Ziauddin et al. 1996). Immunomodulators can be supplemented to a person having active or low active immune response (Puri et al. 1994). Hence we can say that immunomodulator is not used as a booster to response but to maintain immune response. The mode of action of immunomodulator is still not known till date. The beneficial aspects of them are to increase molecules/compounds that are responsible for immunity as well as they can maintain the immunity (Patwardan et al. 1990). Some of the molecules for example such as biobran can also reduce inflammatory cytokines production (Patwardhan et al. 1990). The immune system is well organized defense system that evolved to cure cancer related diseases and also to protect animals from microbial pathogens. It is also capable to produce an enormous variety of molecules and cells which are able to recognizing and eliminating specifically from foreign invaders. These cells and molecules perform together in a complexed network whose rivals that similar as the nervous system (Diaesio and LoBueglio 1996).

The immune system is capable to be aware of chemical differences that can differentiate one foreign particle/pathogen/molecule from another (Ziaudin et al. 1996). Further, the immune system is also able to distinguish between foreign molecules/pathogens and the body’s own cells and enzymatical proteins. When a foreign pathogen has been identified, the body system, it releases, wide variety of cells and molecules to enhance an appropriate response, known as effector response, to neutralize or eliminate the organism (Puri et al. 1994). By this way the immune system can convert the recognized one into a variety of effector response. Afterwards when the same antigen exposessystem induces a memory response (Puri et al. 1994). The efficiency and function of the immune system can be influenced by various external and internal
factors which results in either enhances of immune system or detorirates immune system. Those agents having an activity to regularize or change pathophysiological processes are known as immunomodulatory agents.

2.1 IMMUNOMODULATION

Immunomodulating activity is a biological / pharmacological effect of compounds on cellular or humoral aspect of the immune response (Wagner et al, 1990). Modulation of immune response through stimulation or suppression may help in maintaining a disease free state (Ghule et al, 2006). Apart from being specifically stimulatory or suppressive, certain agents have been shown to possess activity to normalize or modulate pathophysiological processes and are hence called immunomodulatory agents (Bafna et al, 2005). Immunomodulatory agents are of two types:

2.1.1. Immunosuppression

Depending on the mechanism of action, the phenomena of immunosuppression can be either antigen specific or antigen nonspecific.

2.1.1.1 Antigen specific immunosuppression:

Specific suppression of IgE antibodies to haptens and protein antigens is achieved by various methods. Treatment of IgE producing mice with high doses of aceto-acetylated OVA markedly reduced their ability to respond to later immunizations of low doses of OVA (Makare et al, 2001). Ishizaka et al., also showed immunization with urea denatured antigen-E (Ag-E) of rag weed pollen into normal or AgE primed mice resulted in the down regulation of both the primary and secondary responses to AgE (Ghule et al, 2003). Katz et al, reported the abrogation of the anti-hapten IgE antibody response by conjugates of DNP with the nonimmunogenic, synthetic D-Gl, copolymer and demonstrated that in the system a hapten specific unresponsive state in the IgE B cell population of normal and of primed mice had produced. Another group of researchers reported that conjugates of poly-N-methyl glycine and grass pollen allergen extracts suppressed the primary antigen specific IgE antibody response to the grass pollen allergen. It was also reported that the production of cytotoxic T cells were involved in the tolerogenic state in these mice.
2.1.1.2 Antigen nonspecific immunosuppression:

Most of the immunosuppressive agents are antigen nonspecific in action. They mediate immunosuppression over a broad range of antigen sources. Some nonspecific immunosuppression can be due to antigen competition where one antigen inhibits the antibody response to the closely spaced later immunization with a second antigen. Similar effects can be induced by *in vivo* graft versus host reaction and *in vitro* by mixed lymphocyte reaction and mitogen stimulation. There are reports, which showed suppression of *in vitro* response of normal spleen cells to unrelated antigens. Other studies also showed that a variety of nonspecific T cell and B cell responses to mitogens, T cell dependent and independent antigens and cytotoxic T cell responses. Most of the immunosuppressive drugs that are in wide therapeutic usage now have been good examples for nonspecific suppression of immune response (Ghule *et al*, 2003). Examples of immunosuppressants are Prednisolone, Cyclosporine, Azathioprine, cyclophosphamide, Interferon, FK 506, Rapamycin and Deoxyspergualin etc.

