Review on Immunomodulatory Role of Plant Drugs in Indian Traditional Systems of Medicine

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ABSTRACT

Modulation of immune response to cure various diseases has been a very interesting concept and becoming the field of intensive research worldwide. Widespread efforts have been made to identify immunomodulatory agents as prophylactic or therapeutic regimens to combat infections or to enhance the immunocompetence of the host. Immunomodulators have been gaining global significance now days because it is better to prevent disease instances rather to treat them. The use of traditional knowledge and practices as a drug discovery engine is time and cost effective as well as could lead to better success. Although a number of synthetic drugs are being used in immunotherapeutics, the adverse side effects associated with their usage such as nephro toxicity, anaemia, thrombocytopenia, bone marrow suppression etc., has produced an awareness to limit their usage and to search for safe alternatives. Besides their side effects, synthetic immunomodulators also incur heavy economic burden, hence there is a need for an alternative cost effective safe herbal immunomodulator. In this context, the present review focused on the immunomodulatory role of plant drugs which are used in Siddha and Ayurveda system of medicines in India.

Keywords: Immune system, Immune modulation, Plant drugs, Siddha, Ayurveda.

INTRODUCTION

A number of compounds with immunomodulatory potential have been isolated from lower organisms1. Drugs or biological agents capable of modulating single pathways or targets are of limited value in immune related therapies as immunity is interplay between multiple organs, cells and signalling molecules. Since botanicals are chemically complex and diverse, could therefore provide appropriate combinations of synergistic moieties to treat immune dysfunctions. According to the World Health Organization (WHO), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for the primary health care. In fact, herbs and/or plants are the oldest friends of mankind. They not only provide food and shelter but also serve to cure different ailments. Herbal medicine, sometimes called traditional or natural medicine, has always existed in one way or another in different cultures and civilizations, such as Ayurvedic (India), Egyptian, Western, Chinese, Kampo (Japan) and Greco-Arab or Unani-Tibb (south Asia).

Ayurveda, the Indian traditional system of medicine, lays emphasis on promotion of health concept of strengthening host defences against different diseases2. 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 axis3. Several plants from these texts have been studied for their immunomodulatory properties and found to have the potential of providing new scaffolds for safer, synergistic, cocktail immunomodulators4. Thirty four plants have been identified as Rasayanas in the Ayurvedic system of medicine. Besides these, several other medicinal plants which are not included as Rasayana in Ayurveda have also been found to possess immunomodulatory properties5.

Immunity as per Ayurveda and Siddha perspectives

It is a well-known fact that traditional systems of medicines always played important role in meeting the global health care needs. The system of medicines which are considered to be Indian in origin or the systems of medicine, which have come to India from outside India and which got assimilated in to Indian culture are known as Indian systems of medicine6. India has the unique distinction of having such six recognized systems of medicine in this category, like Ayurveda, Siddha, Unani, Yoga, Naturopathy and Homoeopathy. Among these Ayurveda and siddha systems of medicine are considered to be purely indigenous.

Ayurvedic system of medicine

“Ayurveda” literally means the Science of life. It is presumed that the fundamental and applied principles of Ayurveda got organized and enunciated around 1500 BC. Atharvaveda, the last of the four great bodies of knowledge-known as Vedas, forms the backbone of Indian civilization, which contains 114 hymns related to formulations prescribed for the treatment of different diseases. From the knowledge gathered and nurtured over century’s two major schools and eight specializations got evolved. One was the school of physicians called as ‘Dhanvantri Sampradaya’ (Sampradaya means tradition) and the second school of surgeons referred in literature as ‘Atreya Sampradaya’.
These schools had their respective representative compilations - Charaka Samhita for the school of Medicine and Sushruta Samhita for the school of Surgery. The former contains several chapters dealing with different aspects of medicine and related subjects. Around six hundred drugs of plant, animal and mineral origin have been mentioned in this treatise.

Sushruta Samhita contains description of about 650 drugs and discusses different aspects related to other surgery related topics such as anatomy, embryology, toxicology and therapeutics. Vagabhata’s ‘Astanga-Hridaya’ is considered as another major treatise of Ayurveda. The above three documents are popularly known as ‘Brihat trayees’ (the big or major three). Besides these three scholarly and authoritative treatises a vast body of literature exist in the form of compilations covering a period of more than 1500 years.

**The concept of health in Ayurveda**

In India, Ayurveda is considered as a complete medical system that takes in to consideration physical, psychological, philosophical, ethical and spiritual well being of mankind. It lays great importance on living in harmony with the Universe and emphasises harmony of nature and science. This universal and holistic approach makes it a unique and distinct medical system. This system insists the importance of maintenance of proper life style for keeping positive health. This concept was in practice since two millennium and the practitioners of modern medicine have now started recognising the importance of this aspect. Not surprisingly the WHO’s concept of health propounded in the modern era is in close approximation with the concept of health defined in Ayurveda.

**The concept of immunity in Ayurveda**

A definite approach towards understanding the rationale behind the therapy of immune disorders all through these years is still elusive, but its mention is available in the age-old Indian medical expertise - “Ayurveda”. It is pertinent here to explore the concept of immunity existing in this ancient science of life which offers a holistic immunotherapeutic approach. The main purpose and objectives of Ayurveda is maintenance of health in healthy individual and eradication of diseases which are curable. A person who is having balanced proportion of muscles, compactness, and excellent sensory faculties never suffers from diseases. Such people can easily withstand extremes of hunger, thirst, heat of the sun, cold and physical exercises.

Ayurveda narrates the promotion of health through the strengthening of host defences, and to act as a resistive force against day-to-day physiological extremes as well as opportunistic maladies. This force to reckon with, as regards everyday wellness is termed as “Vyadhikshamatva” in Ayurveda. The concept expounds both preventive medicine aspects, along with curative aspects of treatment are self-explanatory terms – “Vyadhibalavirodhitvam” and “Vyadyutpadapratibandhatvam” (Table 1).

The concept of Vyadhikshamatva (immunity) is of tremendous importance in the daily wellness of human beings; for prevention and recovery from diseases. Vyadhikshamatva in Ayurveda is not merely immunity against a specific infectious agent or disease; rather, it implies resistance against the loss of the integrity, proportion, and interrelationship amongst the individual’s doshas (bioenergies) and dhatus (tissues) (Cha. Sam. Sutrasthana 28/7, Cakrapani commentary, p.570). Synonyms for Vyadhikshamatva which appears in Ayurvedic scriptures are: Sleshma, Bala and Ojas.

**Sleshma**

Sleshma in normal state is called Bala and Oja. Sleshma in abnormal state is called Mala (waste) and Papma (diseases) (Charaka Samhita Sutrasthana 17/117, p.366).

