INTRODUCTION

1. INTRODUCTION:

THE R&D thrust in the pharmaceutical sector is focused on development of new drugs, innovative/indigenous processes for known drugs and development of plant-based drugs through investigation of leads from the traditional systems of medicine. In addition, many nutraceuticals are being consumed in unregulated markets for perceived benefits in health care and improvement of quality of life. Natural pharmaceuticals (Naturaceuticals), nutraceuticals and cosmeceuticals are of great importance as a reservoir of chemical diversity aimed at new drug discovery and are explored for antimicrobial, cardiovascular, immunosuppressive and anticancer drugs. Around 80% of all such products are of plant origin; their sales exceeded US $ 65 billion in 2003. Examples of plant products and derivatives used by the pharmaceutical industry include paclitaxel, vincristine, vinblastine, artemisinin, camptothecin, podophyllotoxin, etc.

Globally, there have been efforts to monitor quality and regulate the growing business of herbal drugs and traditional medicine. Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. The future of natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients and the community (Patwardhan and Hooper, 1992).

Lag phase for botanical medicine is now rapidly changing for a number of reasons (Dahanukar et al., 2000 and Patwardhan, 2000).

- Problems with drug-resistant microorganisms, side effects of modern drugs, and emerging diseases where no medicines are

available, have stimulated renewed interest in plants as a significant source of new medicines.

- Pharmaceutical scientists are experiencing difficulty in identifying new lead structures, templates and scaffolds in the finite world of chemical diversity.
- A number of synthetic drugs have adverse and unacceptable side effects.
- There have been impressive successes with botanical medicines, most notably quinghaosu, artemisinin from Chinese medicine.
- Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on Ayurvedic medicinal plants.
- Numerous molecules have come out of ayurvedic experiential base, examples include *Rauwolfia* alkaloids for hypertension, psoralens in vitiligo, *Holarrhena* alkaloids in amoebiasis, guggulsterones as hypolipidemic agents, *Mucuna pruriens* for Parkinson's disease, piperidines as bioavailability enhancers, bacosides in mental retention, picrosides in hepatic protection, phyllanthins as antivirals, curcumin in inflammation, withanolides, and many other steroidal lactones and glycosides as immunomodulators.
- A whole range of chronic and difficult-to-treat diseases such as cancers, cardiovascular disease, diabetes, rheumatism and AIDS, all require new effective drugs.

Most developing countries have relied and will continue to rely on traditional natural medicines due to the deterrence of high costs of modern allopathic medicines. Current estimates indicate that about 80% of people in developing countries still rely on traditional medicine based largely on various species of plants and animals for their primary healthcare. Four out of ten Americans used alternative medicine therapies in 1997; total visits to alternative medicine practitioners increased by almost 50% from 1990 and exceeded the visits to all US primary care physicians (Grabley and Thiericke, 1999).

Every medical system or therapy has certain advantages and limitations. Modern medicine is no exception to this (Goodwin, 1997).

Thirty per cent of the worldwide sales of drugs is based on natural products. Though recombinant proteins and peptides account for increasing sales rates, the superiority of low-molecular mass compounds in human disease therapy remains undisputed mainly due to more favorable compliance and bioavailability properties. Approaches to improve and accelerate the joint drug discovery and development process are expected to take place mainly from innovation in drug target elucidation and lead structure discovery. Therefore, the need for new concepts to generate collection of large compounds with improved structural diversity has been correctly emphasized by Grabley and Thiericke (1999). There are number of problems connected with the search for new prototype drugs of biological origin. Investigations of plants used in traditional and modern medicine in China serve as a source of inspiration and as models for the synthesis of new drugs with better therapeutic, chemical or physical properties than the original compounds (Baerheim and Scheffer, 1982). The World Health Organization (WHO) also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines.

The US National Cancer Institute regularly earmarks large appropriations to screen 50,000 natural substances for activity against cancer cell lines and the AIDS virus. China, Germany, India and Japan, among others, are also screening wild species for new drugs. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines (Akerele, 1993). The mass screening of plants in the search for new drugs is vastly expensive and inefficient. It would be cheaper and perhaps more productive to re-examine plant remedies described in ancient and medieval texts (Holland, 1994). Many higher plants produce economically important organic compounds such as oils, resins, tannins, natural rubber, gums, waxes, dyes, flavours, fragrances, pharmaceuticals and pesticides. Advances in biotechnology, particularly methods for culturing plant cells and tissues, should provide new means for the commercial processing of even rare plants and the chemicals that they produce.

These new technologies will extend and enhance the usefulness of plants as renewable resources of valuable chemicals. In future, biologically active, plant-derived chemicals can be expected to play an increasingly significant role in the commercial development of new products for regulating plant growth and for insect and weed control (Balandrin et al., 1985). In natural products drug discovery it is important to follow systems-theory and systems-biology applications to facilitate the process (Leroy Hood, 2003).

Numerous drugs have entered the international pharmacopoeia through ethnobotany and traditional medicine (De Smet, 1997). There are many similarities in traditional systems of medicine as well as ethnomedicines being connected to each other as 'great traditions and little traditions'. All botanical drugs will have to fulfill the international requirements on quality, safety and efficacy (Vogel, 1991).

Ayurveda remains one of the most ancient and yet living traditions practised widely in India, Sri Lanka and other countries and has a sound philosophical and experiential basis (Dahanukar and Thatte, 2000). *Atharvaveda* (around 1200 BC), *Charak Samhita* and *Sushruta Samhita* (1000–500 BC) are the main classics that give detailed descriptions of over 700 herbs. A scholarly description of the legacy of Charaka in contemporary idiom, best attempted with a commentary from modern medicine and science viewpoint, gives some glimpses of ancient wisdom (Valiathan, 2003). Indian healthcare consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions (Waxler-Morrison, 1988). India has about 45,000 plant species; medicinal properties have been assigned to several thousands. About 2000 are found in the literature; indigenous systems commonly employ about 500–700. Some recent work in drug development relates to species of *Commiphora* (used as a hypolipidaemic agent), *Picrorhiza* (which is hepatoprotective), *Bacopa* (memory enhancer), *Curcuma* (anti-inflammatory) and *Asclepias* (cardiotonic) (Jain, 1994). Currently, with over 400,000 registered ayurvedic practitioners, the Government of India has formal structures to regulate quality, safety, efficacy and practice of

herbal medicine. With unique holistic approach, ayurvedic medicines are usually customized to an individual constitution. Exhaustive information is available in ayurvedic literature that can be converted into a large database giving information of various foods, herbs, medicines and other materials with their taste, actions and utility in different disorders. The basis of traditional medicine is in its use for a number of years and therefore its clinical existence comes as a presumption. However, for bringing more objectivity and also to confirm traditional claims, systematic clinical trials are necessary. In ayurvedic medicine research, clinical experiences, observations or available data becomes a starting point. In conventional drug research, it comes at the end. Thus, the drug discovery based on Ayurveda follows a 'reverse pharmacology' path (Vaidya et al., 2001). Nevertheless, all the critical pharmacopoeial tests such as dissolution time, microbial, pesticide and heavy metals contamination, etc. must be in accordance with global standards. It is important to ensure that all the ayurvedic medicine manufacture is in accordance with current good manufacturing procedures for herbal products (Verpoorte and Mukherjee, 2003). There have been concerns about quality standards and safety issues of herbal medicines. The need for new regulations for botanical medicines has also been frequently stressed and some such regulations are coming into force in different parts of the world (Marcus, 2002).