2.1.1.2.1 Therapeutic uses of immune suppressants:

- It helps in organ transplantation and tissues by suppression of immune cells (liver, kidney, heart, bone marrow etc.)
- In bone marrow transplants, it suppresses graft-versus-host.
- Selective immune suppression for cure of Rh hemolytic disease in newborn children. (Ghule *et al*, 2003)
- It helps in idiopathic thrombocytopenia purpuraeae, some forms of glomerulo nephritis and hemolytic anemia, myastheania gravis, systemic lupus erythrematosus, psoriasis and rheumatoid arthritis.

2.1.2 Immunostimulation

Immunostimulatory agents not only influence the enhanced immune response but also affect the production of the isotype of IgG and cell mediated response (Wagner *et al*, 1990). Nonspecific immunostimulation has progressed from crude substances to various chemically
well defined drugs with selective effect on different components of the immune system. Hadden J H, discussed about many categories of immunostimulants and attempted to explain their mechanism of action. There are many immunostimulants are available today with clinical significance and therapeutic value. Many prominent clinically defined and licensed immunostimulants are presented in table A. Microbial sources provide four categories of products on the basis of structure function relativity (Wagner et al, 1990). Immunostimulants are being used in treatments of various diseases like AIDS(Ghule et al, 2006). Examples of immunostimulants are Levamisole, BCG, Recombinant Cytokines, Interferons, Interleukin-2, Vaccines Immune Globulin, Rho (D) Immune Globulin etc.

2.1.2.1 Therapeutic uses of Immunostimulants:

Therapeutic application of immune stimulants are used in the treatments of diseases like

- Immuno deficiency disorders i.e., AIDS
- Acute and chronic infectious disease
- Cancer and malignant tumors(Ghule et al, 2003)

Below are the some of the examples of immunosuppressants and immunostimulants and their mechanism of action.

2.1.3 Immunosuppressants:

2.1.3.1 Cyclophosphamide:

Cyclophosphamide is a nitrogen alkylating agent from the oxazophorines group. Cyclophosphamide supresses primary and secondary humoral immune response, delayed type of hypersensitivity, animal diseases of autoimmunity and skin graft rejection (Eric R. Hurd, 1973). It is used to treat some autoimmune disorders and various types of cancer. Cyclophosphamide also reduces the immune system's response to various diseases. Hence, it is used in various non-neoplastic autoimmune diseases (Steinberg et al., 1971). It is also used to treat severe rheumatoid arthritis, minimal change disease (Townes et al., 1976), multiple sclerosis (Makhani et al., 2009) and Wegener's granulomatosis (NOVAc and Pearson, 1971). It can be in autoimmune
hemolytic anemia, autoimmune disorders, patients possessing factor- XIII antibodies and bleeding syndromes and Wegener's granulomatosis.

2.1.3.2 Cyclosporin A

Cyclosporin A is a widely used immunosuppressive compound. It is a 11-amino acid cyclic peptide isolated from Tolypocladium inflatum. It was to be very effective in inhibiting the rejection of cardiac, liver, lung, renal and multiple organ transplantation. It is being widely used in treating the autoimmune diseases, asthma, psoriasis, atopic dermatitis, ulcerative coritis and many others. It induces unresponsive state by inhibiting the Ca+2 dependent T cell proliferation induced by antigens, antibodies and mitogens. It selectively inhibits transcription of early phase of T cell activation of lymphokine genes IL-2, IL-4, IL-6, GM-CSF, TNF-α,γ which are important in the transformation of the T cell from resting stage (G0) to active state (G1). The main important function of the Cs A is the inhibition of NF-kB, NFAT, and OCT-1 transcriptional factors of IL-2 gene (Borelet al, 1970; Shevachet al, 1985). Cyclosporin A can be use in organ transplantation of Heart, liver, Kidney and also other organs. Early engraftment, rheumatoid arthritis and psoriasis, extending kidney graft survival.
2.1.3.3 Azathioprine:

Azathioprine (IMURAN) is a purine anti-metabolite which is used as a primary therapy, for treatment of immune-mediated disease. An imidazole derivative i.e., Azathioprine, inhibits production of nucleic acid via both salvage and de novo pathways. The imidazole residue of Azathioprine alkylates thiol groups on surface of T-cell membranes blocks antigen recognition. It induced hepatotoxicity due to endothelial damage and can cause veno-occlusive disease (Sternek et al., 1991). Myelosuppression, mainly leukopenia, is the common side effect of Azathioprine therapy (Rossi et al., 1993). It can be used in organ transplant rejection, allogeneic kidney transplantation.