**Bala**

Acharya Sushruta described bala as “Tatra balen sthirapacitamamsataa sarvachestasvaprotigataah sarvarvarnaprasado bahyanamabhyantararanam cha karananammatmakarya pratipattirbhavati” means Bala imparts firm integrity to the muscles, improves the voice and complexion, and helps the person to perform his natural functions normally (Sus. Sam. Sutrasthana 15/25, p.61). Three types of bala are there in Ayurveda (Figure 1).

**Ojas**

Ojas have direct influence on the body’s defense against decay, degeneration and infections. It provides appropriate substances to the body to destroy the virulent factors of disease. Ojas is the sara of all the Dhatus, being located in Hrudaya, mixes with Rasa and circulates through the blood vessels and capillaries and performs Tarpana of the whole body. Two types of Ojas are Para Ojas or Ashtabindu Ojas (if decreases person will die), and Apara Ojas or Ardhanjali Ojas (if decreases or vitiated manifest abnormalities).
Methods to enhance Immunity-Vyadhiksamatva

In Ayurvedic texts, various codes of conducts are described for each person (from conception to old age) to be followed in different seasons, during healthy and diseased conditions in terms of dietetic (ahara) and mode of life (vihar). These are presented in the following table.

Concept of immunomodulation in Ayurveda

“Rasayana” is made up of two words: ‘rasa’ and ‘ayana’. ‘Rasa’ primarily means essential seven vital tissues (saptadhatu e.g. rasa, rakta, mansa, meda, asthi, majja and shukra). ‘Ayana’ means the path or channel. So, rasayanas are those that bring about proper uptake, growth and improvement of essential dhatus. According to acharya Charaka, use of rasayanas results in disease-free long life (dirghamaayu), smiriti (recapitulating power), medha, aarogyam (healthy well being), tarun vaya (youthfulness), prabha, varna (complexion), voice and strength (Cha. Sam. Chikitisasthana 1/1/7-8, p.5). According to Acharya Sarangdhara, various drugs, diet and regimens which promote longevity by delaying aging and preventing diseases are called Rasayana such as amrita, guggul, and haritaki (Sar. Poorvakhandha 4/13, p.48).

Rasayana is a treatment in which the body constituents are prepared to adapt to a selective tissue endowment program. This concept in modern scientific understanding would mean the enhancement of immune responsiveness of an organism against pathogens by activating the immune system with immunomodulatory plant agents. Rasayana Chikitsa or rejuvenation therapy helps in promoting and maintenance of health and longevity in the healthy, and to cure disease in sick.

Classification of Rasayana
A) As per method of use
- Kutipraveshika (indoor regimen): As per the needs of rasayana, Patient has to be kept in a very specialized manner in a specifically made kuti10.
- Vatatapika rasayana (outdoor regimen): Patients can stay at their residence and take the treatment. Their normal routines will not be disturbed.
- Achara Rasayana (mode of conduct): This line of treatment follows a particular code of conduct in routine life, which keeps oneself to attain good mental and spiritual health. By following achara rasayana, person can be free from anxiety, stress, and thereby prevent all diseases that are generated due to undue stress, anxiety, fear, anger and depression.

B) As per Scope of use
- Kamya Rasayana- used for the attainment of a specific wish11.

- Prana kamya: Aiming to complete desires of vitality and longevity.
- Medhakamya: To promote intellectual power
- Shreekamya: To enhance complexion and luster.
- Naimittika Rasayana- To be used in person suffering from definite disease.
- Ajasrika Rasayana- It can be used daily as diet.

C) According to Prabhava (Effect):-
- Samshodhana Rasayana- Rasayana which eradicate the vitiated Doshas by expelling from body.
- Samshamana Rasayana- Rasayana drugs which have shaman effects on Doshas.

D) As per content of Rasayana:–
- Ahara rasayana- It is based on diet.
- Aushadha rasayana- It is based on herbs and drugs.
- Achara rasayana- It is based on conduct and behaviour.

E) According to Sapta Dhatus (Body tissues):–
- Rasa (Lymphatic)
- Rakta (Blood)
- Mamsa (Muscles)
- Meda (Adipose tissue)
- Asthi (Bone)
- Majja (Bone marrow)
- Shukra (Reproductive element)

F) According to Satmya:-
- Desha Satmya
  - Anupa Desha
  - Jangala Desha
  - Sadharana Desha
- Ritu Satmya
  - Aadana kala- Shishir, basant and grishma ritu.
  - Visarga Kala- Varsha, sharad and he-mant ritu.

G) According to Modern Medical Science:-
- To enhance Immunity.
- To improve and recover metabolism
- To improve secretions (Both exocrine and endocrine).
Plants identified as Rasayana

Rasayana chikitsa means rejuvenation therapy. Administration of rasayana could be useful in improving immunity of a person and prevent him from getting diseases. The possible mechanisms by which action of Rasayana can be interpreted in modern views are to provide nutritive function, immunomodulatory action, antioxidant action, anti-aging action, neuro-protective action, and haemopoietic effect. Thirty four plants have been identified as Rasayasanas in the Ayurvedic system of medicine. These plants possess various pharmacological properties. These are enlisted in Table 4.

Besides these, several other medicinal plants, which are not included as Rasayana in Ayurveda have also been found to possess immunomodulatory potential. ‘Ama’ is immunologically active complexes generated in the intestine due to improper digestion of food, which is the cause of pathogenesis of several diseases like rheumatoid arthritis, ulcerative colitis and liver disease, all of which have an immunological background. Plants can influence ‘ama’ in two ways, those which increase digestion capacity and prevent formation of ‘ama’ (Deepak), and those which digest ‘ama’ (Pachak). Plants having anti-allergic properties can also be categorized as immunomodulators.

Siddha system of medicine

Siddha system of medicine is practiced in some parts of South India especially in Tamilnadu. This system is closely identified with Tamil civilization. The term ‘Siddha’ has come from the word ‘Siddhi’- which means achievement. Siddhars were the men who have obtained supremacy in the field of medicine, yoga or tapa (meditation). The materia medica of Siddha system of medicine depends on drugs of metal and mineral origin. Based on the history of Siddha, eighteen Siddhars were considered to have contributed to the development of Siddha medicine, yoga and philosophy.

According to the Siddha concepts matter and energy are the two dominant entities, which influence the nature of the Universe. They are called Siva and Sakthi. Matter cannot exist without energy and vice-versa. Thus both are inseparable. The universe is made up of five proto-elements.

The concept of five proto-elements and three doshas in this system of medicine is quite similar to Ayurvedic concept. However there are certain differences in the interpretation.

The concepts behind diagnostic measures also show great similarities differing in certain aspects only.

