

# Role of phytochemicals as immunomodulatory agents: A review

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## Abstract

A strong, well-functioning immune system is the cornerstone of good health. Immunity is the balanced state of having adequate biological defenses to fight infection, disease, or other unwanted biological invasion while having tolerance to avoid allergy and autoimmune diseases. Immune responses are the result of an effective interaction between innate (natural and non-specific) and acquired (adaptive and specific) components of the immune system. Over the last three decades, there has been remarkable interest in the immune system as a potential target of toxicity following exposure to drugs, chemicals or environmental pollutants. Immunodeficiencies occur when one or more of the components of the immune system are inactive. Many factors play a significant role in altering the immunocompetence such as age, sex, genetic variability, stress, alcohol/drug abuse, malnutrition, environmental pollution, lifestyle. Immunomodulation is a very broad term which refers to any changes in the immune response and may involve induction, expression, amplification or inhibition of any part or phase in the immune response. In clinical perspective immunomodulators can be classified into the following three categories: Immunoadjuvants, immunostimulants, and immunosuppressants. A diverse array of synthetic, natural and recombinant compounds is available with both merits and demerits. Phytochemicals are naturally occurring compound with bioactive potentials, which have potential immunostimulating activity. The present review focused on the immune-modulating activity of various phytochemicals such as alkaloids, polysaccharides, lectins, glycosides, phenolic compounds, flavonoids, anthocyanins, tannins, saponins, terpenoids, sterols and also explains the role of antioxidants as immune-modulators.

**Key words:** Immune system, immune-stimulation, immunodeficiency, immunomodulators, phytochemicals

## INTRODUCTION

Every human is desirous of longevity, youthfulness, and health. Health is the level of functional or metabolic efficiency of a living organism. In humans, it is the ability of individuals or communities to adapt and self-manage when facing physical, mental or social challenges.<sup>[1]</sup> The World Health Organization defined health in its broader sense in its 1948 constitution as a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity. Other definitions have been proposed, among which a recent definition states that health is that balanced condition of the living organism in which the integral, harmonious performance of the vital functions tends to the preservation of the organism and the normal development of the individual. A strong, well-functioning immune system is the cornerstone of good health. Immunity is the balanced state of having

adequate biological defenses to fight infection, disease, or other unwanted biological invasion, while having tolerance to avoid allergy and autoimmune diseases.

## IMMUNE SYSTEM

Immune system is a remarkably sophisticated defense system to protect the host against invading microorganisms and against malignant cells. It is a very complex and regulated organ system that involves the cooperation and interaction of a number of different cell types, cell products, tissues, and

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organs. Inherent to its function, the immune system is spread over primary and secondary lymphoid organs, particularly present at sites that are exposed to the outside, such as the mucosa of lungs, intestine, and skin. All immune cells are derived from pluripotent hematopoietic stem cells in the bone marrow. During the first step of the differentiation process, myeloid and lymphoid stem cells emerge. Subsequent differentiation into lymphocytes of the T and B lineages occurs within the micro-environment of thymus and bone marrow, respectively.<sup>[2]</sup> Immune responses can be regulated by nervous and endocrine systems.<sup>[3-5]</sup>

## INNATE IMMUNE SYSTEM

Immune responses are the result of an effective interaction between innate (natural and non-specific) and acquired (adaptive and specific) components of the immune system [Figure 1]. The innate immune responses are the first line of defense against invading pathogens which rely on the body's ability to recognize conserved features of pathogens that are not present in the uninfected host. These pathogen-specific molecules are recognized by toll-like receptor proteins, which are present in plants and in invertebrate and vertebrate animals. In vertebrates, microbial surface molecules also activate complement, a group of blood proteins that act together to disrupt the membrane of the microorganism, to target them for phagocytosis by macrophages and neutrophils, and to produce an inflammatory response. The phagocytic cells use a combination of degrading enzymes, antimicrobial peptides, and reactive oxygen species (ROS) to kill the invaders. In addition, they release signaling molecules that trigger an inflammatory response and begin to marshal the forces of the adaptive immune system. Cells infected with viruses produce interferons (IFN), which induce a series of cellular responses to inhibit viral replication and activate the killing activities of natural killer (NK) cells and cytotoxic T-lymphocytes.

## ADAPTIVE IMMUNE SYSTEM

The adaptive immune system exhibits a stronger immune response as well as immunological memory, where each pathogen is "remembered" by a signature antigen.<sup>[6]</sup> The cells of the adaptive immune system are special types of leukocytes, called B- and T-lymphocytes. B-cells are involved in the humoral immune response, whereas T-cells are involved in the cell-mediated immune response. Both B-cells and T-cells carry receptor molecules that recognize specific targets. T-cells recognize a "non-self" target, such as a pathogen, only after antigens (small fragments of the pathogen) have been processed and presented in combination with a "self" receptor called a major histocompatibility complex (MHC) molecule. Killer T-cells recognize antigens coupled to Class I MHC molecules while helper T-cells recognize antigens coupled to Class II MHC molecules. A third, minor subtype

is the gamma-delta T-cells that recognize intact antigens that are not bound to MHC receptors.<sup>[7]</sup>

In contrast, the B-cell antigen-specific receptor is an antibody molecule on the B-cell surface and recognizes whole pathogens without any need for antigen processing. Each lineage of B-cell expresses a different antibody and so the complete set of B-cell antigen receptors represent all the antibodies that the body can manufacture. When B-cells and T-cells are activated and begin to replicate, some of their offspring become long-lived memory cells. Throughout the lifetime of an animal, these memory cells remember each specific pathogen encountered and can mount a strong response in future challenges [Figure 2].