2.1.3.4 FK 506

In 1987, Goto et al., isolated a 23-membered macrocyclic lactone lactum antibiotic, which is more potent immunosuppressive compound than Cs A from the fermentation broths of Streptomyces tsukubaensis, a microorganism found in the soil of Tsukuba Japan. The LC 50 values of FK 506 indifferent experiments revealed that it is a 10-100 fold more potent than Cs A. It produces its effects in the early signaling process since it inhibits T cell proliferation induced by a combination of Ca+2 ionophore ionomycine and protein kinese C activator PHA. It selectively inhibits transcription of early phase of T cell activation of lymphokine genes like Cs A (Liu J et al, 1991; Goto et al., 1987). It is used in pediatric liver transplantation, Prophylaxis of solid-organ allograft rejection and kidney transplantation.

2.1.3.5 Rapamycin

Rapamycin is a lipophilic macrolide and antifungal antibiotic produced by Streptomyceshygroscopicus. Martel first reported its immunosuppressive activity. Unlike Cs A and FK 506, it inhibits cytokine induced proliferation. It can inhibit Ca+2 dependent independent responses. It blocks the clonal expansion of the helper and cytotoxic T cells induced by alloantigen and in vitro thymocyte proliferation and results in the involution of thymus in vivo. Studies showed that expression of aldolase, a gene responsible for activation of T cell and B cell and the stimulation was suppressed by rapamycin at both enzymatic and m-RNA levels (Tocciet al, 1989; Sehgalet al., 1975). It helps in Graft rejection, incorporated into stents to inhibit local cell proliferation and blood vessel occlusion, organ transplant inhibitor.
2.1.3.6 Deoxyspergualin

Deoxyspergualin was isolated from the soil bacterium *Bacillus laterosporus* by Takuechi *et al.*, 5-Deoxyspergualin (DSG) (1-amino-19-guanidine-11-hydroxy-4, 9, 12- triazo-nonadecane- 10, 13- dione) is a synthetic analogue of spergualin. It was found to inhibit; i) mitogen or antigen induced T cell proliferation ii) the expression of IL-2 R; iii) the antibody formation and B cell differentiation. It modulates the IL-1 production, down regulates class I and II MHC molecules and inhibits the kappa light chain expression. DSG has been found to inhibit generation of super oxide radicals, hydrolytic enzymes. Hoeger *et al.*, reported that DSG inhibits the antigen processing and presentation of monocytes and macrophages and conventional antigen driven lymphocyte proliferation and many glucocorticoids are also used in the immunosuppression (Fujii *et al.*, 1990; Waaga *et al.*, 1996; Peter *et al.*, 1994).

2.1.3.7 Mycophenolate Mofetil

A 2-morpholinoethyl ester of mycophenolic acid is also known as Mycophenolate mofetil (CELCEPT). The mode of action is to inhibit inosine monophosphate dehydrogenase (IMPDH), enzyme used in the de novo pathway for guanine nucleotide synthesis. B and T lymphocytes depend on this pathway for cell proliferation. Hence, MPA can be used selectively for inhibition of lymphocyte proliferation (U.S. Patil *et al.*, 2012). It is clinically used renal transplantation, prophylaxis of transplant rejection.

2.1.3.8 Antithymocyte Globulin (ATG)

Antithymocyte globulin is a purified gamma globulin from the serum of rabbits immunized with human thymocytes (U.S. Patil *et al.*, 2012). It contains cytotoxic antibodies that can bind to CD molecules and HLA class I and II molecules on the human T cellsurface. The antibodies reduce lymphocytes circulating by direct cytotoxicity (both cell-mediated and complement) and lymphocyte will be blocked by adhering to cell surface molecules that are involved in the normalized cell function. It is used in recovery from ischemic reperfusion injury, acute renal transplant rejection.

2.1.4 Immunostimulants:
2.1.4.1 Levamisole (ERGAMISOL) was to "restore" dejected immune function of macrophages, B-cells and T-cells. It is chiefly used as adjuvant (Arutjuniaan VM, Grigorian EG, 2003). Based on present research levamisole can augment immune response towards Th1 development by activation of T cell aspects and dendritic cells. Th2 when suppressed the immune response and eliciting Th1 immune response is clinically more important, particularly in the prevention and curing of atopic diseases (Chen et al., 2008). This adjuvant is used mostly to cure Duke’s stage C colon cancer.

2.1.4.2 Live bacillus Calmette-Guérin (BCG) is an attenuated vaccine that is live culture of the strain of *Mycobacterium bovis* which induces a granulomatous reaction. This is active against tumors and is used for treatment of carcinoma (Paterson and Patel, 1998). It is most appropriate key therapy for high-risk patients. BCG is used in treatment of prophylaxis of Ta and/or T1 papillary tumors and treatment of the urinary bladder carcinoma.