Diagnosis in Siddha system is carried out by the well known ‘asthasthana pareeksha’ method (examination of eight sites) that encompasses examination of nadi (pulse), kan (eyes), swara (voice), sparisam (touch), varna (colour), na (tongue), mala (faeces) and neer (urine). These examination procedures are provided in greater detail in classical Siddha literature.

Concept of health in Siddha system of medicine

According to the siddha system, diet and lifestyle play a major role in health and in curing diseases. It is assumed that when the normal equilibrium of the three body humors-vaadham, pittham and kabam is disturbed, disease occurs. The factors assumed to affect this equilibrium are environment, climatic conditions, diet, physical activities and stress. Under normal conditions the ratio between vaadham, pittham and kabam should be 4:2:1 respectively.

Immunity as per Siddha practices

Siddha drugs that are helpful in making our body strong and healthy are called Kayakalpa drugs. In this group 108 herbs are mentioned and are recommended for normal individuals to boost their immunity, to promote general health, and for preventing diseases. Herbal health foods and nutraceuticals are grouped under general kayakalpa and can be used as prophylactic agents. Besides being preferred as powerful medicaments to fight against degenerative diseases, these drugs are also useful in enhancing memory, complexion and power of sense organs. Thus Kayakalpa are drugs that increases longevity, improves immunity and enhances beauty.

Kayakalpa herbs

Oryza sativa or the paddy rice is a kalpa drug which is to be ground and mixed with cow’s ghee and taken for 40 days for rejuvenation. Presence of phenolic content possesses higher antioxidant activity and prevents bowel cancer. Nelumbo nucifera stamens powder is mixed with sugar and honey and taken (1 to 2 g) daily in the early morning for 40 days helps in curing deafness and male sterility. The isolated glycosides from this botanical source such as isorhamnetin glycosides and isorhamnetin rutinoside possess antioxidant activity. Phyllanthus amarus whole plant is prepared as paste and taken with milk/butter milk for 40 days for ophthalmic diseases, liver disorders, giddiness and vomiting. Aqueous extract showed a significant reduction in plasma lipid peroxidation. It is protective and effective against lymphocyte DNA damage thereby it reduces the oxidative stress.

Eclipta prostrata (Karisalai) whole plant is dried and prepared as chooranam (powder) and taken with tender coconut water for one month followed by one month with honey. This treatment gives lustre to the body, brightness to eyes and enhances intelligence. Karisalai karpam possess free radical scavenging activity. Further it has enzymatic and non-enzymatic antioxidants such as ascorbic acid and rutin. Dry fleshy leaves of 1-2 g of Aloe barbadenses (Katrazhai) are to be taken for 40 days for premature greying of hair, chronic piles and anti-ageing therapy. Aloe barbadensis contain polysaccharides and flavanoids which are potent antioxidants. Another study
revealed that polysaccharides could help in preventing cancer and oxidative damage significantly. In *Zingiber officinale* (Ginger) the rhizome’s outer skin is removed, sliced and soaked in honey of which 3-5 slices are to be taken in the early morning for 40 days to prevent senility, promote good eye sight and to strengthen the body. The rhizome has phytochemical constituents such as di-arylheptanoid a potent antioxidant and vanillloid that induces cytochrome-C mediated apoptotic pathway. *Ocimum sanctum* (Basil) leaf or root juice is boiled with oil and applied over head and used in oil bath for a period of 40 days helps in treating disorders of kapha, vatha and delirium. It significantly increases the level of superoxide dismutase, glutathione and total thiols which are proven antioxidants.

*Semecarpus anacardium* (Soapnut) nut is prepared as *legium* (powdered and mixed with ghee and honey) as per the method of *Siddha* literature. *Theyran karisal* taken internally 2-5 g twice daily for easy digestion, lustre, good voice and also used as an appetizer. This serves as an antioxidant because of the presence of flavanoids which scavenge free radicals. *Withania sominifera* (*Amukara*) root powder 500 mg is mixed with 2 ml of ghee and taken twice a day for 40 days. It provides lustre, reduces nervous debility, strengthens the body and enhances longevity of life. Its extract is effective in preventing DNA damage and in scavenging active radicals generated by mutagens. *Limonia acidissima* (Vila), all parts including leaf, unripe fruit, fruit, bark, root are consumed as curry or in a decoction form to prevent ageing and death.

The various parts of the plant extract contain antioxidants and free radical scavenging activity.

*Azaadrastra indica* (*Vembu*) tender leaves are ground and added with *Trachyspermum ammi* (*omam*) and salt. This powder is to be taken starting from rohini nakshathra day for 40 days to maintain good health. Leaves and flowers have strong antioxidant and antitumor activity.

*Citrus limon* (*Elumichai*) fruits are to be used as juice or pickles for 6 months to prevent senility, grey hair and ascites, Both juice and peel demonstrated antioxidant properties.

*Centella asiatica* (*Vallarai*) leaf can be used as curry daily. It is used in liver disorders, in enhancing memory, intelligence and strengthening the body. It is an adaptogenic agent used in the treatment of cancer.

*Basella alba* (*Kodi Pasalai*) leaf can be prepared as curry and taken along with diet for 40 days. This will strengthen the body. It has high content of total phenol, flavonoids and ascorbic acid content and hence possesses antioxidant activity.

*Terminalia chebula* (*Kadukai*) epicarp is made into powder and 500 to 1000 mg is taken with water, especially in the evening for 40 days to prevent premature greying of hair, senility, pitha diseases and liver disorders. It rejuvenates the body. It has greater triterpenoid content and exhibits good antioxidant activity.

**Methods used to detect the immunomodulatory compounds**

A number of assays, both *in vitro* and *in vivo* have been developed to test immunomodulatory activities of plant extracts. Basic research on natural substances with immunomodulating properties is performed by assays primarily carried out on the stimulation of non specific immunity of the innate responses, such as the efficiency of granulocytes, macrophages, complement and natural killer cells and their effect on phagocytes, lymphocyte proliferation and T-lymphocyte migration. More recent research on immunomodulating substances includes studies on cytokine production by macrophages such as IL-1, IL-6 and TNF-α. Simple tests used to assay immunomodulation include estimation of total WBCs and differential WBCs. These cells usually include the eosinophils, basophils, monocytes, neutrophils and lymphocytes. Increase in their number or activity would be a good indicator of enhanced immunity.