## DISORDERS RELATED WITH IMMUNITY

Over the last three decades, there has been remarkable interest in the immune system as a potential target of toxicity following exposure to drugs, chemicals or environmental pollutants, collectively referred to as xenobiotics [Figure 3]. Since cells of the immune system undergo continual proliferation and differentiation for self-renewal to maintain immune-competence is also often affected by xenobiotics resulting in a cellular imbalance. The interaction of xenobiotics with the immune system may result in immune toxic alterations. Immuno-suppression leads to alterations

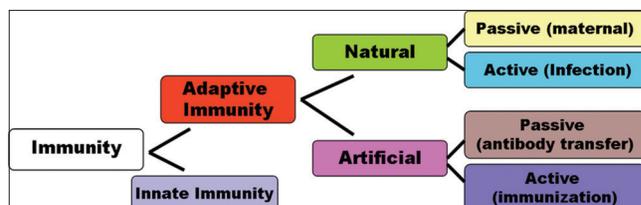


Figure 1: Types of immunity

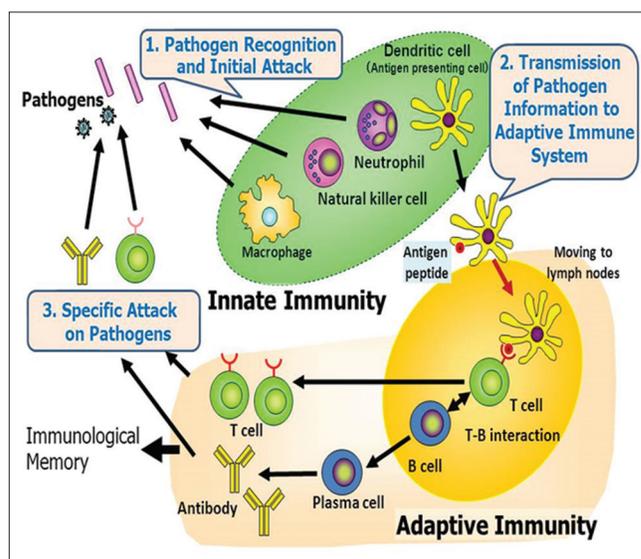


Figure 2: Overview of immune response

in host defense mechanisms against pathogens or neoplasia or dysregulations of the immune response causing allergy, hypersensitivity, and autoimmune reactions.<sup>[8]</sup>

## IMMUNODEFICIENCY

Immunodeficiency occurs when one or more of the components of the immune system are inactive. Immunodeficiency can be inherited or “acquired.” Chronic granulomatous disease, where phagocytes have a reduced ability to destroy pathogens, is an example of an inherited, or congenital, immunodeficiency. Infectious diseases, acquired immune deficiency syndrome (AIDS), and some types of cancer result from acquired immunodeficiency.<sup>[9,10]</sup>

## AUTOIMMUNITY

Overactive immune responses comprise the other end of immune dysfunction, in particular, the autoimmune disorders. Here, the immune system fails to properly distinguish between self and non-self, and attacks part of the body. Under normal circumstances, many T-cells and antibodies react with “self” peptides.<sup>[11]</sup> One of the functions of specialized cells (located in the thymus and bone marrow) is to present young lymphocytes with self-antigens produced throughout the body and to eliminate those cells that recognize self-antigens, preventing autoimmunity.

## HYPERSENSITIVITY

Hypersensitivity is an immune response that damages the body’s own tissues. They are divided into four classes (Type I-IV) based on the mechanisms involved and the time course of the hypersensitive reaction [Figure 4].

## FACTORS AFFECTING IMMUNE FUNCTION

Many factors play significant role in altering the immune-competence.

### Age

The Aging process weakens the immune system and predisposes geriatric individuals to a higher rate of immune insults, often more severe than in young age group. Due to immune-senescence, both innate and adaptive responses are compromised.

### Sex

Sex-dependent factors influence the susceptibility and progression of disease, compared to females; males experience

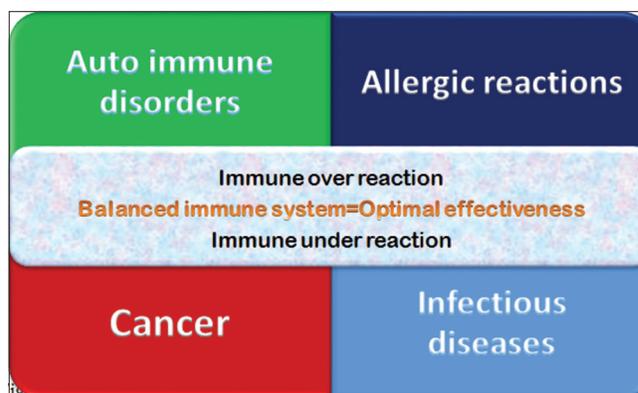


Figure 3: Disorders related with immunity

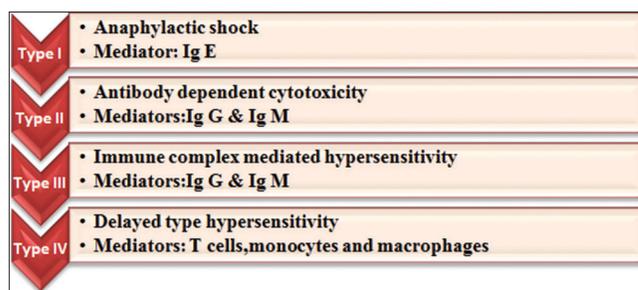


Figure 4: Types of hypersensitivity

higher severity and prevalence of many infectious diseases.<sup>[12]</sup> Although females mount greater immune responses and faster infection clearance, they more frequently develop immune-mediated pathologies.<sup>[13]</sup> The risk of death from all malignant cancers is 1.6 times higher for men.<sup>[14]</sup>

### Genetic Variability

Genetic variability among individuals play an important role in producing variation in interindividual immune response.