2.1.4.3 Interferons

Interferons (alpha, beta, and gamma) originally identified by their antiviral activity, these also possess immunomodulatory activities (Tilg and Kaser, 1999). They bind to specific receptors on cell surface that begin a series of reactions inside the cells such as, increase in cell proliferation and immune activity. It also increases the production of macrophages thus by increase in phagocytosis and cytotoxicity by T lymphocytes which is specific. Used in treatment of chronic hepatitis B, follicular lymphoma, hairy cell leukemia, malignant melanoma, condylomata acuminata and AIDS related Kaposi's sarcoma.

2.1.4.4 Immune Globulin

Immunoglobulin is prepared commercially from pooling human plasma from different samples and it has good antibody titers against common bacterial, fungal and viral pathogens. The antibodies are provided passively to immunodeficient person. It is used in hematological disorders, multiple myeloma and chronic lymphocytic leukemia.

2.1.4.5 Interleukin-2

Human recombinant interleukin-2 is produced by recombinant DNA technology in *E. coli* (Taniguchi and Minami, 1993). It is used to enhance proliferation of lymphocyte, increase in
activity of interfons (Whittington and Faulds, 1993). Aldesleukin was immunized by vivo mode in animals which results in numerous immunologic effects in a dose-dependent manner. Thrombocytopenia, eosinophilia and lymphocytosis were activated by cellular immunity which further enhances in production of cytokines (e.g., TNF, interferon, IL-1). Used in Melanoma and Metastatic renal cell carcinoma.

2.1.4.6 Thalidomide:

The circulation of TNF-α in patient with nodosum leprosum, erythemas was decreased by using Thalidomide has been reported. Simultaneously, this compound known to effect angiogenesis. It is used in chronic refractory rheumatod arthritis

2.1.4.7 Isoprinosine:

The pacetmidobenzote salt of N, N-dimethylamino-2-propanol is known as Isoprinosine. Its mode of action is to increase cytokines production i.e., IL-1, IL-2 and IFN-γ. It also causes rising in lymphocyte proliferation when exposed to antigen/mitogen, induces T-cell surface markers on protymocytes and activates T-cell rosettes. It is used in treatment of Epstein-Barr, acute viral encephalitis caused by measles viruses, subacute sclerosing panencepalitis and Herpes simplex infections.

In addition to immunostimulators and immunosuppressants we can induce the immune response by a group of compounds known as Adjuvants. These compounds act upon immune system along with antigen to induce immune response.

2.1.5 Adjuvants

Adjuvants are the compounds that to help or aid, refers to any compound/agent that enhances the ongoing antigen induced cellular or humoral response.

Adjuvants can be used:

- To booster the immune response of several antigens by delivering in native form.
- To decrease the multiple immunization protocol for defensive immunity. Also used to develop one step vaccination that can lessen the cost of vaccination.
To augment the immune response in immuno compromised adults and also in children with weakened immune system, to obtain cytotoxic T cell response (Sivakumare et al, 2011).

2.1.5.1 Mode of Action of Adjuvants

To maintain an antibody response, a supply of antigens is needed. Adjuvant may support the immune response by forming a depository of antigens at the site of injection that results in the release of minute quantities of antigens for long period of time. A booster dose of antigens may be given.

Alternatively, an adjuvant can help to deliver the antigens to the lymph nodes or spleen, where antigen is trapped by the dendritic cells. This results most of the required cell to cell interactions take place to produce plasma cells. For example, an antigen containing oil i.e., microdroplets formed in an oil-in-water emulsion of adjuvant, are readily engulfed by macrophage and delivered to spleen or lymph nodes.

2.1.5.1 Types of Adjuvants

Adjuvants are conventionally classified into the following categories: Mineral compounds, Bacterial products, Oil-based emulsions, ISCOMs and Liposomes. Of these, the aluminium based mineral compounds are the most widespread (Cox et al, 1997) and the most preferred for humans (Jansen et al, 2006).

2.1.5.2 Aluminium based minerals

Aluminium based adjuvants, like Aluminium hydroxide and Aluminium phosphate have been known to induce early, long lasting, high titre, protective immunity (Lindblad et al, 2004). However, aluminium is a weak adjuvant for antibody induction to recombinant protein vaccines (Rajput et al, 2007).