To evaluate neutrophils for their enhanced activity, neutrophil adhesion test is used. It is determined by getting an initial count of total WBCs and differential WBCs from a blood sample. The blood sample is then incubated in sterile nylon fiber column packed in a silicnazed Pasteur pipette. After a brief incubation period, the blood sample is again analyzed for total WBCs and differential WBCs. The product of total and differential WBCs gives the neutrophil index of the blood sample. The activity of neutrophils or macrophages can further be evaluated by utilizing the nitroblue tetrazolium reduction test. This test relies on the generation of bactericidal enzymes in neutrophils during intracellular killing. These enzymes are necessary for normal intracellular destruction of foreign antigens. During intracellular killing, the cellular oxygen consumption increases and glucose metabolism reduces the colourless NBT to blue formazan. Other assays that have been used to monitor *in vitro* activity of neutrophils and macrophages include Candida, SRBC and bacterial phagocytosis.

*In vivo*, phagocytic activity can be evaluated by intraperitoneal injection of SRBCs in laboratory rodents. Intraperitoneal cells are then washed with saline, fixed on slides and stained with Giemsa’s solution. The percentage of macrophages that ingest SRBC (phagocyte ratio) and the number of SRBC in the macrophage (phagocytic index) are calculated by counting 100 macrophages under a microscope. Alternatively carbon clearance test can be used. It involves injecting standard dose of colloidal carbon, liposomes, denatured albumin or Indian black ink into the tail vein of laboratory rodents. Blood is then drawn out at different time intervals and the concentration of residual particles determined spectrophotometrically. Assay of cytokines has also
been used and involves measuring the concentration of plasma soluble IL-2 receptor and the concentration of TNF, IL-6, IL-10 and IFN by ELISA technique. It gives an indication about the extent of activation of macrophages and lymphocytes. Flow cytometric determination of various cell types can be determined to ascertain the percentage of T-cells and B-cells.

Lymphocyte proliferation assay is a test that measures the influence of compounds on T-lymphocyte cell population. Lymphocyte transformation test reflects the degree of blastogenesis of T-lymphocytes after having contacted with mitogen. Stimulation rate can be measured by $^3$H thymidine incorporation in T-lymphocytes, using phytohemagglutinin or Concanavalin A as control mitogen. Using monoclonal antibodies and fluorescence activated cell sorting; it is even possible to monitor T-lymphocyte subpopulations. These measures allow prediction of whether a substance displays predominantly either stimulating or suppressing activity.

**Medicinal plants investigated for immunomodulatory potential**

Plants are considered as the key reservoirs of natural entities having tremendous therapeutic value. It is estimated that around 2, 50,000 flowering plant species are found on the earth, of which around 1, 25,000 occur in the tropical forests and around 2000 species of higher plants are used for medicinal purposes throughout the world. India harbours more than 45,000 plant species, among these thousands have been claimed to possess medicinal properties. Large numbers of tropical plants have not yet been exploited in detail for their pharmacological properties or chemical constituents. The compounds isolated from medicinal plants will serve as lead molecules for development of safe and better drugs against various diseases and in enhancing the efficacy of presently available synthetic drugs when used in combination.

One of the fast developing area of drug development involves the search for novel immunomodulatory agents having either immune-stimulatory or immune-suppressant activity that could be used for the treatment of various immune dysfunctions or in case of residual cancer. Number of plants used in Indian traditional systems of medicines has been shown to stimulate immune responses and several active substances have also been isolated. From these plants, a list of plants sourced from our traditional medicinal literature investigated for immunomodulatory activity are presented in the Table 2 and 3.

**Table 1:** Codes of conducts to be followed to enhance immunity

<table>
<thead>
<tr>
<th>Karma to be followed</th>
<th>Phalashruti-Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumsavana karma</td>
<td>Healthy foetus</td>
</tr>
<tr>
<td>Lehana karma</td>
<td>Enhances immunity and reduces infection episodes from childhood</td>
</tr>
<tr>
<td>Dinacharya</td>
<td>To perform the daily activities in a healthy way</td>
</tr>
<tr>
<td>Ritucharya</td>
<td>Taking care of health in different seasons</td>
</tr>
<tr>
<td>Ahara, Nidra and Brahmacharya</td>
<td>To gain complete physical and mental health</td>
</tr>
<tr>
<td>Sadwritta and AcharaRasayana</td>
<td>For promoting mental and spiritual strength</td>
</tr>
<tr>
<td>Panchakarma</td>
<td>To eliminate the vitiated Doshas from the body</td>
</tr>
<tr>
<td>Rasayana</td>
<td>Promotes and rejuvenate the physiology of body, produce resistance against disease both physically and mentally</td>
</tr>
</tbody>
</table>

**Table 2:** Plants identified as Rasayanas and their pharmacological properties

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Rasayanas</th>
<th>Properties</th>
<th>Parts used</th>
<th>Mechanism of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acorus calamus</td>
<td>Neurostimulant, Antiaging</td>
<td>Rhizome</td>
<td>Increase the production of IL-2,TNF-α</td>
<td>98-100</td>
</tr>
<tr>
<td>2.</td>
<td>Allium sativum</td>
<td>Antibacterial, Antiviral</td>
<td>Fruits</td>
<td>Stimulates humoral immune response, Inhibits growth of cancer cell lines, Enhances oxidative burst, Augments NK cells, stimulates T-cells and IL-2 production</td>
<td>101-104</td>
</tr>
<tr>
<td>3.</td>
<td>Aloe vera</td>
<td>Antiseptic</td>
<td>Leaves</td>
<td>Enhances IL-6,TNF-α &amp; NO release, Inhibits wound healing, Causes regression of tumor</td>
<td>105-107</td>
</tr>
<tr>
<td>4.</td>
<td>Argyreia speciosa</td>
<td>Anti-rheumatic, Tonic</td>
<td>Root &amp; Seeds</td>
<td>Enhance the production of circulating antibody titre, increase in DTH reaction</td>
<td>108</td>
</tr>
<tr>
<td>5.</td>
<td>Asparagus racemosus</td>
<td>Anti-stress, Anti-cancer</td>
<td>Root</td>
<td>Stimulates RE system and PMN cells Prevents leucopenia induced by cyclophosphamide</td>
<td>109, 110</td>
</tr>
<tr>
<td></td>
<td>Plant Name</td>
<td>Medical Use</td>
<td>Part Used</td>
<td>Description</td>
<td>References</td>
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<td>--------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>6</td>
<td>Azadirachta indica (Nimba, Neem)</td>
<td>Antiseptic, Anti-eczema</td>
<td>Leaves</td>
<td>Stimulates the production of interleukins, Attenuation of stress and xenobiotic induced suppression of humoral and cell mediated immunity Reduces erythema, desquamation and infiltration of psoriatic lesions</td>
<td>3, 111-113</td>
</tr>
<tr>
<td>7</td>
<td>Bacopa monnieri (Brahmi)</td>
<td>Antiasthmatic, Antiaging</td>
<td>Saponin rich fraction</td>
<td>Stimulatory effect on release of immune mediators from murine peritoneal cells and proliferative effects on immune cells in vitro</td>
<td>114</td>
</tr>
<tr>
<td>8</td>
<td>Boerhaavia diffusa (Sant)</td>
<td>Anti-inflammatory, Anti-stress, Adaptogenic, Antiaging</td>
<td>Root</td>
<td>Inhibits production of NO, IL-2, TNFα</td>
<td>115</td>
</tr>
<tr>
<td>9</td>
<td>Cissampelos pareira (Akanadi)</td>
<td>Antiviral, Effective in urinary tract infections</td>
<td>Alkaloidal fraction of roots</td>
<td>Immunosuppressive activity</td>
<td>116</td>
</tr>
<tr>
<td>10</td>
<td>Commiphora mukul (Guggul)</td>
<td>Antiseptic</td>
<td>Gum</td>
<td>Immunostimulant, Increases WBC count</td>
<td>117</td>
</tr>
<tr>
<td>11</td>
<td>Convolvulus pluricaulis (Shankhapushp)</td>
<td>Tonic</td>
<td>All parts</td>
<td>Neurotonic, antioxidant, anti microbial anthelmintic</td>
<td>118</td>
</tr>
<tr>
<td>12</td>
<td>Curculigo orchoides (Krishna Musali)</td>
<td>Antiasthmatic, Tonic</td>
<td>Ethyl acetate soluble fraction of menthol extract of roots</td>
<td>Increases HA titre, DTH response and plaque forming cells.</td>
<td>119</td>
</tr>
<tr>
<td>13</td>
<td>Curcuma longa (Haldi)</td>
<td>Antiasthmatic, Anti-inflammatory, Tonic</td>
<td>Rhizome</td>
<td>Increases mitogenic response of lymphocytes Helps in rheumatoid arthritis and cancer</td>
<td>120, 121</td>
</tr>
<tr>
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27. **Semecarpus anacardium** (Bhilawa)  
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   - Extract of Nuts  
   - Cures arthritis, inhibits production of pro-inflammatory cytokines  
   - Reference: 135, 136