### Stress

Chronic psychological factors produce low to moderate degrees of immune suppression and increased incidence of infectious disease.<sup>[15]</sup> Immune testing in chronically stressed individuals has also provided insights into the relationship between mild to moderate immune suppression and disease.<sup>[16,17]</sup> In chronic stress population showing an increased rate of infections total circulating T-cell numbers can be reduced to as much as 20% below mean control values CD4: CD8 ratios can be reduced as much as 40% and NK cell activity by 10-25% below mean control value.<sup>[18,19]</sup>

### Drug/Alcohol Abuse

Extensive alcohol/drug abuse can directly suppress a wide range of immune responses by impairing T-and B-lymphocytes, NK cells, monocytes and macrophages

decreasing inflammatory response altering cytokine production and causing free radical damage.<sup>[20,21]</sup>

### Malnutrition

Nutrition plays a crucial role in the establishment and maintenance of healthy immune system. Protein-calorie malnutrition, deficiency of micronutrients, stringent self-imposed dieting as in anorexia robs the body of its defensive capabilities, depleting white blood cells as well as crucial immune system proteins. A lack of micronutrients, particularly iodine, iron, vitamin A, and zinc produce adverse effects on the immune function.

### Environmental Pollution

Environmental pollution has become an important risk factor in causing detrimental effects on the immune function. Even short-term exposure to air pollutants affects respiratory health, resulting in upper respiratory tract infections and asthma. Many environmentally persistent xenobiotics such as insecticides, herbicides, fungicides as well as several industrial chemicals (Polybrominated biphenyl, styrenens, lead, mercury), may mimic or antagonize endogenous hormones and adversely affect not only the endocrine and reproductive systems but also the immune system.<sup>[22,23]</sup>

### Unhealthy Life Style

Consumption of processed foods, sedentary lifestyle and many other factors impose a greater risk on the immune function.

### Immunomodulation

The concept of immunomodulation has been gaining much significance worldwide as people started realizing the indispensable role of the immune system in maintaining a disease-free state. In the recent past, the frequency of life-threatening infections has increased dramatically among cancer patients, transplant recipients, AIDS patients and in those receiving broad-spectrum antibiotics, corticosteroids, and cytotoxic drugs.<sup>[24]</sup> In the last two decades, to further complicate matters, there has been an upsurge in the number of strains of infectious agents that no longer succumb to antibiotics has been observed.<sup>[25]</sup> It is apparently clear that antibiotics have lost their magic touch after decades of incautious prescription, improper use and inevitable spread of bacterial genes that confer drug resistance.<sup>[26]</sup> Selective pharmacological action on an individual component of a complex immune response is seen as a particularly attractive approach to the therapy of immunologically mediated diseases.<sup>[27]</sup> The control of disease by immunologic means has two objectives: The development of immunity and prevention of undesired immune reactions.

Modulation of the immune system can be addressed through a variety of specific and non-specific approaches. Many agents of synthetic and natural origin have stimulatory, suppressive and regulatory activity. Immunomodulation is a very broad term which refers to any changes in the immune response and may involve induction, expression, amplification or inhibition of any part or phase in the immune response. It may involve strengthening or suppression of the indicators of cellular and humoral immunity.

The essence of immunomodulation is that where a pharmacological agent acting under various dose and time regimens displays an immunomodulating effect. A stimulation of the immune response is desired for certain people such as immunocompromised patients, whereas suppression of the immune response is desired for others such as transplant recipients or patient with autoimmune or inflammatory diseases.<sup>[28,29]</sup>

## CLASSIFICATION OF IMMUNOMODULATORS

In clinical perspective immunomodulators can be classified into three categories [Figure 5] as given below:

### Immunoadjuvants

An adjuvant is an agent that stimulates the immune system, increasing the response to a vaccine, while not having any specific antigenic effect. Adjuvants perform one or more of three main functions.<sup>[30-33]</sup>

- They provide a “depot” for the slow release of antigen;
- Facilitate targeting of the antigen to immune cells and enhance phagocytosis,
- Modulate and enhance the type of immune response induced by the antigen alone.

Adjuvants may also provide the danger signal the immune system needs to respond to the antigen as it would to an active infection. One of the best known examples is Freund’s adjuvant. In addition to the above use, the immunoadjuvants hold the promise of being the true modulators of the immune response. It has been proposed to exploit them for selecting between cellular and humoral, Th1 and Th2, immunoprotective and immunodestructive, and reagenic (immunoglobulin E)

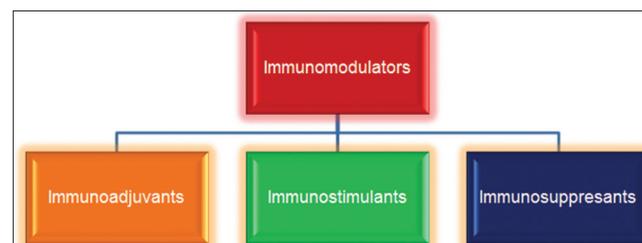


Figure 5: Types of immunomodulators

versus immunoglobulin G type of immune responses. These attempts pose to be a real challenge to vaccine designers.<sup>[34]</sup>

### Immunostimulants

These agents are envisaged to enhance body's resistance against infections (and may be against allergy, autoimmunity, and cancer as well), can act through both the innate and adaptive arms of the immune response. In healthy individuals, the immunostimulants are expected to serve as prophylactic agents such as immune potentiators by enhancing the basic levels of immune response and in individuals with immunocompromized conditions (primary and secondary immune deficiencies) as immunotherapeutic agent.<sup>[35-40]</sup> These agents do not affect immunological memory cells. Their pharmacological efficacy fades away quickly and must, therefore, be renewed by administering the drug either in intervals or continuously.<sup>[41]</sup>

### Immunosuppressants

These agents could be used for control of pathological immune response in autoimmune diseases, graft rejection, graft versus host disease, hypersensitivity (immediate or delayed type), and immune pathology associated with infections. The maximum use of these agents is for prevention of graft rejection and treatment of autoimmune diseases.<sup>[42]</sup> Rejection processes remain an important cause of morbidity and graft loss. Hence, the goal of immunosuppression in organ transplantation is to blunt the immune response of the patient to the allograft, while maintaining sufficient resistance to avoid opportunistic infections and malignancy.