1.1 Ayurveda: a new discovery engine

Combining the strengths of the knowledge base of traditional systems such as ayurveda with the dramatic power of combinatorial sciences will help in the generation of structure–activity libraries. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, money and toxicity – the three main hurdles in drug development. These records are particularly valuable, since effectively these medicines have been tested for thousands of years on people. Efforts are underway to establish pharmaco-epidemiological evidence base regarding safety and practice of ayurvedic medicines. Development of standardized herbal formulations is underway as an

initiative of the Council for Scientific and Industrial Research (CSIR) and New Millennium Indian Technology Leadership Initiative (NMITLI). Randomized controlled clinical trials for rheumatoid and osteoarthritis, hepatoprotectives, hypolipidemic agents, asthma, Parkinson's disease and many other disorders have reasonably established clinical efficacy. A review of some exemplary evidence-based researches and approaches has now resulted in wider acceptance of ayurvedic medicines (Vaidya et al., 2001 and Chopra et al., 2000). Thus the ayurvedic knowledge database allows drug researchers to start from a well-tested and safe botanical material. With ayurveda, the normal drug discovery course of 'laboratories to clinics' actually becomes from 'clinics to laboratories' – a true reverse pharmacology approach (Vaidya, 2002). In this process safety remains the most important starting point and efficacy becomes a matter of validation.

Globally, there is a positive trend towards holistic health, integrative sciences, systems biology approaches in drug discovery and therapeutics that has remained one of the unique features of ayurveda (Nityanand, 2003). A golden triangle consisting of ayurveda, modern medicine and science will converge to form a real discovery engine that can result in newer, safer, cheaper and effective therapies. It will be in the interest of pharmaceutical companies, researchers and ultimately the global community to respect the traditions and build on their knowledge and experiential wisdom.

1.2 The *Rasayana* concept of Ayurveda

The modulation of immune response by using medicinal plants as a possible therapeutic measure has become a subject of active scientific investigations. The basic concept has, however, existed in the ancient Vedic scripture, the Ayurveda, and has been practiced in Indian traditional medicine for many centuries. The two main approaches to illness in Ayurveda are preventive and curative.

According to Ayurveda theory, a harmonious balance between three humors of the body viz. 'Vayu', 'Pitta' and 'Kafa' is needed for positive health; imbalance of these may cause diseases. A significant part

of Ayurvedic therapeutics is preventive in nature. It aims to promote positive health. An entire section of materia medica of ayurveda termed 'Rasayana' is devoted to enhancement of body's resistance against infections. The prescribed procedure includes not only drugs but also daily routine including exercise, diet and nutrition besides mental attitude and discipline. One of the therapeutic strategies in Ayurvedic medicine is to increase body's natural resistance to the disease causing agents rather than directly neutralizing the agent itself. In practice this is achieved by using extracts of various plant materials called *rasayanas.* This term 'Rasayana' has been spilt up into 'Rasa' and 'Ayana' meaning the path 'Rasa' takes. It is believed, in Ayurveda, that the qualities of the *rasa-dhatu* influence the health of other *dhatus* (tissues) of the body. Hence any medicine that improves the quality of *rasa (rasayanas)* should strengthen or promote the health of all tissues of the body.

These *rasayana* plants are said to possess the following properties:

- prevent ageing,
- re-establish youth,
- strengthen life and brain power and prevent disease (Sharma, 1983; Ghanekar, 1981),
- increase the resistance of the body against any onslaught.

Traditionally, these agents are used against a plethora of seemingly diverse disorders with no pathophysiological connection according to modern medicine. Looking at these diverse applications there appeared to be a possibility of identifying adaptogenic agents from this group of *rasayanas.* Knowing that the central nervous system, endocrine system and the immune system participate in intense cross-talk (Ader *et al.,* 1990; Glaser and Kiecolt-Glaser, 1994), it was easy to hypothesize that by acting on the immune system these *rasayanas* could exert broad-based effects by initiating a massive cascade of events involving various neurotransmitters, hormones and amines of the stress response. These plants, labelled as 'rasayana', have been endowed with multiple properties like delaying the onset of senescence and improving mental functions by strengthening the psycho-neuro-immune axis (Katiyar et al., 1997). Hence, an attempt was made to scientifically validate these claims.

1.3 Immunomodulation:

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of non specific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immunosuppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulation and immunosuppression both need to be tackled in order to regulate the normal immunological functioning. 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 (Patwardhan et al., 1990).

Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulative agents. But there are major limitations to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system. In general, immunomodulators are biological response modifiers (BRM) that affect the immune response in either a positive or a negative fashion. Whereas the field of pharmacological immunostimulation is still at a very early stage of development, immunosuppressive therapy has become a part of everyday medical practice, either to prevent allograft rejection in transplanted patients or in treating a variety of autoimmune diseases, asthma and allergy. With the exception of a monoclonal antibody, all the immunosuppressive drugs used in medicine are exogenous molecules, either microbial secondary metabolites (CsA, FK506, rapamycin, etc.) or synthetic substances like leflunomide, azathioprine, brequinar, etc. [Hausen and Morris, 1997; Thomson and Starzl, 1993 and Gummert et al., 1999]. Nonspecific immunostimulation has progressed from crude microbial substances to chemically defined drugs with selective effects on different components of the immune system. An ever-increasing array of immunopotentiators are being examined for therapeutic benefit in a variety of disorders including malignancies, immunodeficiencies such as

AIDS, viral or prion infections such as epidemic influenza or Creutzfeld–Jakob disease, and inflammatory diseases [Masihi, 2000; Chen and Hasumi, 1993]. Microbial products (e.g., BCG, bacterial lysate cocktail), drugs of natural (e.g., glucan) and synthetic (e.g., MDP, Isoprinosine) origin, and peptides/proteins derived from the immune system (e.g., thymic hormones, cytokines) represent some of the immunostimulators that are currently in use [Ooi and Liu, 2000; Werner and Jolles, 1996 and Zlotta et al., 2000]. The widespread occurrence of dysfunctions of the immune system requires new approaches. Immunomodulation through natural or synthetic substances may be considered an alternative for the prevention and cure of infections and of neoplastic diseases, respectively (Azuma and Jolles, 1987; Hadden, 1994).