28. **Sida spinosa** (Gulsakari)  
   - Tonic  
   - Leaves  
   - Anti asthmatic, anti gonorrhoeal  
   - Reference: 137

29. **Solanum nigrum** (Makoi)  
   - Anti-Psoriasis  
   - Polyaccharide from leaves  
   - Significant increase in INF-γ, potent antitumor activity  
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30. **Spheronthus indicus** (Mundi)  
   - Tonic  
   - Petroleum ether extract of flower heads  
   - Effective in increasing phagocytic activity, hemaggulitation antibody titer and delayed type hypersensitivity  
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31. **Terminalia belerica** (Bahera)  
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   - Mitogenic activity of epilallocatechin on B-cells  
   - Increased phagocytosis  
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32. **Terminalia chebula** (Haritaki)  
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33. **Tinospora cordifolia** (Guduchi)  
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   - Inhibits myelosuppression induced by cyclophosphamide  
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34. **Withania somnifera** (Ashwagandha)  
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<td>18</td>
<td><em>Chrysanthemum indicum</em></td>
<td>Aerial parts</td>
<td>Increases DTH reaction, antibody generation</td>
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<td><em>Cinnamomum tamala</em></td>
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<td>Immunostimulant at low doses, immunosuppressive at doses higher than 800mg/kg</td>
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<td>Inhibits proliferation of PHA activated mononuclear cells</td>
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<td><em>Cleome gynandra</em></td>
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<td>Methanol extract of whole plant</td>
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<td><em>Evolvulus alsinoides</em></td>
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<td>27</td>
<td><em>Ficus benghalensis</em></td>
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<td>Enhances phagocytic activity of neutrophils, increase the antibody titer value</td>
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<td><em>Ficus carica</em></td>
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Leucocytes

Leucocytes (white blood cells) are the main cells of the immune system that provide either innate or specific adaptive immunity. They are motile with specialized functions. The number of leucocytes in normal blood ranges between 4,500 and 11,000 per cubic millimeter.

Granulocytes, monocytes, and natural killer cells. There are three types of granulocytes: neutrophils (50 to 70%), eosinophils (1 – 4%), and basophils (0.5%).

Plant extracts and plant derived constituents have been known to affect leucocytes by increasing or reducing their numbers resulting in leucocytosis or leucopenia. Aqueous extract of *Fodogia agrestis* stem was found to significantly increase WBC. Aqueous root extract of *Harungana madagascariensis* was found to significantly increase total leukocyte in acitamiphen injured rats. Nwineka demonstrated that aqueous extract of stem bark of *Mangifera indica* was able to increase leukocyte levels in normal albino rats. Ethanolic extract of stem bark *Mammea africana* has been found to significantly decrease white blood cell counts in extract treated rats. They investigated several plants belonging to the genus *Euphorbia* and found that some were able to cause leucopenia while others caused leucocytosis. Administration of ethanolic leaf extract of *Croton zambesicus* to rats has been demonstrated to produce a reduction in packed cell volume and WBC.

Neutrophils

Neutrophils are one of the major types of cells that are recruited to ingest, kill and digest pathogens. These are important components in the surveillance and protection systems of host defenses. They play key role as effectors or killer cells for many types of antigenic challenges especially for infections. The primary function of the neutrophils in host resistance is the migration towards the challenge which is called chemotaxis and the intracellular killing of microorganisms by the formation of oxygen radicals. Phagocytosis by neutrophils constitutes an essential arm of the host defense against foreign antigens. Neutrophils have receptors for complement component (C3b) which are involved in the uptake of foreign antigens. Neutrophils engulf and digest bacteria and other microorganism and microscopic particles.

Plant extracts and plant derived compounds have been demonstrated to have diverse effect on the activity of neutrophils. In some cases they are known to increase the number of neutrophils resulting in neutrophilia while in other cases increases the chemotaxis, neutrophil adherence and phagocytic capacity of neutrophils. In some other instances, plant extracts seem not to have an effect on neutrophils. Aqueous and ethanol extracts of *Aeginetia indica* Roxb had no effect on the number and activity of neutrophils following oral administration in female B6C3F1 mice. Myeloperoxidase (MPO) is one of the antimicrobial enzymes produced by neutrophils and changes in the activity of MPO indicate the functional alteration of neutrophils. Exposure to the extracts of

Mechanism of action of plant extracts in immunomodulation

Effect of plant extracts on the specialized cells of the immune system

The immune system is a network of specialized tissues, organs, cells, and chemical mediators. Plant extracts and plant derived constituents have been known to modulate the components of immune system which are presented and discussed in sequel.