## EXISTING SYNTHETIC IMMUNOMODULATORS-MERITS AND DEMERITS

A diverse array of synthetic, natural and recombinant compounds are available. Of the synthetic immunomodulators, levamisole, isoprinosine, pentoxifylline, and thalidomide are some of the most significant.<sup>[43]</sup> Microbial immunomodulators, such as Bacille Calmette-Guérin (BCG), have been in use for years for non-specific activation of the immune system in some forms of cancer (bladder) and infectious diseases.<sup>[44]</sup> The discovery of cytokines has led to evaluation of efficacy of various interleukins and IFN in immunotherapy of cancer.<sup>[45]</sup> The use of immunostimulants as adjunct to chemotherapy for control and prevention of infections holds great promise.

The intralesional application of BCG<sup>[46]</sup> and systemic use of chemical non-specific immunomodulator levamisole<sup>[47,48]</sup> have shown promising results, however, break-through in the field of immunomodulators was achieved when a strong immunosuppressant drug, cyclosporine<sup>[49]</sup> was discovered that prevented the rejection of graft and had use in other

auto-immune diseases. Cyclophosphamide is another synthetic immunosuppressant which has also been extensively used. A recent review by Patil *et al.*<sup>[50]</sup> provides useful information about various synthetic immunomodulators. While these synthetic immunomodulating drugs have numerous benefits, their adverse side effect profile and generalized effect throughout the immune system poses a major limitation to the general deliberate use of these drugs and warrants the search for a more effective and safer agents exerting immunomodulatory activity. This research focus is becoming a field of major interest all over the world. Existing synthetic immunostimulants, suppressants, their therapeutic applications and adverse effects are presented in Tables 1 and 2.

## PHYTOCHEMICALS AS IMMUNITY REGULATORS

Plants are the biosynthetic laboratory of phytochemicals. Phytochemicals are naturally occurring compound with bioactive potentials. The prefix "Phyto" is from a Greek word meaning plant. These chemicals are often referred to as "secondary metabolites". These are several classes of compounds that include alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes, and terpenoids.<sup>[51,52]</sup> In addition to compounds that are necessary for the growth and reproduction, plant cells synthesize a number of secondary metabolites, which do not appear to be strictly necessary for the survival of the plant. These secondary metabolites or phytochemicals are produced as a response to external stimuli such as infection, nutritional or climatic changes and they may be accumulated in only certain parts of the plant.<sup>[53]</sup> In plants, phytochemicals act as a natural defense system for host plants and provide color, aroma and flavor. More than 4000 of these compounds have been discovered until date.

Besides, protection of plants these phytochemicals also possess therapeutic potentials such as anti-oxidant, anti-diabetic, memory enhancing, cholesterol lowering effects, adaptogenic property, anticancer, and immunomodulatory activity. The thousands of phytochemicals that have been discovered are grouped based on function and sometimes source. Natural compounds with potential immunostimulating activity can be classified as high- and low-molecular compounds. Terpenoids, phenolic compounds, and alkaloids dominate among low-molecular immunomodulatory compounds, polysaccharides dominate among the high-molecular weight compounds.<sup>[29]</sup>

## ALKALOIDS

Alkaloids rank among the most efficient and therapeutically significant plant substances.<sup>[54]</sup> Nearly 5,500 alkaloids are known and comprise the largest single class of secondary plant substances which contain one or more Nitrogen atoms, usually

**Table 1:** Existing novel synthetic immunostimulants<sup>[50]</sup>

Types	Chemical nature	Mode of action	Therapeutic uses	Adverse effects
Bacillus Calmette-Guerin (Natural BRM)	Live mycobacteria	Induces granulomatous reaction at the site of administration	Carcinoma of the urinary bladder, prophylaxis of primary and recurrent stage Ta and/or T1 papillary tumors after transurethral resection	Hypersensitivity, shock, chills, fever, malaise, and immune complex disease
Levamisole	Phenyl imidothiazole	Restore depressed immune function of B-lymphocytes, T-lymphocytes, monocytes and macrophages	Adjuvant with 5-fluorouracil after surgical resection in patients with Duke's stage C colon cancer, agranulocytosis	Flu-like symptoms, allergic manifestation, nausea and muscle pain
Thalidomide	$\alpha$ -(N-phthalimido) glutarimide	Decrease circulating TNF- $\alpha$ in patients with erythema nodosum leprosum, but to increase it in patients who are HIV-seropositive	Refractory rheumatoid arthritis	Teratogenicity
Recombinant cytokines	IFN, Alpha, beta, gamma	Induction of certain enzymes, Inhibition of cell proliferation and enhancement of immune activities like increased phagocytosis by macrophages and augmentation of specific cytotoxicity by T-lymphocytes	Hairy cell leukemia, malignant melanoma, follicular lymphoma, AIDS related Kaposi's sarcoma, chronic hepatitis B	Hypotension, arrhythmias, rarely cardiomyopathy, myocardial infarction, GI distress, anorexia, weight loss, myalgia and depression
	IL aldesleukin, Des alanyl-1, Serine 125 human IL-2	Cellular immunity is profoundly activated with lymphocytosis, eosinophilia, thrombocytopenia and release of multiple cytokines	Metastatic renal carcinoma and melanoma	Capillary leak syndrome, hypotension, reduced organ perfusion and death
	Colony stimulating factors	Increases the number and differentiation of myeloid progenitors	Leucopenia, gancyclovir induces neutropenia	Myocardial infarction, anorexia
Isoprinosine	Inosine salt complex	Augment production of cytokines such as IL-1, IL-2 and IFN- $\gamma$ . It increases proliferation of lymphocytes in response to mitogenic or antigenic stimuli, increases active T-cell rosettes and induces T-cell surface markers on prothymocytes	Herpes simplex infections, subacute sclerosing panencephalitis, acute viral encephalitis caused by herpes simplex, Epstein-Barr and measles viruses	Minor CNS depression, transient nausea, rise of uric acid in serum and urine

BRM: Biological response modifiers, TNF- $\alpha$ : Tumor necrosis factor alpha, HIV: Human immunodeficiency virus, AIDS: Acquired immune deficiency syndrome, GI: Gastrointestinal, IL: Interleukin, IFN: Interferon, CNS: Central nervous system

in combination as part of a cyclic structure.<sup>[51]</sup> Examples include nicotine, cocaine, morphine and codeine, quinine, reserpine, and they have a large demand worldwide. They exhibit marked physiological activity when administered to animals.<sup>[55]</sup> Alkaloids possess anti-tumor activity (vinblastine and vincristine), antimicrobial (cepharanthine), analgesic activity (morphine) and are also known to enhance immune response and a large number of alkaloids are being investigated for their immunostimulating properties [Table 3].