1.3.1 Plants as Immunomodulators:

Modulation of the immune response through stimulation or suppression may help in maintaining a disease free state. Agents that activate host defence mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy. Upadhyay (1997) has highlighted the therapeutic potential of immunomodulatory agents from plant products. They have evaluated Indian medicinal plants for immunomodulatory activity (Katiyar et al., 1997).

Thatte and Dahanukar, (1997) have described how clues from the description of ancient writings can lead to the development of new immunostimulatory agents. The experiments carried out to prove the rasayana concept of Ayurveda have demonstrated that *Asparagus racemosus, Tinospora cordifolia* and *Withania somnifera* protected animals against infections in normal and immunosuppressed states induced by hemisplenectomy or surgery (Dahanukar and Thatte, 1997). These plants also produced leucocytosis with predominant neutrophilia and prevented, to varying degrees, the leucopenia induced by cyclophosphamide. They were found to activate the polymorphonuclear and monocyte-macrophage systems. Only those rasayanas which produced sweet (madhur) vipaka *Tinospora cordifolia, Asparagus*

racemosus, Emblica officinalis, Terminalia chebula and *Withania somnifera* were found to stimulate the reticulo-endothelial system, but not those like *Acorus calamus, Commiphora mukul and Picorrhiza kurroa*, which produced bitter (katu) vipaka (Dahanukar and Thatte, 1997).

Among the immunostimulant rasayanas, *Tinospora cordifolia* has been extensively studied by Dahanukar et al (1997). It has been found to activate the mononuclear cells to release cytokines like GMCSF (Thatte et al., 1994) and IL-1 in a dose dependent manner (Dahanukar and Thatte, 1997). Whole aqueous extract of *Tinospora cordifolia*, standardized using HPTLC, has been evaluated as an adjuvant in clinical conditions like obstructive jaundice, tuberculosis and cancer chemotherapy and has been found to increase the efficacy of conventional therapy (Dahanukar and Thatte, 1997). Active principles of *Tinospora cordifolia* were found to possess anti-complementary and immunomodulatory activities. Syringin (TC-4) and cordiol (TC-7) inhibited the *in vitro* immuno-haemolysis of antibody coated sheep erythrocytes by guinea pig serum by inhibiting the C3-convertase of the classical complement pathway. The compounds also gave rise to significant increases in IgG antibodies in serum. Both humoral and cell-mediated immunity were dose-dependently enhanced. Macrophage activation was reported for cordioside (TC-2), cordiofolioside A (TC- 5) and cordiol (TC-7) and this activation was more pronounced with increasing incubation times (Kapil and Sharma, 1997). The effect of *Asparagus racemosus, Tinospora cordifolia, Withania somnifera* and *Picorrhiza kurroa* on macrophage function obtained from mice treated with the carcinogen, ochratoxin (OTA) was evaluated by Dhuley (1997). Treatment with these plants significantly attenuated the OTA-induced suppression of chemotactic activity as well as IL-1 and TNF-□ production by macrophages. Moreover, *Withania somnifera* potentiated macrophage chemotaxis and *Asparagus racemosus* induced excessive production of TNF-□ as compared to controls.

Ray et al (1996) demonstrated that ovalbumin immunized mice treated with *Azadirachta indica* leaf extract had higher IgG and IgM levels and anti-ovalbumin antibody titres as compared to control (humoral response). *Azadirachta indica* also induced cell mediated response as

seen from the enhancement of macrophage migration inhibition and footpad thickness. These findings were supported by Ansari et al, (1997). They found that *Azadirachta indica* potentiated the antibody titres following typhoid H. antigen immunization and induced delayed hypersensitivity following administration of tuberculin to animals. In human volunteers, it stimulated humoral immunity by increasing antibody levels and cell mediated immunity by increasing total lymphocyte and T-cell count in 21 days. Oral pretreatment with leaf extract of *Azadirachta indica* reversed the inhibitory effect of restraint stress on formation of anti-sheep RBC antibody titres in rats immunized with sheep RBC and also the increase in foot pad thickness. It reversed the DDT induced suppression of antibody response and leukocyte migration inhibition in tetanus toxoid immunized rats. Restraint stress along with administration of DDT in sub threshold doses resulted in an inhibition of the immune response. *Azadirachta indica* attenuated the immunotoxicity of environmental and xenobiotic stressors (Ray, et al., 1997).

The alkaloidal fraction of *Boerrhiva diffusa* significantly restored the suppressed humoral response in stressed rats as observed by Mungantiwar et al, (1997) wherein *Boerrhiva diffusa* increased the suppressed antibody titres following immunization by sheep RBCs in rats subjected to restraint stress. It also significantly reversed the depleted adrenal cortisol level and the elevated plasma cortisol level in the stressed rats, thus appearing to have a corticosteroid sparing effect in experimental stress. Immune-21, a polyherbal natural product, has been shown to exhibit significant immunopotentiating and immuno-prophylactic activity, both *in vitro* and *in vivo* (De et al., 1998).

1.3.2 Screening methods for immunomodulatory agents

Numbers of *in vitro* and *in vivo* test systems are available for screening immunomodulatory activity. For selection of proper test models, the understanding of methodologies is required which can enable the researcher to thoroughly evaluate such agents. In general most of the models used for studying inflammatory processes including measurements of paw edema can be used preliminary test models. However following are few selected methods widely employed for evaluation of immunological factors.

1.3.2.1Carbon clearance test

Among the *in vivo* test this is the simplest model to test phagocytosis. It is the test of phagocytosis efficiency and can be correlated with in vitro granulocyte test. The carbon clearance i.e. rate of elimination of carbon from blood is determined spectrophotometrically at 650 nm (Biozzi et al., 1953).