### Leucocytes

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### Neutrophils

Neutrophils are one of the major types of cells that are recruited to ingest, kill and digest pathogens. These are important components in the surveillance and protection systems of host defenses. They play key role as effectors or killer cells for many types of antigenic challenges especially for infections. The primary function of the neutrophils in host resistance is the migration towards the challenge which is called chemotaxis and the intracellular killing of microorganisms by the formation of oxygen radicals. Phagocytosis by neutrophils constitutes an essential arm of the host defense against foreign antigens. Neutrophils have receptors for complement component (C3b) which are involved in the uptake of foreign antigens. Neutrophils engulf and digest bacteria and other microorganism and microscopic particles.

Plant extracts and plant derived compounds have been demonstrated to have diverse effect on the activity of neutrophils. In some cases they are known to increase the number of neutrophils resulting in neutrophilia while in other cases increases the chemotaxis, neutrophil adherence and phagocytic capacity of neutrophils. In some other instances, plant extracts seem not to have an effect on neutrophils. Aqueous and ethanol extracts of *Aeginetia indica* Roxb had no effect on the number and activity of neutrophils following oral administration in female B6C3F1 mice. Myeloperoxidase (MPO) is one of the antimicrobial enzymes produced by neutrophils and changes in the activity of MPO indicate the functional alteration of neutrophils. Exposure to the extracts of

### Effect of plant extracts on the specialized cells of the immune system

The immune system is a network of specialized tissues, organs, cells, and chemical mediators. Plant extracts and plant derived constituents have been known to modulate the components of immune system which are presented and discussed in sequel.

#### Leucocytes

Leucocytes (white blood cells) are the main cells of the immune system that provide either innate or specific adaptive immunity. They are motile with specialized functions. The number of leucocytes in normal blood ranges between 4,500 and 11,000 per cubic millimeter. They are further classified into lymphocytes, granulocytes, monocytes, and natural killer cells. There are three types of granulocytes: neutrophils (50 to 70%), eosinophils (1 – 4%), and basophils (0.5%).

Plant extracts and plant derived constituents have been known to affect leucocytes by increasing or reducing their numbers resulting in leucocytosis or leucopenia. Aqueous extract of *Fodogia agrestis* stem was found to significantly increase WBC. Aqueous root extract of *Harungana madagascariensis* was found to significantly increase total leukocyte in acetaminophen injured rats. Nwineka demonstrated that aqueous extract of stem bark of *Mangifera indica* was able to increase leukocyte levels in normal albino rats. Ethanolic extract of stem bark *Mammea africana* has been found to significantly decrease white blood cell counts in extract treated rats. They investigated several plants belonging to the genus *Euphorbia* and found that some were able to cause leucopenia while others caused leucocytosis. Administration of ethanolic leaf extract of *Croton zambesicus* to rats has been demonstrated to produce a reduction in packed cell volume and WBC.

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<table>
<thead>
<tr>
<th>Plant Extracts</th>
<th>Effect on Leucocytes</th>
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<tbody>
<tr>
<td>Emblica officinalis</td>
<td>Increased the macrophage activity and their number, enhancement of non-specific immune response and reduction of chances of infection. Protected against Cyclosporine A induced immunosuppression</td>
</tr>
<tr>
<td>Lantern sycamore (Piper cubeb), Myristica officinalis</td>
<td>Oral administration of Triphala stimulated the neutrophil functions in the immunized rats. Stress induced suppression in the neutrophil functions were also significantly prevented by Triphala.</td>
</tr>
<tr>
<td>RA-01 Cardiospermum halicacabum, Tinospora cordifolia, Alpinia officinarum, Withania somnifera, Glycyrrhiza glabra, Linga chenduram</td>
<td>Stimulatory effect on both humoral and cell mediated immunity</td>
</tr>
<tr>
<td>Saya churnam, Fruits of Terminalia chebula, Piper longum, Piper cubeb, Myristica officinalis and Rhizome of Alpinia galanga</td>
<td>Triggers both specific and non-specific responses to a greater extent. The study showed significantly increased results in Heme agglutination (HA) titer, DTH response, Ig E test, biochemical and hematological analysis.</td>
</tr>
</tbody>
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Aeginetia indica for 28 days did not result in an increased MPO levels.

Eosinophils and Basrophils

These are also motile, phagocytic, and migrate into the tissues. They are particularly important in the defence against parasites and participate in hypersensitivity and inflammatory reactions. Their cytotoxicity is mediated by cytoplasmic granules. When aggravated, they release histamine and other mediators that are involved in allergic reactions. Basophils display high affinity surface membrane receptors for IgE antibodies. It is not known how plant extracts affect the number and activity of these types of granulocytes and there is scarcity of information and in most cases; differential leukocyte count in animals reveals that the numbers of these granulocytes remain unchanged following administration of plant extracts.

Monocytes / Macrophages

Monocytes are produced in the bone marrow, constitute up to 10% of the blood leucocytes. However, most of them leave the blood after few hours and migrate into almost all tissues, where they develop into macrophages. Both monocytes and macrophages are highly adherent, motile and phagocytic. They marshal and regulate other cells of the immune system. They also serve as antigen processing-presenting cells and act as cytotoxic cells when armed with specific IgG antibodies. Macrophages play an important role in host defense by phagocytizing foreign invaders, undergoing oxidative burst, antigen presentation, and secreting cytokines such as tumor necrosis factor – α and interleukin-1. Through TNF – α and IL -1 macrophages control the proliferation, differentiation and effector functions of lymphocytes.

It seems that monocytes/macrophages are one of the immune cells that are highly affected by plant extracts. Benny and Vanitha reported that poly saccharides isolated from Astragalus membraneus increased macrophage count as well as enhanced their activity. A polyphenol extract from Geranium sanguineum induced a rise in the number and migration of alveolar macrophages in both influenza virus infected mice and healthy mice. Methanolic extract of Poteria cambodia was demonstrated to enhance lysosomal enzyme activity in macrophage indicating stimulation effect. The inhibitory activity on nitric oxide production by the extracts of Chinese medicinal plants in activated macrophages was demonstrated by Ryu. Peritoneal macrophages from male BALB/c mice orally treated with 500-1500 mg/kg of Anoectochilus formosanus were shown to exhibit high phagocytic activity.

Natural killer (NK) cells

These cells resemble macrophages and are also called large granular lymphocytes. They have little phagocytic function and no specialized lymphoid functions. They kill many virus infected cells and cancer cells, with the help of T lymphocytes. In in-vitro assays utilizing PBMCs from three groups of subjects (Healthy, with AIDS, or with chronic fatigue syndrome), Echinacea purpurea whole plant extract enhanced natural killer cell function in all groups. Polysaccharides from Ganoderma lucidum have been reported to increase natural killer cell count.