## POLYSACCHARIDES

Botanical polysaccharides exhibit a number of beneficial therapeutic properties, and it is thought that the mechanisms

involved in these effects are due to the modulation of innate immunity more specifically macrophage function. Thus, the scientific evaluation of botanical polysaccharides provides a unique opportunity for the discovery of novel therapeutic agents and adjuvants that could act as a beneficial immunomodulatory agent.<sup>[56]</sup>

## LECTINS

A plant lectin from *Viscum album* has been shown to increase the number and cytotoxic activity of NK cells and to induce antitumor activity in animal models. Lectin-sugar interactions on the cell surface of immunocompetent cells can induce cytokine gene expression and protein synthesis.<sup>[57]</sup> Lectin

**Table 2: Existing synthetic Immunosuppressants<sup>[50]</sup>**

Mode of action	Example	Therapeutic uses	Adverse effects
Inhibitors of Lymphocyte gene expression	Corticosteroids	Acute transplant rejection, graft-versus-host disease in bone-marrow transplantation, rheumatoid and other arthritides, systemic lupus erythematosus, systemic dermatomyositis, psoriasis and other skin conditions, asthma and other allergic disorders, inflammatory bowel disease, inflammatory ophthalmic diseases	Growth retardation in children, avascular necrosis of bone, osteopenia, increased risk of infection, poor wound healing, cataracts, hyperglycemia, and hypertension
Inhibitors of lymphocyte signaling	Calcineurin inhibitors Cyclosporine Tacrolimus	Kidney, liver, heart, and other organ transplantation, rheumatoid arthritis and psoriasis, early engraftment, extending kidney graft survival, cardiac and liver transplantation, Behcet's acute ocular syndrome, endogenous ileitis, atopic dermatitis Solid-organ allograft rejection, kidney transplantation, paediatric liver transplantation	Renal dysfunction, tremor, hirsutism, hypertension, hyperlipidemia, gum hyperplasia, hyperuricemia, hyper-cholesterolemia, nephrotoxicity, hypertension, diabetogenic, Elevated LDL cholesterol Nephrotoxicity, neurotoxicity (tremor, headache, motor disturbances and seizures), GI complaints, hypertension, hyperkalemia, hyperglycemia, and diabetes
	mTOR inhibitors Sirolimus	Graft rejection, incorporated into stents to inhibit local cell proliferation and blood vessel occlusion	Dose-dependent increase in serum cholesterol and triglycerides, impaired renal function, prolong delayed graft function, lymphocele, anemia, leukopenia
Cytotoxic agents	Antimetabolites Azathioprine Mycophenolate mofetil	Allogeneic kidney transplantation, organ transplant rejection	Bone marrow suppression including leukopenia (common), thrombocytopenia (less common), and/or anemia (uncommon), increased susceptibility to infections (especially varicella and herpes simplex viruses), hepatotoxicity, alopecia, GI toxicity, pancreatitis Leukopenia, diarrhoea, and vomiting, sepsis associated with cytomegalovirus
	Alkylating agents Cyclophosphamide	Autoimmune disorders (including systemic lupus erythematosus), in patients with acquired factor XIII antibodies and bleeding syndromes, autoimmune hemolytic anemia, antibody-induced pure red cell aplasia, and Wegener's granulomatosis	Pancytopenia and hemorrhagic cystitis, graft-versus-host disease syndrome, nausea, vomiting, cardiac toxicity and electrolyte disturbances
Cytokine inhibitors (anticytokine antibodies)	TNF $\alpha$ inhibitors IL-1 inhibitors IL-2 inhibitors	Rheumatoid arthritis and psoriatic arthritis, Cronh's disease	
Antibodies against specific immune cell molecules	Polyclonal antibodies	Acute renal transplant rejection, recovery from ischemic reperfusion injury	Fever and chills, hypotension, serum sickness, glomerulonephritis, leukopenia and thrombocytopenia, increased risk of infection and malignancy especially when multiple immunosuppressive agents are combined

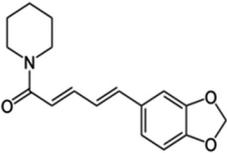
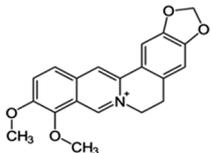
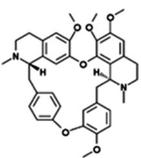
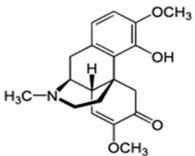
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Table 2: Contd....

Mode of action	Example	Therapeutic uses	Adverse effects
	Monoclonal antibodies	Acute organ transplant rejection	Cytokine release syndrome, high fever, chills/rigor, headache, tremor, nausea/vomiting, diarrhea, abdominal pain, malaise, myalgias, arthralgias, and generalized weakness. Less common complaints include skin reactions and cardiorespiratory and CNS disorders, including aseptic meningitis. Potentially fatal severe pulmonary edema, acute respiratory distress syndrome, cardiovascular collapse, cardiac arrest
Inhibitors of immune cell adhesion	Efalizumab	Survival of murine skin and heart allografts and monkey heart allografts, psoriasis, renal transplantation	Less common side effects include skin and other infections
Miscellaneous	Rho(D) immunoglobulin	Hemolytic disease of the newborn	Bloody urine, decreased frequency of urination, increased blood pressure

LDL: Low-density lipoprotein, GI: Gastrointestinal, mTOR: Mechanistic target of rapamycin, TNF- $\alpha$ : Tumor necrosis factor alpha, IL: Interleukin, CNS: Central nervous system