1.3.2.2 *In vitro* phagocytosis

a) Microscopic smear test is carried out with human granulocytes fiscal density centrifugation from heparinized blood. Granulocyte fraction is incubated for 30 minutes with yeast and substance to be analyzed. Phagocytosis is terminated with EDTA and the suspension is distributed on microscopic slides. After staining according to Pappenheim, the phagocytosis index is determined. In order to differentiate between cytotoxicity and immunosuppression it is stained with trypan blue (Brandt, 1967).

b) Chemoluminescence test with human granulocytes macrophages. This method measures the quantity of oxygen radicals produced during granulocytes phagocytosis. Luminol is used as an indicator which is excited to chemoluminescence by the oxygen radicals, OH^- and O^{2-}. Zymosan, a complex polysaccharide is used as phagocytosis bait with the help of biolumate, the oxygen production of granulocytes suspension of

known cell density can be followed in parallel in six channels over a period of 1-2 hours, by measuring photons with photomultiplier. The resulting luminescence curves are integrated and the % luminescence is recorded against control. This test can be performed with macrophages, monocytes or kupffer cells from liver (Allen, 1981).

1.3.2.3 Mitogen induced lymphocyte proliferation

Cultured lymphocytes can be stimulated to a proliferative response and to DNA synthesis by various mitogens. Measurement of DNA synthesis can be accomplished by pulse-labeling the culture with tritiated thymidine (^{3}H-thymidine), a nucleoside which is incorporated into the newly synthesized DNA. Immunomodulating properties can be detected either by pretreatment of the animals *in vivo* or by adding the test drug to the cultured lymphocytes (Elves, 1972 and Sensi et al., 1984).

1.3.2.4 Immune induced cytotoxicity test

Macrophages can be transformed into effector cells by induction and stimulation to release cytotoxic effector substances. Tumor necrosis factor (TNF) is one of the important effectors, which necrotizes or lyses tumor tissue. This test depends on whether the tumor cells can be radioactively labeled with Chromium or H-Thymidine. Active test substances promote release of TNF from the macrophages. Quantity of TNF is correlated with the radioactivity in the supernatant. This test can also be performed with the macrophages stimulated *in vivo* (Ruff and Gifford, 1961).

1.3.2.5 Complement test

Complement plays important role in antigen processing i.e. it is involved in defense against viruses and tumors. Activation of complement implies increased destruction and lysis of these cells that possess a lipoprotein membrane (bacteria, viruses, tumor cells). In other terms complement is responsible for some inflammatory processes that occur in

a state of hyper-reactivity where complement inhibition is of considerable interest. *In vivo* test is performed with sensitized sheep erythrocytes and guinea pig complement in a buffer system containing Ca^{2+} and Mg^{2+}. After incubation at 37°C the degree of hemolysis in the supernatant is determined spectrophotometrically, and is compared with the untreated control to determine the decrease in lysis which may result from either activation or inhibition of complement system (Kabat and Mayer, 1969).

1.3.2.6 Inhibition of histamine release from mast cells

Hypersensitivity reactions can be elicited by various factors: either immunologically induced, i.e. allergic reactions to natural or synthetic compounds mediated by IgE, or non-immunologically induced, i.e. activation of mediator release from cells through direct contact, without the induction of, or the mediation through immune responses. Mediators responsible for hypersensitivity reactions are released from mast cells. An important preformed mediator of allergic reactions found in these cells is histamine. Specific allergens or the calcium ionophore 48/80 induce release of histamine from mast cells. The histamine concentration can be determined with the *o*-phthalaldehyde reaction (Church and Young, 1983).

1.3.2.7 Inhibition of T-cell proliferation

Activation and/or proliferation of clonal populations of cells are critical for the initiation of an antigen-specific immune response. Thus, inhibition of T cell activation provides a potent means for suppressing specific immune response. A number of immunosuppressive agents exhibit the ability to suppress T cell activation (Chong et al., 1993a and b).

1.3.2.8 PFC (plaque forming colony) test *in vitro*

Identification of antibody producing cells is based on the ability of the secreted IgM antibody to fix complement and thereby lyse the indicator erythrocytes. Spleen cells or peripheral blood lymphocytes, previously incubated with antigen, are mixed with sheep red blood cells

(SRBC). After addition of compliment and incubation, plaques (clear areas) caused by the lysis of SRBC appear in the otherwise cloudy layer. Antibody forming cells can be detected by the appearance of plaques. The number of plaques obtained is proportional to the number of antibody producing lymphocytes in the cell population (Cunningham and Szenberg, 1983).

1.3.2.9 Inhibition of dihydro-orotate dehydrogenase

Dihydro-orotate dehydrogenase catalyzes the fourth committed step in the de novo biosynthesis of pyrimidines. As rapidly proliferating human T cells have an exceptional requirement for de novo pyrimidine biosynthesis, small molecule dihydro-orotate dehydrogenase inhibitors constitute an attractive therapeutic approach to autoimmune diseases, immunosuppression and cancer. The main mode of action of the immuno-suppressive compound leflunomide and its active metabolites is considered to be the inhibition of the enzyme dihydroorotate dihydrogenase (Bruneau et al. 1998; Graul and Castañer 1998; Knecht and Löffler 1998 and Herrmann et al. 2000).

1.3.2.10 Arthus type immediate hypersensitivity

The immune complex induced Arthus reaction comprises inflammatory factors that have been implicated in the acute responses in joints of rheumatic patients. Complement and polymorphonuclear neutrophiles are activated via precipitating antigen-antibody complexes leading to an inflammatory focus characterized by edema, hemorrhage and vasculitis. Arthus reaction of the immediate type becomes maximal 2–8 h after challenge (Horvat et al., 1990).

1.3.2.11 Delayed type hypersensitivity

Delayed type hypersensitivity (DTH) is a reaction of cell mediated immunity and becomes visible only after 16–24 h. The same methods as for testing immediate type hypersensitivity can be used (Titus and Chiller, 1981).

1.3.2.12 Reversed passive arthus reaction

In the reversed passive Arthus reaction the antigen is injected intravenously followed by a local injection –either intradermally or into the pleural space – of the respective antibody. Generation of an immune-mediated reverse passive Arthus reaction in the rat pleural cavity results in a classic acute inflammatory response. The methods are used to evaluate new anti-inflammatory agents (Bailey and Sturrn, 1983).