2.1.5.3 Oil-based emulsions

These are popular immune potentiators for inactivated vaccines (Jansen et al, 2006). Saponins: Saponins are steroid or triterpenoid glycosides, which occur in different plant species. In Triterpenoid saponins are predominant in cultivated crops, whereas steroid saponins are frequent in plants that are used as herbs for the health provoking properties. These have the
unique ability to stimulate cell-mediated immunity, as well as to enhance antibody production (Jansen et al., 2006). Certain traditional Chinese medicinal research on herbs such as, Astraglus species, Panax ginseng, Panax notoginseng have gained attention as candidates for plant derived saponins. Quillaja saponaria extract as adjuvants, first described in the 1930s have been the most prominent of the saponins used as adjuvants to feature in the respective literature (Sun et al., 2009; Francis et al., 2002).

2.1.5.4 Immunostimulating complexes (ISCOMS)

Iscoms are cage like structure (~40 nm) formed by the interaction of saponins and cholesterol. Iscoms are being used in the preparation of veterinary vaccines. They induce strong Th1 Th2 responses, CTL responses. They are also better adapted to targeting and antigen presentation. It is important to incorporate immunogen into iscom to unleash an effective CTL response Barr et al., 1996; Ronnberget al., 1995; Gregoriadiset al., 1989).

2.1.5.5 Liposomes

They are single or multi lamellar bilayer membrane vesicles (20nm to 3 µm) comprising cholesterol and phospholipid. Many antigens are being used by embedding in liposomes for induction of immune response and effective antigen presentation and processing. These are potential agents for good targeting and CTL responses (Alvinget al., 1992; Powerset al., 1995; Relyveldet al., 1986).
2.1.5.6 Bacterial products

Due to their potent immunostimulatory capacity, bacterial products are considered a good source of immunological adjuvants. Bacterial products were effective adjuvant for CD4+ T cells in vivo (Mcsorley et al, 2002). Heat shock proteins (HSPs) are conserved proteins that are highly immunogenic and function as adjuvants that may play a vital role in adaptive and innate immunity (Ebrahimiet al, 2011). Cytokines Cytokines like IFNg or GM-CSF have been popular for over a decade as effective adjuvant molecules (Songet al, 2009). Induction of local delayed hypersensitivity (DTH) is commonly observed after the use of Proinflammatory cytokines and interleukins.

Plants have been used since early historic times in the treatment of diseases associated with mankind. Many plants are reported to be involved in immunomodulatory activity (Juang et al, 2004). Some of the plants were listed below that were used in this investigation.

2.2 Terminalia chebula

*Terminalia chebula* is mainly used in traditional folk medicine. It belongs to the family combretacae. It is widely used in homeopathy, unani, andayurvedic medicines. *Terminalia chebula* is commonly used in traditional medicine not only used in India but also in other parts of Africa and Asia. This is widely used because of broad spectrum of pharmacological activities.

2.2.1 Taxonomic classification

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<td>Kingdom</td>
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<td>Order</td>
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Family : Combretaceae
Genus : Terminalia
Species : Chebula

It is mainly found in India, Bangladesh, Myanmar, Egypt, Iran, China, Turkey etc. In India this tree is grows in deciduous forests.

*T. chebula* is rich in tannins (about 32%-34%) (Anwesa Bag *et al*.) and its content varies with geographical distribution (Jayaramkumar *et al.*, 2006). The tannins that are present in *T. chebula* are of pyrogallol type. Recently researchers found that 14 components of pyrogall tannins (chebulagic acid, unicalagin, neochebulinic acid, chebulanin, corilagin, ellagic acid, chebulinic acid, terchebulingallic acid etc) from *T. chebula* fruits (Juang *et al.*, 2004). Anthraquinones, ellagic acid and chebulinic acid are phenolics that are present. Polyphenols such as galloyl glucose, Corilagin, maslinic acid, punicalagin and terflavin A (Williamson *et al.*, 2002). Apart from these constituents, anthraquinone like succinic acid, amino acids, fructose, betasitosterol, and resin are also present (Tubtimdee *et al.*, 2011; Thakuret *et al.*, 2008). A 12 fatty acids were isolated from *T. chebula* of which, oleic acid, linoleic acid and palmitic acid were major constituents (Zhanget *et al.*, 1997). Arjunin, chebulosides I and II, 2α- hydroxyursolic acid, 2α-hydroxymicromiric acid and arjunguloside are some of triterpenoid glycosides have been reported by Mammenet *et al.*, 2012. Polyphenols that are terflavins B, C, and D punicalagin, punicalin were present in the leaves (Juang *et al.*, 2004; Han *et al*.; Anwesa Bag *et al.*, 2013).