Table 3: Alkaloids as immunomodulators

Phytochemical and botanical source	Structure	Mechanism of action	Reference
Piperine <i>Piper longum</i>		Increases total WBC count, bone marrow cellularity, total antibody production	[82]
Berberine <i>Hydrasti canadensis</i>		Significant reduction of plasma TNF- $\alpha$ , IFN- $\gamma$ and NO levels	[83]
Tetrandrine <i>Stephania tetrandra</i>		Suppress cytokine production Inhibits NF- $\kappa$ B mediated release of inflammatory factors	[84,85]
Sinomenine <i>Sinomenium acutum</i>		Graft survival	[86]

WBC: White blood cell, TNF- $\alpha$ : Tumor necrosis factor alpha, IFN- $\gamma$ : Interferon gamma, NO: Nitric oxide, NF- $\kappa$ B: Nuclear factor-kappaB

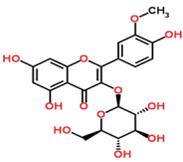
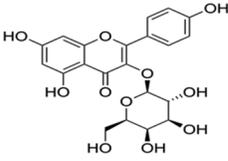
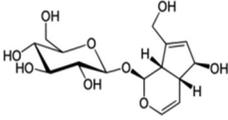
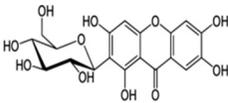
alters mitochondrial transmembrane potential and increases intracellular levels of ROS.<sup>[58]</sup>

## GLYCOSIDES

These include a wide range of chemical sub groups containing a glycan (sugar) and an aglycan (non-sugar).

These are polar compounds which consist of at least one sugar molecule linked to another moiety. Pharmaceutically important glycosides include saponins and anthracin derivatives. Glycosides mainly participate in the stimulation of cardiac system, central nervous system stimulation and immune system, they also possess antimicrobial activity [Table 4].

**Table 4:** Glycosides as immunomodulators

Phytochemical and botanical source	Structure	Mechanism of action	Reference
Isorhamnetin-3-O-glucoside <i>Urtica dioica</i>		<i>In vitro</i> immunomodulatory potential	[87]
Eupalitin-3-O-β-D-galactopyranoside <i>Boerhaavia diffusa</i>		Inhibited PHA-stimulated proliferation of peripheral blood mononuclear cells IL-2 and TNF-α	[88]
Aucubin <i>Plantago major</i>		Enhances lymphocyte proliferation and secretion of IFN-γ	[89]
Mangiferin <i>Mangifera indica</i>		Enhances the production of IgG1 and IgG2b	[90]

PHA: Phytohaemagglutinin, IL: Interleukin, TNF-α: Tumor necrosis factor alpha, IFN-γ: Interferon gamma, IgG: Immunoglobulin G

## PHENOLIC COMPOUNDS

These are wide range plant substances possessing an aromatic ring bearing one or more hydroxyl substituents. They are water soluble as they most frequently occur combined with sugar as glycosides. They are usually located in the cell vacuole. The presence of phenols is considered to be potentially toxic to the growth and development of pathogens [Table 5]. This group includes flavonoids, tannins, and other phenols.<sup>[55]</sup>

isolates and anthocyanin-rich mixtures of bioflavonoids may provide protection from DNA cleavage, estrogenic activity (altering development of hormone-dependent disease symptoms), enzyme inhibition, boosting production of cytokines (thus regulating immune responses), anti-inflammatory activity, lipid peroxidation, decreasing capillary permeability and fragility, and membrane strengthening [Table 7].<sup>[63-65]</sup>

## FLAVONOIDS

These are among the most widely distributed natural products in plants occurring both in the free-state and as glycosides. They are mainly water-soluble compounds. Their chemical structure is based upon C6-C3-C6 carbon skeleton. Flavonoids are potent water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage have strong anti-cancer activity and protect against all stage of carcinogens. Flavonoids in the body are known to reduce the risk of heart diseases.<sup>[59]</sup> In terms of anti-cancer activity, they inhibit the initiation, promotion, and progression of tumors.<sup>[52,59]</sup> In recent times, plant flavonoids have attracted attention of researchers as a potentially important dietary supplement for cancer patients as they act as chemoprotective agents.<sup>[60,61]</sup> Some isoflavones act as allelochemicals which is widely used as insecticides [Table 6].<sup>[62]</sup>

## ANTHOCYANINS

The colorful anthocyanins are the most recognized, visible members of the bioflavonoid phytochemicals. Anthocyanin

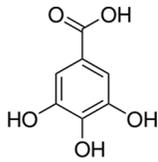
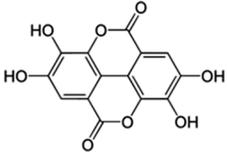
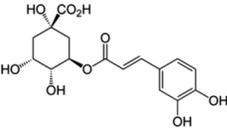
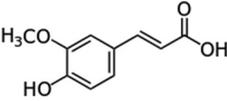
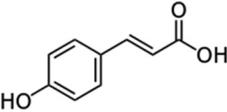
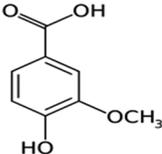
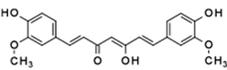
## TANNINS

These are amorphous, rarely crystalline substances, soluble in water and alcohol and have an astringent and bitter taste. Chemically, they are complex phenolic substances classified on the basis of hydrolysis product. They occur widely in vascular plants; in angiosperms their occurrence is associated with woody tissues. Hydrolysable tannins are based on gallic acid, usually as multiple esters with D-glucose, while the numerous condensed tannins (often proanthocyanides) are derived from flavonoid monomers.<sup>[51,54]</sup> Many physiological activities such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective action have been assigned to tannins [Table 8].<sup>[55]</sup>

## SAPONINS

Saponins are common in a variety of higher plants and usually found in roots, tubers, leaves, blooms or seeds. Based on the carbon skeletons, saponins were classified into triterpenes and steroids. Their glycone parts were mostly oligosaccharides, arranged either in a linear or branched fashion, attached