Lymphocytes

Lymphocytes constitutes about 25-50% of the blood leucocytes. They are non-motile and enter the circulation through lymphatic channels. They are found in large numbers in the secondary lymphoid organs. T – Lymphocytes which are produced in the bone marrow pass through thymus and mature there. The other class is the B-lymphocytes; they do not pass through thymus but mature in bone marrow.

T-lymphocytes

There are two major classes of T-lymphocytes: T - helper (Th) and T - cytotoxic lymphocytes (Tc). T helper (Th) lymphocytes regulate the antibody-forming function of B – lymphocytes and participate in rejection of transplants. They posses CD4 surface molecules (also called CD4 cells). T helper cells are functionally further subdivided into at least two types, Th-1 and Th-2. The other T lymphocytes are those involved in the defense against viral infections, and include T – cytotoxic lymphocytes (Tc) and T – suppressor (Ts). Cytotoxic T (Tc) cells are capable of destroying a target cell, that is infected with virus or that expresses some form of foreign antigen. These cells are the major immune effectors of the cellular immune response. They express CD8 molecules. T – Suppressor (Ts) cells act to diminish helper T – cell activity; they directly kill virus infected or cancer cells when the battle is over. They express CD8 molecules. In contrast to helper T-cells, Ts cells down-modulate immune responses. Thus, the combination of helper and suppressor cells determines the level of the immune response to any specific antigen.

Methanolic seed extracts of Citrullus colocynthis was shown to be able to increase lymphocyte numbers in rabbits at a dose of 100 mg/kg. Ocimum basilicum aqueous and methanolic extracts were found to induce high lymphoproliferative response. Methanolic extract of Euphorbia cheirenia was demonstrated to have a significant stimulatory effect on T lymphocytes by increasing IL-2 secretion in cultures treated with the extract. This was an indication of the ability of the extract to enhance of T cells and release IL-2. Methanolic extracts of Justica gendarussa, Plumbago indica, Aloe vera and Aegle marmelos inhibited lymphocyte proliferation.

B Lymphocytes

These have a relatively short life span compared to T – cells. As B- cells mature, they turn into antibody-producing plasma cells found in lymph nodes and in the spleen. Once the B-cells have created a specific antibody
to attack a specific pathogen, their primitive intelligence remembers this information and will know it later should it encounter it. Two major functions of B cells are to secrete immunoglobulins and to bind, process and present antigen to T cells. Each B cell produces immunoglobulins with a single specific antigen binding site. By virtue of having immunoglobulin on their surface, B cells bind specific antigens which then deliver a signal to these cells to proliferate and clonally expand into memory cells or to differentiate into Ig-secretion cells. Since only one in $10^{5}$–$10^{8}$ normal B cells have specificity for a particular antigen, the number of potential antigen-binding B cells are too few in number to provide an appropriate model to study the mechanisms of cell activation.

Studies by Amirghofran demonstrated stimulatory effects of *Euphorbia cheiradenia* methanolic extracts on the humoral immune response. Immunized mice showed a significant increase in anti-SRBC hemagglutinin titre. A Japanese plant, *Cistanche salsa* extract was shown to enhance antibody production in human lymph node memory cells or to differentiate into lg-secreting cells. These cells to proliferate and clonally expand into memory cells. Activation of the integrins stops the flow of leukocytes. Many of the activators are produced by the endothelial cells themselves, and by monocytes or bacteria. Basaran reported a number of plants extract that enhanced chemotactic capacity of neutrophils and macrophages. These plant extracts include *Viscum album*, *Arctium minus*, *Momorica charantia* and *Urtica dioica*.

**Opsonization**

Microorganisms are phagocytosed after they have been opsonized. Opsonins are substances that facilitate the phagocytosis process. There are two main classes of opsonin IgG and IgA antibodies and the third component of complement. Opsonin binds to the surface of the phagocytosed particle with one end and, via specific receptors to the other end. There are two main classes of receptors, Fc receptors specific for the Fc region of IgG, IgA and IgE antibodies; and complement receptors specific for C3b fragments. Upon binding of immune complexes containing the antibodies of appropriate isotypes these receptors induce phagocytosis and other activating reactions in phagocytosis. Investigations on the role of plant extracts and plant derived compounds on the process of opsonization are lacking and there is need to initiate more research into this area. It is possible to hypothesize that some plant extracts could by themselves act directly as opsonin, or increase the levels of IgG secretion, activating the third complement factor, thereby enhancing opsonization.

Peritoneal macrophages from male BALB/c mice orally treated with 500-1500 mg/kg of *Anoectochilus formosanus* were shown to exhibit high phagocytic activity. Extracts of *Echinacea* and *Terminalia bellerica* were demonstrated to have a stimulatory effect on macrophage phagocytic activity. Three Peruvian plants, *Pistia stratiotes*, *Piper peitatum* and *Uncaria tomentosa* were demonstrated to enhance phagocytosis. Shokri showed that the Iranian herb *Zataria multiflora* affects function of the innate immunity in mice by increasing phagocytosis and TNF-α secretion. Pycnogenol was demonstrated to enhance phagocytosis of fluorescein-conjugated Escherichia coli particles by 1774 cells. The mechanisms involved in killing after phagocytosis and ingestion are discussed in sequel.

**Respiratory Burst (Killing)**

The most important mechanism contributing to the killing of the phagocytosed microorganism is the respiratory burst in which the oxygen metabolites are produced. *Zataria multiflora* extract was found to increase respiratory burst in BALB/c mice following intraperitoneal injection. Pycnogenol, a procyanidins extracted from the bark of French maritime pine (*Pinus pinaster*) has been reported to inhibit macrophage...
oxidative burst\textsuperscript{56}. PMNs are able to kill microorganisms by two distinct mechanisms, one of which is oxygen dependent, while the other is oxygen-independent.

**Oxygen-dependent killing mechanism**

The oxygen-dependent antimicrobial mechanisms are initiated when PMNs undergo a ‘respiratory burst’. It is believed that G protein serves as intermediate between cell surface receptors and effector enzymes responsible for generating second messengers\textsuperscript{58}. The interaction with G protein leads to changes in configuration of the G-protein and activation of adenylcyclase and phospholipase. This process, results in the activation of second messengers such as cAMP, inositol triphosphate, Ca\textsuperscript{2+} and, arachidonic acid. High cAMP and Ca\textsuperscript{2+} levels lead to activation of protein kinase A (PKA) and protein kinase C (PKC) respectively which play a role in the activation of NADPH oxidase and other bacterialid enzymes in the cell membrane\textsuperscript{32,63}. This results in the reduction of O\textsubscript{2} to superoxide. Reduction of O\textsubscript{2} is the first step in a series of reactions that produce toxic oxygen species during the respiratory burst. Superoxide can react with hydrogen peroxide to produce hydroxyl radical (OH\textsuperscript{•}), which causes the peroxidation of membrane lipids, breakage of DNA strands, and inactivation of enzymes in cells\textsuperscript{67}. Macrophages are known to produce NO which in high concentration, acts as inflammatory mediator\textsuperscript{67,69}.