Many pharmacological studies for various biological activities of *Terminaliachebula* in *in-vivo* and *in-vitro* test models have been carried out to study the presence of chemical constituents. Some of these brief findings of pharmacological studies were mentioned below. Anticancer activity reported by Salem A *et al.*, 2002; antibacterial activity identified by Kannan P *et al*., 2009, Malekzadeh F *et al*., 2001; antifungal activity reported by Dr. Saheb L Shinde *et al*., 2011, Vivek K *et al*., 2010; antioxidant activity identified by Suchalata *et al*., 2009, Chia-lin chang *et al*., 2010; antiviral activity reported by Hongbo *et al*., 2010; Kim *et al*., 2001; antiulcer activity identified by Raju *et al*., 2006; wound healing activity reported by Manish Pal Singh *et al*., 2009; anti diabetic activity reported by Gandhipuram Periyasamy *et al*., 2006, Rao N.K *et al*., 2006; cardio protective effect identified by Suchalata *et al*.; antimitagenic activity reported by
Grover et al., 1992; Kaur et al., 2002; radiation protective effect reported by Jagetia et al.; immunodulatory activity reported by Vaibhav et al.

2.3 Punica granatum

Pomegranate is a very important pharmacological fruit. The wide adoption of the pomegranate is due to recent studies that have shown that the fruit contains a high amount of antioxidants that are beneficial to our health in many ways.

*Punica granatum* is a deciduous tree scattered throughout the world. It is commonly known as pomegranate.

From ancient times, pomegranate is used significantly for many purposes because of its pharmacological constituents. All parts of the fruit extracts possess beneficial properties (Naqvi et al., 1991) and some other reports shown that the leaves, roots, and bark have medicinal benefits (Lansky et al., 2007). The constituents mainly found are punicic acid, anthocyanidins, flavonoids, anthocynins, elagic acid and estrogenic and flavonols.

Although pomegranates have wide-range of therapeutic benefits such as may antioxidant, anticarcinogenic, and anti-inflammatory properties. Antioxidant activity reported by (Kulkarni et al., 2007; Zahin et al., 2010); anti-carcinogenic activity reported by (Balkwillet et al., 2005; Malik et al., 2006; Khan et al., 2007; Malik et al., 2006), anti-inflammatory activity reported by (Simmonset al., 2005; Eatock et al., 2000), immunomodulatory activity reported by Gracious Ross et al., 2001.

2.4 Syzygium jambolanum

It is a common tree found found all over India. *Syzygium jambolanum/ cumini* are native to the Indian Subcontinent and adjoining regions of Southeast Asia. Its fruit are very sweet, appetizing to all taste and soothing. Leaves bark and seeds are used for the medicine. It has a decided action in checking and curing diabetes mellitus.

The species ranges across India, Bangladesh, Pakistan, Nepal, Sri Lanka, Malaysia, the Philippines, and Indonesia.
Compounds like myrecetin, ellagic acid, glucoside, kaemferol, isoquercetin, and anthocyanins rich in Jambolan. An alkaloid, jambosine and antimellin were found in the seeds Morton, 1987. The seeds of jambolin were found to be rich in antioxidants, flavonoids which are responsible for scavenging activity for the defensive effect on antioxidant enzymes (Ravi et al., 2004). Reports also found that this plant have elevated levels of phenolics with considerable antioxidant activity. It is also rich in vitamin C, sugars mineral salts and flavonoids (CSIR, 1948).

Research was focused on chemopreventive effects activity reported by (Aggarwal et al., 2009). Jamun leaf also possesses radioprotective effects (Jagetia et al., 2004) and antineoplastic effects activity reported by (DeVita et al., 2004; Arun et al., 2010; Yun et al., 2009)

### 2.5 Aeglemarmelos

*Aegle marmelos* belongs to Indian origin possessing medicinal value; it belongs to family Rutaceae (Sharma et al., 2007).

*A. marmelos* is a subtropical plant and grows up to an altitude of 1,200 m altitude from sea level.

It grows well in the dry forests on hilly and plain areas. *A. marmelos* is a widely distributed plant and found in India, China including south-east Asian countries. In India it found in Sub-Himalayan tracts from Jhelum eastwards to West Bengal, in central and south India. It found almost in all the states of India (Dhankhar et al., 2011)

The various organic fractions of *A. marmelos* contain cardiac glycosides, tannins, alkaloids, terpenoids, steroids, flavonoids and saponins (Venkatesan et al., 2009; Sivaraj et al., 2011). *Aegle marmelos* fruit pulp possess flavonoids, phenolic compounds, fat and oil, lignin, proteins, inulin, carbohydrates (Rajan et al., 2011).