**Table 5:** Phenolic compounds as immunomodulators

Phytochemical and botanical source	Structure	Mechanism of action	Reference
Gallic acid		B-cell proliferation, inhibition of mast cell degranulation	[91,92]
Ellagic acid <i>Punica granatum</i>		Anti-proliferative and antioxidant	[93]
Chlorogenic acid <i>Plantago major</i>		Enhances lymphocyte proliferation and secretion of IFN	[89]
Ferulic acid <i>Plantago major</i>		Enhances lymphocyte proliferation and secretion of IFN	[89]
P-Coumaric acid <i>Plantago major</i>		Enhances lymphocyte proliferation and secretion of IFN	[89]
Vanillic acid <i>Plantago major</i>		Enhances lymphocyte proliferation and secretion of IFN	[89]
Curcumin <i>Curcuma longa</i>		Enhances bone marrow cellularity, $\alpha$ esterase positive cells and phagocytic activity. Inhibits IL-2 expression and NF- $\kappa$ B	[94,95]

IFN: Interferon, IL: Interleukin, NF- $\kappa$ B: Nuclear factor-kappaB

to hydroxyl groups through an acetal linkage.<sup>[66]</sup> Modern researchers found that saponins have antitumor effect on many cancer cells. Several saponins inhibit tumor cell growth by cell cycle arrest and apoptosis with  $IC_{50}$  values up to 0.2 mM. Saponins in combination with conventional tumor treatment strategies can result in improved therapeutic success rate [Table 9].<sup>[67]</sup>

## TERPENOIDS

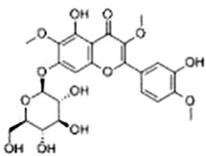
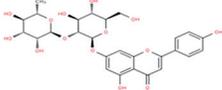
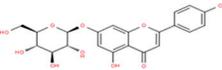
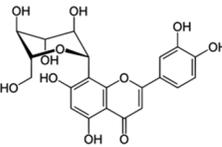
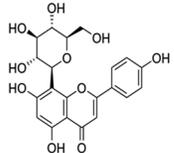
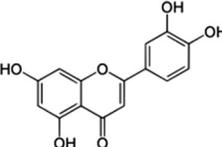
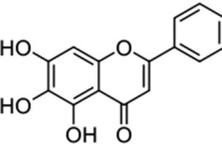
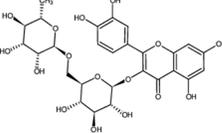
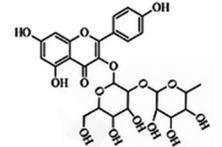
These are natural products whose structure may be divided into isoprene units, hence also called as isoprenoids. Triterpenoids are compounds with a carbon skeleton based on six isoprene units and are derived biosynthetically from the cyclic C30 hydrocarbon, squalene. They are colorless, crystalline, often have high melting points and are optically active substances. The essential triterpenoids are saponins, steroids and cardiac glycosides which occur mainly as glycosides. Triterpenes occur especially in the waxy coatings

of leaves and on fruit such as apple and pear, and they may serve a protective function in repelling insects and microbial attack.<sup>[51]</sup> Several terpenoids are reported to possess antiarthritic or antiphlogistic activity, and their biological activities appear to be mediated by immunological processes. Effect of these compounds on immune system appears to be two-fold; first to enhance antibody production and second to suppress T-cell response [Table 10].

## STEROLS

The mixture of sterols and sterolins enhances the cytotoxic ability of NK cells against the target cell line NK 562. It has also been postulated that the sterols in a specific ratio could reinstate a balance between the Th1-Th2 cells, a delicate balance that determines the final outcome of the immune response. The phytosterols,  $\beta$ -Sitosterol, and its glycoside enhanced the *in vitro* proliferative response of T-cells stimulated by sub-optimal concentrations

**Table 6:** Flavonoids as immunomodulators

Phytochemical and botanical source	Structure	Mechanism of action	Reference
Centaurein <i>Bidens pilosa</i>		Augmentation of IFN- $\gamma$ promoter activity	[96]
Apigenin 7-o- $\beta$ -D-Neohesperidoside <i>Jatropha curcas</i>		Stimulation of humoral and cell mediated immune response	[97]
Apigenin 7-O- $\beta$ -D-Galactoside <i>Jatropha curcas</i>		Stimulation of humoral and cell mediated immune response	[97]
Orientin <i>Jatropha curcas</i>		Stimulation of humoral and cell mediated immune response	[97]
Vitexin <i>Jatropha curcas</i>		Stimulation of humoral and cell mediated immune response	[97]
Luteolin <i>Plantago major</i>		Enhances lymphocyte proliferation and secretion of IFN	[89]
Baicalein <i>Plantago major</i>		Enhances lymphocyte proliferation and secretion of IFN	[89]
Quercetin-3-O-rutinoside <i>Urtica dioica</i>		Immunomodulation	[87]
Kaempferol-3-O-rutinoside <i>Urtica dioica</i>		Immunomodulation	[87]

IFN- $\gamma$ : Interferon gamma

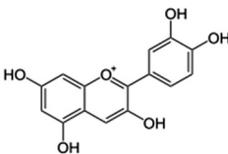
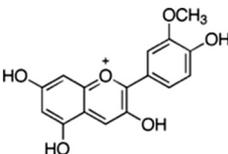
of phytohaemagglutinin several-fold at extremely low concentrations [Table 11].<sup>[68]</sup>

## ANTIOXIDANTS AS IMMUNOMODULATORS

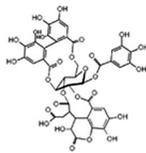
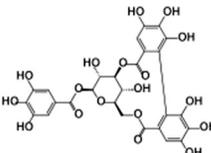
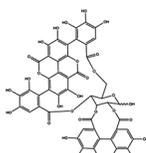
In recent years, there is an upsurge in the areas related to newer developments such as prevention of diseases

through free radicals scavenging using antioxidants. Free radicals have been implicated in the etiology of several human diseases as well as ageing.<sup>[69,70]</sup> However, it has to be emphasized that ROS and RNS are both produced in a well-regulated manner so as to help in the maintenance of homeostasis at the cellular level in the normal healthy tissues and to play an important role as signaling molecules. Most cells can produce superoxide ( $O_2^{\cdot-}$ ), hydrogen peroxide ( $H_2O_2$ ) and nitric oxide (NO) on demand. Hence, it is worth