1.3.2.13 Adjuvant arthritis in rats

Adjuvant arthritis in rats has been described by Pearson and Wood (1959) exhibiting many similarities to human rheumatoid arthritis. Injections of complete Freund's adjuvant into the rat paw induce inflammation as primary lesion with a maximum after 3 to 5 days. Secondary lesions occur after a delay of approximately 11 to 12 days which are characterized by inflammation of non-injected sites (hindleg, forepaws, ears, nose and tail), a decrease of weight and immune responses. The procedure has been modified by several authors in order to differentiate between anti-inflammatory and immunosuppressive activity (e.g. Perper et al. 1971). Anti-inflammatory compounds do not inhibit secondary lesions, which are prevented or diminished by immunosuppressive agents. Two protocols, termed "preventative" (or "prophylactic") and "therapeutic" (or "established") adjuvant arthritis, have gained wide usage for assessing a drug's potential anti-arthritic activity (Schorlemmer et al. 1999).

1.3.2.14 Collagen type II induced arthritis in rats

As reported by Trentham et al. (1977) intradermal injection of homologous or heterologous type II collagen in incomplete Freund's adjuvant results in an inflammatory polyarthritis in rats. The demonstration of antibodies to collagen in patients with rheumatic polyarthritis suggests that autoimmunity may contribute to the pathophysiology of synovitis and joint destruction. Because of the similarities of the symptoms in rats to human disease the test is

considered to be useful to detect anti-inflammatory and immunosuppressive properties of test compounds.

1.3.2.15 Experimental allergic encephalomyelitis

Experimental allergic encephalomyelitis was first produced in laboratory animals by Rivers et al. in 1933. This pathological model is an immunologic disease arising from a delayed hypersensitivity reaction to nervous tissue. In many respects, the model resembles autoimmune diseases, especially demyelinating diseases, in man. The method is used for evaluation of immunosuppressive properties of drugs.

1.3.2.16 Acute graft versus host disease (GVHD) in rats

The intravenous injection of a mixture of parental splenocytes into healthy inbred F1-rats results in graft versus-host (GVH) induced immune abnormalities. This is due to T-lymphocytes in the donor inoculum that recognize the major histocompatibility alloantigens expressed by the F1-animals. The host F1 T-cells are genetically unable to recognize antigens of the parental donor as foreign, thus the response involves only donor recognition of host and not host recognition of donor. The ensuing immune abnormalities lead to clinical symptoms of an acute, lethal GVH-disease (GVHD), i.e. profound immunodeficiency, anemia, hypogammaglobulinemia and runting (Ford, 1970 and Gelpi et al., 1994).

1.3.2.17 Inhibition of allogenic transplant rejection

Transplantation of allogenic organs to recipients results in rejection of the transplants. This effect can be suppressed or delayed by immunosuppressive agents. Various organs are used for allogenic transplantation in animal experiments, such as skin pieces (Schorlemmer et al. 1993), kidney (Lee 1967; Küchle et al. 1991), rat heart, rat small intestine (Xiao et al. 1994) and corneal buttons (Coupland et al. 1994). The immunosuppressive activity can be evaluated either by using a major histocompatibility complex variant strain combination or a strong allogenic system.

1.4.1 *Rasayana* drugs as antioxidants

As plants produce a lot of antioxidants to control the oxidative stress, they can represent a source of new compounds with antioxidant activity. Ayurveda, the Indian traditional health care system is the oldest medical system in the world, which exploits the potential of various herbs generally in polyherbal formulations as drugs (Dash and Kashyap, 1980). A number of plants and plant isolates have been reported to protect free-radical induced damage in various experimental models.

One of the clinical specialities of Ayurveda is Rasayana. Rasayana is not only a drug therapy but is a specialized procedure practiced in the form of rejuvenating recipes, dietary regimen promoting good habit. With regard to the rasayana drug therapy the strong antioxidant activity of any rasayana has been reported: these compounds were found to be 1000 times more potent than ascorbic acid, α-tocopherol and probucol. *Emblica officinalis, Tinospora cordifolia, Asparagus racemosus, Tribulus terristris, Withania somnifera, Mangifera indica* are some examples of rasayana drugs claimed to have antioxidant properties (Scartezzini and Speroni, 2000).

Rasayanas are reputed to promote physical and mental health, improve defence mechanisms of the body and enhance longevity. These attributes are similar to the modern concept of adaptogenic agents, which are, known to afford protection of the human physiological system against diverse stressors (Bhattacharya et al., 2000).

Even the most conservative medical fields nowadays accept the importance of antioxidants and people find great benefits from these nutritional ingredients in achieving optimum health.

Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) ravage and restore the optimal balance by neutralizing the reactive species. Thus, herbal drugs containing antioxidants are gaining immense importance by virtue of their critical role in disease prevention.

1.4.2 *In vitro* methods for screening antioxidant activity

A free radical is any species that contains one or more unpaired electrons and is capable of independent existence (Halliwell et al., 1995). Free radicals such as trichloromethyl ($CCl^{\bullet}_3$), superoxide ($O^{\bullet-}_2$), hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$), and nitric oxide ($NO^{\bullet}$) are known to be produced metabolically in living organisms. In addition, some non-radical derivatives of oxygen molecules (hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl)), can be generated in foods and biological systems. All of these reactive oxygen species participate in the chain reaction of free radicals, thus tests of the ability of a substance to scavenge radical species may be relevant in the evaluation of antioxidant activity (Halliwell, 1990; Halliwell et al., 1995).

1.4.2.1 Scavenging of Hydrogen Peroxide (H_2O_2)

The generation of hydrogen peroxide (H_2O_2) by activated phagocytes is known to play an important part in the killing of several bacterial and fungal strains. Additionally, H_2O_2 is generated in vivo by several oxidase enzymes. There is increasing evidence that H_2O_2, either directly or indirectly via its reduction product OH^-, acts as a messenger molecule in the synthesis and activation of inflammatory mediators. Hydrogen peroxide-scavenging activity is easily and sensitively measured by using peroxidase-based assay systems. The most common employs horseradish peroxidase, which uses H_2O_2 to oxidize scopoletin into a non fluorescent product. In the presence of a putative scavenger, the oxidation of scopoletin is inhibited and the H_2O_2 scavenging can be monitored (Halliwell, 1990). Following this assay, Martý´ nez-Tome´ et al. (2001a, b) evaluated the antioxidant activity of broccoli amino acids, and of Mediterranean spices in an aqueous medium.

1.4.2.2 Scavenging of Hypochlorous Acid (HOCl)

Another source of strong oxidants *in vivo* is neutrophil myeloperoxidase (MPO), which catalyzes oxidation of chloride ions by H_2O_2, resulting in hypochlorous acid (HOCl) production. The cytotoxicity of this reaction contributes to the killing of bacteria in the host defense

system. However, HOCl generated by MPO might also inactivate □1-antiproteinase and contribute to proteolytic damage of healthy human tissues in inflammatory disease (Halliwell and Gutteridge, 1990; Hippeli and Elstner, 1999). Antioxidants can be tested for their potential to interfere with tissue damage caused by HOCl. Scavenging of HOCl can be examined using MPO/ H_2O_2/sodium chloride as a source of this substance. In this reaction system, Lavelli et al. (1999, 2000) evaluated the inhibition by tomato extracts of different polarities of HOCl production in the presence of 1-aminocyclopropane-1-carboxylic acid (ACC). The reaction is followed by measurement by GLC of ethene release from ACC by HOCl.