The following are the studies carried out on this plant:

Antioxidant activity (Sharmila et al., 2011; Rajan et al., 2011); antimicrobial activity (Gheisari et al., 2011; Sivaraj et al., 2011; Gavimath et al., 2008), antidiarrheal activity (Poonkothai et al., 2008), antidiabetic activity (Arumugam et al., 2008), antiproliferative
activity (Lampronti et al., 2003), cytoprotective effect (Vinodhini et al., 2009), hepatoprotective effect (Singanan et al., 2007), analgesic activity (Shankarananth et al., 2007), anti-arthritis activity (Trivedi et al., 2011), anti-inflammatory activity (Rao et al., 2003), toxicity studies (Veerappan et al., 2007)

2.6 Nyctanthes arbortristis

Nyctanthes arbortristis Linn. commonly known as ‘Night Jasmine’ due to the fact that its flowers release a very pleasant fragrance during night. (Siddiqui et al., 2006; Rout et al., 2007). The genus name ‘Nyctanthes’ has been derived from two Greek words ‘Nykhta’ (Night) and ‘anthos’ (flower) (Vats et al., 2009; Meshram et al., 2012). The species name ‘arbortristis’ meaning ‘the sad tree’ is supposedly derived from dull looks of the tree during daytime (Bansal Gulshan et al., 2015).

Native to Thailand and Indonesia, growing wild in the sub-Himalayan region from the Chenab River to Nepal southwards through Central India to northern Karnataka and Andhra Pradesh. In its natural habitat it grows gregariously on dry steep hill slides and rocky terrain and is often found in the understorey of dry deciduous forests, sometimes forming dense thickets. It is cultivated in gardens almost throughout India and in many other tropical countries for its flowers, which are valued for making garlands and as votive offerings in temples.

Several compounds belongs to various chemical classes such as alkaloids, steroids, flavonoids, terpenes, aliphatic compounds and glycosides have been derived and characterized from various plant parts of N. arbortristis. The bark contains two alkaloids and a glycoside. The roots comprises of glucosides, tannins and alkaloids. (Bansal Gulshan et al., 2015)

Researchers have tested this plant for antibacterial activity (Vats M et al., 2009; Rathore A et al., 1990), antiviral activity (Gupta P et al., 2005; Khatu et al., 2001), anti-inflammatory activity (Saxena RS et al., 1984; Rathore B et al., 2007; Omkar A et al., 2006; Saxena RS et al., 1987, antioxidant activity Paul et al., 1997), antifilarial activity (Kusum S et al., 2009), antiallergic activity (Saxena RS et al., 2002), antitryptaminergic activity (Chatterjee SK et al., 2007), hepatoprotective activity (Rathee JS et al., 2007), anticholinesterase activity (Bhatia A et al., 2001; Chatterjee SK et al., 2007), anti-inflammatory activity (Saxena RS et al., 1984; Rathore B

2.7 Annona squamosa

*Annona squamosa* L., this plant belongs to Annonaceae family, also called custard apple, is mostly found in deciduous forests. This plant is widely cultivated in various regions of southern and northern India. This plant is mainly belongs to West Indies. Many research reported that every parts of this plant possess therapeutic property (Veeramuthu et al., 2006).

This plant is widely cultivated in various regions of southern and northern India. This plant is mainly belongs to West Indies.

This plant extracts have shown the presence of secondary metabolites such as tannins, phenols, flavonoids, proteins, carbohydrates etc (Raman, 2006).

The following activities were reported in this plant


2.8 Acalypha indica

*Acalypha indica* L. belongs to family *Euphorbiaceae* and it is a weed distributed throughout India.

*Acalypha* is the fourth largest generic of its family with approximately 460-560 species. Numerous species are used as remedial plants in the Mascarene Islands and Africa. Each and every part of this plant like roots, stem and leaves are used as traditional medicines.
Acalypha indica mainly useful in Homeopathy, anti-inflammatory activity, anti bacterial and anti fungal activity, antioxidant activity (D. Jagatheeswari et al, 2013)

2.9 Zea mays

Corn, commonly known as maize (Zea mays L.), is annual crop that belongs to the family of grass i.e. Poaceae. Maize is also recognized by different synonyms such as zea, corn, silk corn etc.

Maize is native of South America but extensively cultivated in various other countries as well like India, Thailand, Pakistan and China, and in several parts of Philippines. It is considered as staple article of food in some islands and provinces. It is widely grown in temperate and tropic regions with well drained and fertile soil (Mills, 1994).