Polyphenol extract from Geranium sanguineum was shown to modulate the excessive production of oxygen radicals, hydrogen peroxide and NO in mice infected with influenza virus thereby alleviating disease symptoms and reducing mortality\textsuperscript{69}. Several crude extracts from Brazilian plants down modulated nitric oxide production in murine macrophages validating their use as anti-inflammatory agents\textsuperscript{67}.

**Oxygen-independent killing mechanism**

PMN granules contain additional antimicrobial agents that are released into phagolysosomes and do not require the production of oxidants for activity. These agents include proteases, other hydrolytic enzymes, such as phospholipases, glycosidases and lysozymes, and other proteins and peptides that disrupt microbial functions or structural components. These agents must bind to certain microbial cell surface for their antimicrobial activity. Three well-characterized granule components, bactericidal permeability increasing protein (BPI) and defensins have shown microbialid activity \textit{in vitro}\textsuperscript{68}. BPI is 58 kDa protein of the primary (azurophil) granules and contributes to killing mainly gram-negative bacteria\textsuperscript{69}. Plant extracts and plant derived compounds have also been demonstrated to have an effect on the oxygen independent killing mechanisms by enhancing the release of certain enzymes such as lysosomal and myeloperoxidase. Certain anti-inflammatory and pro-inflammatory cytokines are also released in the presence of plant extracts and participate in the oxygen independent killing mechanism.

**Role of plant extracts on the production of Cytokines**

Cytokines are a group of low molecular weight regulatory proteins secreted by leucocytes and a variety of other cells, in response to a number of inducing stimuli. Cytokines in general act as immune “messanger molecules” that modulate, educate, stimulate, and regulate various aspects of the immune response by acting on cells. Cytokines bind to specific receptors on the surface of target cells. Cytokines that are secreted from lymphocytes are termed lymphokines, whereas those secreted by monocytes or macrophages are termed monokines. Many of the lymphokines are also known as interleukins (ILs), since they are not only secreted by leukocytes but also have an effect on leukocytes. Cytokines include interferons, colony stimulating factors and tumor necrosis factor\textsuperscript{55,73,90}. There is good evidence to suggest that the systemic immune response component of this defense mechanism to communicate with each other and which are also capable of directing the nature of the response. Some of these molecules such as TNF-\textalpha is predominantly synthesized and secreted by activated macrophages. On the other hand, IL-2, IL-4, IL-5, IL-10 and IFN-\textgamma are produced by activated T-lymphocytes. These 2\textsuperscript{nd} group of cytokines have now been classified into 2 subsets (Th1 – type and Th2-type) based on the types of cells that prouce them. Th1 cells secrete mostly IL-2 an IFN-\textgamma, and Th2 cells secrete IL-3, IL-4, IL-5, IL-6 and IL-10\textsuperscript{55}. Th1 cells are responsible for cell mediated immunity, phagocyte-dependent host response, cytotoxicity, and macrophage activation. IL-2 has effect on several other immune cells, including natural killer cells, B-cell monocytes/macrophages and neutrophils\textsuperscript{81}.

Bouic\textsuperscript{92} demonstrated that beta sitosterol from Hypoixis spp caused a release of more growth factors specifically IL-2 and IFN-\textgamma in their supernatants. Maruyama\textsuperscript{80} showed that the expression of IL-6R was increased in B cell surface following treatment with Cistanche salsa extract. However, the cells treated with the extracts for a 7 days period did not express up regulated IL-6R. These results suggested that the Cistanche salsa extract induced activation is not as simple as mitogen induced activation which is mainly dependent on cell growth. \textit{Eurphobia cheiradenia} significantly increased IL-2 secretion in lymphocyte\textsuperscript{77}. Saponins and acidic polysaccharides isolated from the roots of Panax ginseng were reported to stimulate the production of IL-1, IL-6, IL-12, and TNF-\textalpha and TNF-\textbeta\textsuperscript{46}. \textit{Ocimum basilicum} crude methanolic extract was shown to inhibit the key pro-inflammatory cytokines and mediators therefore accounting for its anti-inflammatory effect\textsuperscript{55}. \textit{Anoctochilus formosanus} fraction was found to significantly increase the IFN – \textgamma (Th-1-type) production but not IL-4 (Th 2-type)\textsuperscript{72}. It was suggested that \textit{Anoctochilus formosanus} fraction activated macrophages by up regulating the synthesis and production of this cytokine. Pyconogenol isolated from the bark of French maritime pine was demonstrated to enhance secretion of TNF-\textalpha and IL-1 by macrophages\textsuperscript{95}.
Investigations by Lyu and Park\textsuperscript{11} showed that some flavonoids repressed NO production and TNF-α secretion while others increased or decreased IL-2 secretion indicating the capacity of these flavonoids to modulate the immune response in addition to their anti-inflammatory potential.

Benny and Vanitha\textsuperscript{18} have reported that polysaccharides, saponins, flavonoids, peptides and glycoproteins are known to induce production of different types of cytokines.

**Herbal formulations investigated for immunomodulatory potentials**

Drug formulation in *Ayurveda* is based on two principles: use as a single drug and use of more than one drug, in which the latter is known as PHF (Table 4). This key traditional therapeutic herbal strategy exploits the combination of several medicinal herbs to achieve extra therapeutic efficacy, usually known as polypharmacy or polyherbalism.

Historically, the *Ayurvedic* literature “*Sarangdhar Samhita*” which dates back to 1300 A.D. has highlighted the concept of polyherbalism in this ancient medicinal system. It is known that *Ayurvedic* herbs are prepared in a number of dosage forms, and mostly all of them are PHF.\textsuperscript{7,9} Even though the active phytochemical constituents of individual plants have been well established, they are usually present in minute amount besides, they are insufficient to achieve the desirable therapeutic effects. Phenomenon of positive herb-herb interaction known as synergism produces a greater effect as compared to single herb. Certain pharmacological actions of active constituents of herbs are significant only when potentiated by other plants, and not evident when used alone.\textsuperscript{97}

**CONCLUSION**

There are a few *Ayurvedic* herbal combinations for example combination of ginger with black pepper and long pepper enhances their heating and mucous-reducing effects; bitter and cold herbs are combined with warmer herbs (combination of neem and ginger) to positively offset any extreme effects. Cumin, black pepper and asafoetida are used together traditionally to reduce bloating due to weak digestion; whereas guduchi and turmeric combination enhances one's immunity. Several ayurvedic poly herbal formulations were evaluated in the experimental animals for their immunomodulatory potential.

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