1.4.2.3 Scavenging of the Stable Radical 1,1-diphenyl-2-picrylhydrazyl – DPPH assay

This assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$). The free radical DPPH$^{\bullet}$ is reduced to the corresponding hydrazine when it reacts with hydrogen donors (Contreras-Guzma´ n and Strong, 1982). This ability is evaluated using electron spin resonance spectroscopy on the basis that the DPPH$^{\bullet}$ signal intensity is inversely related to the test antioxidant concentration and to the reaction time (Chen et al., 2000), but the more frequently used technique is the decoloration assay, which evaluates the absorbance decrease at 515–528 nm produced by the addition of the antioxidant to a DPPH$^{\bullet}$ solution in ethanol or methanol. Different authors use different initial radical concentrations and different reaction times. DPPH$^{\bullet}$ assay is considered a valid and easy assay to evaluate scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated as in other radical scavenging assays.

1.4.2.4 Scavenging of Peroxynitrite (ONOO$^{\bullet}$)

Peroxynitrite (ONOO$^{\bullet}$) is formed by the reaction of nitric oxide and superoxide. ONOO$^{\bullet}$ is a cytotoxic reactive species that can be generated by endothelial cells, neutrophils, and macrophages (Balvoine and Geletti,

1999). $ONOO^-$ scavenging by the oxidation of dihydrorhodamine (DHR) 123 to fluorescent rhodamine 123 is measured in the presence of potential antioxidant with a microplate fluorescent spectrophotometer with excitation and emission wavelength of 485 and 530 nm, respectively. The assay measures the potency of marine algae and green tea tannin extracts in the inhibition of DHR 123 oxidation by $ONOO^-$ (Chung et al., 1998, 2001).

1.4.2.5 Assay for superoxide radical scavenging activity

The assay was based on capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-nitro blue tetrazolium (NBT) system. The reaction medium contains phosphate buffer (pH 7.6) 2.5 ml, 100µl riboflavin (20µg), 200µl EDTA (12mM), 100µl NBT (0.1 mg) and different concentration of sample contained in 100µl of methanol. The reaction was started by illuminating the reaction mixture for 5 minutes. The absorbance was measured at 590 nm (Beuchamp and Fridovich, 1971).

1.4.2.6 Assay for nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions which can be estimated by use of Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Sreejayan and Rao, 1997).

1.4.2.7 Determination of reducing power

The reducing power can be determined according to the method of Oyaizu (1986). Samples were mixed with 5 ml phosphate buffer (2M, pH 6.6) and 5 ml potassium ferricyanide (1%), the mixture was then incubated at 50^0 C for 20 minutes, 5 ml trichloroacetic acid (10%) was added an the mixture was centrifuged at 4000 rev./ min. The upper 5 ml solution was then mixed with 5 ml distilled water and 1 ml ferric chloride

(0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

1.4.2.8 Measurement of effect on lipid peroxidation on rat liver homogenate

Rat liver homogenate was prepared by homogenizing the tissue in chilled Tris buffer (10mM, pH 7.4) at a concentration of 10% w/v; peroxidation was induced in liver tissue by Iron-ADP complex in the presence of ascorbic acid. The incubation medium constituted 0.5 ml of the liver homogenate (10% w/v), 100 µM $FeCl_3$, 1.7 µM ADP, 500 µM of ascorbate and different concentrations of samples in 2 ml of total incubation medium. The medium was incubated for 20 min. at 37°C. Extent of lipid peroxidation was measured by estimation of malondialdehyde (MDA) content. Results were expressed in terms of decrease in MDA formation by the sample extract. Ascorbic acid was used as positive control (Slater and Sawyer, 1970).

1.5 LITERATURE REVIEW ON SELECTED PLANTS

The seriousness of immunocompromises of varied aetiologies, and associated susceptibility to opportunistic intra-cellular infections, needs no emphasis. The conventional approaches involving immune-based therapeutics, with interventions such as passive immunity, cytokine treatments, adoptive immunotherapy and therapeutic vaccination have not yet yielded a cost-effective and safe therapy. As against this background, in the ancient science of Indian medicine, the Ayurveda, a number of herbs are mentioned which are known for their restorative and rejuvenative properties. Studies revealed that these agents promote positive health and counter stressors of varied origin-whether biological, physical, environmental or psychological challenges.

In our efforts to screen herbal drugs with potential immunomodulatory activities, leads were taken from Ayurveda and three plants viz., ***Sphaeranthus indicus*** (flowerheads), ***Curculigo orchioides*** (rhizomes) and ***Cissampelos pareira*** (roots) were selected from rasayana category and screened for their immunomodulatory potential. The

available information on these plants collected and presented below showed paucity of data pertaining to immunomodulatory properties of these drugs.

1.5.1 *Sphaeranthus indicus* Linn.

Common (Indian) Names

Sanskrit:	Mahamundi, Mundi.
Hindi, Bengali, Marathi & Gujarati:	Mundi, Gorkhmundi,
Telugu:	Boddatarupa, Boddasoramu
Tamil:	Kottak aranthai
Malayalam:	Mirangani
Punjabi:	Ghundi, Khamadrus

Family: *Asteraceae* (*Compositae*)

Habitat: Common rabi weed found in rice fields.

Distribution: Throughout India, Sri Lanka, Africa and Australia.

Related Species: *Sphaeranthus africanus* L. (Sanskrit-Sveta Hapusa; Malyali-Velutha adakkamaniyan)

Useful Parts: Root, bark, leaves, flowers, and seeds.

Traditional uses: According to Ayurveda, this herb is hot, used as bitter stomachic, stimulant, alterative, pectoral and demulcent, and externally emollient. Distilled water prepared like rose water from the herb is recommended by local physicians for bilious affections and for dispersions of various kinds of tumours. Flowers (flower heads) are highly esteemed as alteratives, depuratives, refrigerants and tonics, useful as blood purifiers in skin diseases. The drug is also useful in urethral discharges and in jaundice (Nadkarni, 1976).

Preparations: Mundi Churna, Mundi panchang swarasa, Mundi kvatha.