Reports have been showed that corn has analgesic activity (Owoyele et al, 2010). Corn silk is used to conquer urinary tract infections and kidney stones (Lans, 2006). In some places decoction of corn silk and parched corn is extremely useful in nausea and vomiting. (Dilip Kumar et al, 2013)
2.10 *Momordica charantia*

*Momordica charantia* L. belongs to family cucurbitaceae which is commonly called bitter melon, a member of, tendril climbing. Bitter melon is a general food of the tropics and is widely used for the treatment of cancer and diabetes (Cefaleu *et al*, 2008; Leung *et al*, 2009; Modaek *et al*, 2007; Nahaas *et al*, 2009). It is an effective hypoglycemic agent (Baseh *et al*, 2003; Singh *et al*, 2011).

This plant is supposed to be originated in the tropical region. It is extensively found in India and other parts of the Indian subcontinent as food and as well as medicine (Kumar *et al*, 2010).

Mixture of steroid saponins called insulin-like peptides, charantins (Singh *et al*, 2011), alkaloids such as momordin, momorcharins, momordenol, momordicin, diosgenin, eleostearic acids, momordicins, momordicinin, goyglycosides, momordolol, charantin, charine, cryptoxanthin, erythrodiol, cucurbitins, cucurbitacin, cucurbitanes, cycloartenols, galacturonic acids, gentisic acid, were found (Husain *et al*, 1994; Xie *et al*, 1998; Yuan *et al*, 1999; Parkash *et al*, 2002).

2.11 Tylophora indica

*Tylophora indica* belongs to family *Asclapiadaceae* and is an important medicinal plant from the repository of valuable plant species of Indian subcontinent. Due to its huge medicinal properties, this plant is exploited and it is considered as an endangered plant species.

The plant was found to be growing in Assam, Uttar Pradesh, Orissa, Bengal, Himalayas and sub Himalayas in India (Joshi, 2000). The plant inhabits up to an elevation of 1,250m in the sub Himalayan tract.

Major alkaloids such as tylophorine (C_{24}H_{27}O_{4}N), tylophorinine (C_{23}H_{25}O_{4}N), tylophrinidine (C_{22}H_{22}O_{4}N) and septidine have been isolated from the leaves and roots of *T. indica* by number of workers (Bhutani *et al*., 1985; Mulchandani *et al*., 1971). Set of seven additional phenanthroindolizidine alkaloids known as tyloindicines A–E isolated from *Tylophora indica*, bore novel structural features (Ali *et al*., 1989).

In a follow-up study, another set of tyloindicines from *T. indica* with even more intriguing structural features were also isolated (Bhutani *et al*., 1985). Of this set, tyloindicines F and G featured a unique tertiary hydroxy group and were screened for anti-tumor potential.

Other major alkaloids include tylophorindine, desmethyltylophorine, desmethyltylophorinine, des-methyltylophoridine, anhydrous dehydrotylo-phorinine (Gupta, 2003). Apart from these, some rare alkaloids namely tyloindicines H, I and J, desmethyltylophorine, des-methyltylophorinine, isotylocrebrine, 4, 6- des-methylisodroxy-o-Methyltylophorinindine have been reported.

The non-alkaloidal derivatives isolated from *T. indica* are quercetin, kampferol, amyrins, tetratriacontanol, octaosanyl, sigmasterol octacsanoate, β-sitosetrol, tyloindane, cetyl-alcohol, wax, tannins, glucose, calcium salts, potassium (Gupta *et al*., 2010).

The following activities were reported in this plant, hepatoprotective activity (Gupta *et al*., 2010; Mujeeb *et al*., 2009), lysosomal enzyme inhibiting activity (Nayampalli *et al*., 1979), antiallergic activity (Dhananjayan *et al*., 1979), diuretic activity (Gupta *et al*., 2010), mast cell stabilisation activity (Bhavan, 1992), anti-Cancer activity (Nadkarni, 1976; Gopalakrishnan *et al*., 1979), anti-tumor activity (Gopalakrishnan *et al*., 1980), antifeedant and antimicrobial activity...
Ayurveda is one of the ancient traditional medicines that are originated in India. According to ayurveda, charaka, rasayana drugs acts on human immune system. On administrating the right formulation of the rasayana in the suitable season under the direction of a practiced ayurvedician, the advantageous effects of rasayana were observed. Narsimha rasayana, Brahma rasayana and amrutha prasham were found to be enhances the proliferation of T-cells in response to mitogens. Activity of NK cell is reported to be giving better response in tumor infected animals. By employing rasayana, many dreadful diseases are being negotiated.