Folklore uses: Various parts (principally flower-heads) of this plant are widely used by tribes of many states of India as folk medicines. The Kondh tribes of Dhenkanal district of Orissa utilizes boiled decoction of the plant for the treatment of diarrhea (Girach et al., 1994).

An ethnobotanical survey of Rayalseema of Andhra Pradesh reveals that this plant is utilized by the tribal as well as non-tribal inhabitants for treating different ailments. Juice of flowers is used as eye drops for the treatment of inflamed eyes and other eye diseases and also in treatment for filariasis. Fruits and roots are used for treatment of stomach worms and filariasis respectively (Nagraju and Rao. 1990).

Mundas and Asurs tribes of Neterhat plateau of Bihar uses this plant for treating different diseases. In cases of eye pains, night blindness and asthma, the flowers are taken with water. In cases of jaundice, about 5 flowers are soaked in water overnight and taken together with 50 g of sugar candy (Jain et al., 1994).

Phytochemistry and Pharmacology: A few phytoconstituents from the plant have already been isolated and also studied for pharmacological actions.

The cherry colored essential oil obtained from shade dried leaves of the plant was found active against *Vibrio cholerae* and *Micrococcus pyrogenes* var. *aureus* (Wealth of India, 1976). Another study conducted on essential oil showed that the oil possesses very good anti-bacterial activity against *S.enteritides, Sh. Flexneri, S.typhimurium* and *S.paratyphi A* (Garg and Kasera, 1983).

A bicyclic sesquiterpene lactone isolated from the petroleum ether extract of the aerial part of the plant showed strong antimicrobial activity against *Staphylococcus aureus, S. albus, Escherichia coli, Fusarium* sp., *Helminthosporium* sp. and other microorganisms (Singh et al., 1988).

The chloroform extract of the air dried, powdered flower tops afforded three crystalline sesquiterpene lactones all with an unusually located tertiary hydroxyl group. Two of the lactone also contains á-methylene-ã-lactone moiety and the third was a saturated lactone with a methyl group (Gogte et al., 1986).

A new sesquiterpene glycoside, sphaerantholide, has been isolated from flower heads and was found as immunostimulating agent when tested by plaque forming cell assay (Shekhani et al., 1990).

Three new eudesmanolides, 11 á, 13-dihydro-3 á, 7 á-dihydroxyfrullanolide, 11 á, 13-dihydro-7 á, 13-dihydroxyfrullanolide, 11 á, 13-dihydro-7 á-hydroxy-13-methoxyfrullanolide, were isolated from the flower heads (Shekhani et al., 1991).

Three new eudesmanolides along with two sesquiterpenoids, cryptomeridiol and 4-epicryptomeridiol has been reported from flower heads (Supada et al., 1992).

A novel isoflavone glycoside isolated, 5,4'-dimethoxy-3'-prenylbiochanin 7-*O*-â-D-galactoside, was isolated from the leaves of the plant (Yadava and Kumar, 1999). Also a new flavone glycoside from stem of the plant isolated (Yadava and Kumar, 1998).

Three new eudesmanolides have been isolated from the whole plant and their structures were established as 11 á, 13-dihydro-3 á, 7 á-dihydroxy-4,5-epoxy-6 â,7-eudesmanolide, 11 á, 13-dihydro-7 á-acetoxy-3 â-hydroxy-6 â,7-eudesm-4 enolide and 3-keto- â-eudesmol (Pujar et al., 2000).

An antimicrobial sesquiterpene lactone, 7-hydroxyfrullanolide, was isolated from plant (Atta-Ur-Rahman et al., 1989). Ethanol extract of *S. indicus* screened for cytostatic activity and was found to possess good cytostatic activity (Smit et al., 1995). It was also screened for its nematocidal activity on larva of *Toxicaria canis* (Kiuchi et al., 1989). In Unani medicine it is mentioned for treatment of tuberculosis (Zafarullah et al., 1980).

1.5.2 *Cissampelos pareira* Linn.

Common (Indian) Names

Sanskrit:	Patha
Hindi:	Harjari, Pahadvel.
Marathi:	Venivel
Gujarati:	Phang, Akanadi.
Oriya:	Akanabindu

Family: *Menispermaceae*

Habitat: Woody climber shrub.

Distribution: Throughout Asia, East Africa, and America.

Related Species: *Cissampelos sympodialis*

Useful Parts: Root, rootbark, leaves, whole plant and seeds.

Traditional uses: The roots are reported to have found use as a diuretic, febrifuge, remedy for heart trouble, and against dysentery and soars (Chopra, 1958). The roots of *C.pareira* are used to prevent a threatened miscarriage, and the herb is also used to stop uterine hemorrhage (Lewis, 1977).

Preparations: Candanasava, Shrikhandasava, Usirasava, Vidangarista, Pusyanuga churna, Aragradhadhi kwatha churna, Cangari ghrita, Pañchatikta guggulu ghrita etc.

Folklore Uses: In Assam many tribes use this plant for birth control, immediately after birth this plant is used in combination with *Piper nigrum* L. (Tiwari et al., 1982). As an ethnomedicine it is also used as anti-snake venom in South India (Selvanayahgam et al., 1994). It is one of the agents used in fevers as a primary health care folklore medicine in India (Singh and Ali, 1994).

Phytochemistry and Pharmacology: *Cissampelos* plants, including *C.pareira*, contain a group of plant chemicals called *isoquinoline alkaloids* which have received a great deal of attention and research.

C.pareira showed antileukemic activity, and a novel tropoloisoquinoline alkaloid named pareirubrine A, was reported (Morita et al., 1993).

Pradhan et al, (1953), carried out pharmacological and clinical studies on hayatin methiodide from *C.pareira* for its muscle relaxant

properties. Basu et al., (1970) reported curariform activity of hyatinin methochloride from *C.pareira*.

Cissamperine and other four bisbenzylisoquinoline alkaloid isolated from *C.Pareira* were found to show significant and reproducible inhibitory activity against human carcinoma of the nasopharynx carried in cell culture (KB) (Kupachan et al., 1965).

Out of 38 alkaloids discovered in *C.pareira* thus far, one called *tetrandrine* is the most well documented. In clinical research over the years, tetrandrine has been documented with analgesic, anti-inflammatory, and fever reducing properties. Over 100 recent clinical studies also describe this chemical's promising actions against cancer and leukemia cells, and research is ongoing. The therapeutic dosages of tetrandrine in these animal studies, however, are reported at much higher dosages than can be obtained reasonably in natural *C.pareira* root or vine. (About 2 pounds of *C.pareira* root would need to be taken daily by the average weight person to provide the therapeutic dosage of tetrandrine used in the animal studies.) Other recent published studies concern tetrandrine's cardioactive and hypotensive effects through numerous pathways and mechanisms of action at much smaller dosages. Another well known alkaloid chemical, berberine, has been documented to be hypotensive, antifungal, and antimicrobial. This chemical has been used for the treatment of irregular heartbeat, cancer, candida, diarrhea, and irritable bowel syndrome (Bruneton, 1995; Werbach, 1994 and Blumenthal, 1997).

A root extract was reported to have a diuretic effect in other animal studies, which also confirms another of its traditional medicine uses (Caceres et al., 1987).

The root evidenced a mild ability to lower blood sugar levels when given in high dosages (5 g per animal). The root was also shown to have anticonvulsant actions in mice and, in dogs it showed marked hypotensive actions (Tripathi et al., 1979 and Adesina, 1982).

In vitro studies over the years have reported that *C.pareira* has antioxidant properties (Sanchez Medina et al., 2001); antibacterial actions against *Staphylococcus*, *Pseudomonas*, *Salmonella*, and *Klebsiella*

(George and Pandalai, 1949) as well as antimalarial effects (Gessler et al., 1994).

1.5.3 *Curculigo orchioides* Gaertn.

Common (Indian) Names

Sanskrit:	Krishna musali
Hindi, Marathi, Gujarati :	Kalimusali.

Family: *Hypoxidaceae, Amaryllidaceae*

Habitat: Perennial, geophilous, Scapigerous monsoon herb.

Distribution: Indigenous to India, China.

Useful Parts: Root stocks, rhizomes.

Traditional uses: Musali is a powerful drug of Ayurvedic system, agreeable and bitter in taste, a nourishing tonic which gives strength and destroys all ailments pertaining to the anal region i.e. piles. It suppresses 'vat' and 'pitta', increases vigor and vitality; gives long life. It is pleasing and increase secretions of body fluids and keeps one healthy and robust. It is useful in fever, joint pains, cuts, diseases of nerves, vomiting, dyspepsia, diabetes, and keeps the three 'doshas' in proper balance. It removes the burning sensation due to hyperacidity, cures diseases of blood, jaundice, asthma, diarrhea and is a restorative. The rhizomes are considered edible and a cooling medicine. The juice of rhizomes mixed with garlic is used as eye drop to cure blindness and white spots on the eyeball.

Preparations: Ashwabal, Musalipak, Vigorex etc.

Phytochemistry and Pharmacology: Phytochemical investigations on *C.orchioides* revealed the presence of a novel pentacyclic triterpenoid (Mehta and Gawarikar, 1991).

Four phenol glycosides have been reported and identified as curculigoside, orcinol glucoside, curculigine A and corchioside (Kubo et.al., 1983 and Garg et.al., 1989). Along with these constituents cycloartane type glycosides and their glycosides also reported (Xu JP et.al., 1992 a).

Pharmacological study on ethanol extract of *C.orchioides* showed enhancing tolerance towards high temperature and hypoxia. It also had sedative, anti-convulsant and androgen like effect, besides it increased immunological activity of mice (Chen et al., 1989).

Another study on isolated Curculigo saponins C and F showed increased proliferation of spleen lymphocytes but no marked influence was observed on antibody formation. Compound 7 increased the weight of thymus when administered at 10 mg/kg intraperitonially for 5 days to mice (Xu JP et.al., 1992 b).

Methanolic extract of the roots has been shown to enhance phagocytic activity of macrophages and an active principle of the extract identified as a curculigoside (5-hydroxy-2-*O*-â-d-glucopyranosyl, benzyl 2,6-dimethoxy benzoate), which has been reported to possess adjuvant activity (Saike et al., 1981).

Powdered drug and its aqueous extract were found to possess hepatoprotective and anti-inflammatory activity (Rao and Mishra, 1996). It also possesses good antioxidant activity (Venukumar and Latha, 2002). Curculigol and Curculigenin A isolated from drug were reported for antihepatotoxic activity against galactosamine on isolated rat hepatocytes (Rao and Mishra, 1997).

1.6 RESEARCH ENVISAGED

In industrialized nations some fifty percent of all prescribed drugs are derived or synthesized from natural products, the only available sources for which are animals, marine, plants and micro-organisms. It is considered that because of the structural and biological diversity of their constituents, plants offer a unique and renewable resource for the discovering of potential new drugs and biological entities. Between 1983 and 1994, 41% of new approved drugs have natural products as their

source, which indicates that natural products still play a very important role in the development of new medicine.

The present work therefore aimed to evaluate and standardize the therapeutic efficacy of selected plant drugs such as ***Sphaeranthus indicus***, ***Cissampelos pareira*** and ***Curculigo orchioides*** for the claims made under traditional systems for their immunomodulatory and antioxidant activities on the following lines:

1. Pharmacognostic studies:

- Collection and identification of plant material
- Macroscopic and microscopic examination
- Proximate analysis

2. Phytochemical studies:

- Preliminary phytochemical screening of successive extracts using qualitative chemical tests and TLC profiles.
- Preparation of selective extracts for biological screening.
- Fractionation of extracts to identify bioactive fraction.
- HPTLC finger print profiles of extracts and /or fractions.
- Isolation and characterization of phytoconstituents from fractions.

3. Biological screening:

3.1 Immunomodulatory activity

The extracts and /or fractions selected for biological screening shall be subjected to immunomodulatory activity using following methods in mice and the active one shall be assigned as bioactive extracts and /or fractions.

- **Carbon clearance test**
- **Humoral antibody titre**
- **Delayed type hypersensitivity**
- **Cyclophosphamide induced myelosuppression assay**

The bioactive extract and /or fraction shall then be subjected to screen immunomodulatory activity in drug induced immunosuppression using following methods-

- Effect of extract and /or fractions and cyclophosphamide on HA titre and DTH response using SRBCs as an antigen in mice- 7 days pretreatment.
- Effect of extract and /or fractions on HA titre and DTH response using SRBCs as an antigen in mice- 15 days pretreatment.

3.2 *In-vitro* antioxidant activity:

The bioactive extracts and /or fractions shall also be subjected to evaluation of *in-vitro* antioxidant activity using following methods-

- **Assay for antiradical activity with DPPH.**
- **Assay for superoxide radical scavenging activity.**
- **Assay for nitric oxide scavenging activity.**
- **Determination of reducing power.**
- **Measurement of effect on lipid peroxidation on rat liver homogenate.**