**Table 7: Immunomodulatory potentials of anthocyanins**

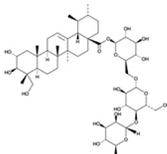
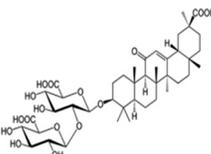
Phytochemical and botanical source	Structure	Mechanism of action	Reference
Cyanidin-3-glycoside <i>Blackberry</i>		Antioxidant and anti-inflammatory mechanism	[98,99]
Peonidin <i>Blackberry</i>		Antioxidant and anti-inflammatory mechanism	[98,99]

**Table 8: Immunomodulatory potentials of tannins**

Phytochemical and botanical source	Structure	Mechanism of action	Reference
Chebulagic acid <i>Terminalia chebula</i>		Down regulation of TNF- $\alpha$ and IL-6	[100]
Corilagin <i>Terminalia chebula</i>		Neuroprotection	[101]
Punicalagin		Free radical scavenging and immunosuppressive action	[102]

TNF- $\alpha$ : Tumor necrosis factor alpha, IL: Interleukin

**Table 9: Saponins as immunomodulators**

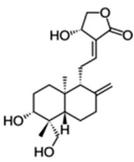
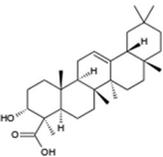
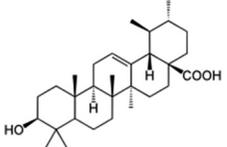
Phytochemical and botanical source	Structure	Mechanism of action	Reference
Asiaticoside <i>Centella asiatica</i>		Enhances phagocytic index and total WBC count	[103]
Glycyrrhizin <i>Glycyrrhiza glabra</i>		Inhibits classical complement pathway	[104]

WBC: White blood cell

understanding the important beneficial role of free radicals. Free radical help in the following processes.<sup>[71]</sup>

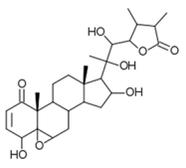
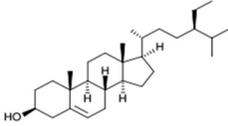
- Generation of adenosine triphosphate (universal energy currency) from adenosine diphosphate in the mitochondria: Oxidative phosphorylation
- Detoxification of xenobiotics by Cytochrome P450 (oxidizing enzymes)
- Apoptosis of effete or defective cells
- Killing of microorganisms and cancer cells by macrophages and cytotoxic lymphocytes
- Production of oxygenases (e.g., cyclo-oxygenases, lipoxygenase) for the generation of prostaglandins and leukotrienes, which have many regulatory functions.

**Table 10: Terpenoids as immunomodulators**

Phytochemical and botanical source	Structure	Mechanism of action	Reference
Andrographolide <i>Andrographis paniculata</i>		Enhances the expression of IL-2 Inhibition of NO in endotoxin stimulated macrophages	[105]
Boswellic acid <i>Bowella serrata</i>		Significant inhibition of mast cell degranulation	[106]
Ursolic acid		Activates intracellular killing effect of macrophages during Mycobacterium tuberculosis infection	[107]

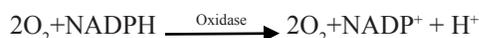
NO: Nitric oxide, IL: Interleukin

**Table 11: Immunomodulatory potentials of sterols**

Phytochemical and botanical source	Structure	Mechanism of action	Reference
Withanolide <i>Withania somnifera</i>		Activates murine macrophages, phagocytosis and lysosomal enzyme activity	[108]
$\beta$ -Sitosterol		Activates human peripheral lymphocyte proliferation	[109]

## GENERATION OF FREE RADICALS IN THE IMMUNE SYSTEM

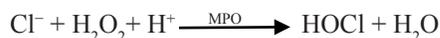
During normal biochemical reactions in our body, there is generation of ROS and reactive nitrogen species (RNS). This gets enhanced during pathophysiological conditions creating "oxidative stress." During this phenomenon cellular constituents get altered resulting in various diseased states. This may be effectively neutralized, by enhancing the cellular defenses, in the form of antioxidants.<sup>[72]</sup> Reactive species are also generated during phagocytosis, a manifestation of innate immunity. The migration of leukocytes at an inflammatory site results in phagocytosis with the release of enzymes and cytokines from both macrophages and neutrophils. Phagocytosis stimulates various independent processes, especially "respiratory burst," which results from activation of Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme normally inactive in resting cells. The generation of ROS begins with the rapid uptake of oxygen and activation of NADPH oxidase and the production of the superoxide free radical.<sup>[73]</sup>



Superoxide is then rapidly converted to hydrogen peroxide by superoxide dismutase (SOD)



These ROS can act either through two oxygen-dependent mechanisms resulting in the destruction of the microorganism or other foreign matter. The reactive species can also be generated by the myeloperoxidase (MPO)-halide- $H_2O_2$  system. The neutrophil cytoplasmic granules contain the enzyme MPO. In the presence of chloride ion, which is ubiquitous, hydrogen peroxide is converted to hypochlorous acid (HOCl) a potent oxidant and antimicrobial agent.<sup>[74]</sup>

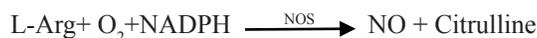


The MPO-independent mechanism, though not as important as the previous one, is still essential. ROS is generated from

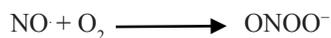
superoxide and  $H_2O_2$  produced via respiratory burst by Fenton (A) and/or Haber-Weiss (B) reactions.<sup>[75]</sup>



RNS are also important. The free radical NO, first described as endothelium-derived relaxation factor, is produced from arginine by NO synthase.



An inducible NOS is capable of continuously producing large amounts of NO. In activated immune cells, it acts as a killer molecule.<sup>[76]</sup> Although the direct toxicity of NO is modest, it gets greatly increased when it reacts with superoxide to form peroxynitrite, a very strong oxidant.



Peroxynitrite can react with aromatic amino acid residues to form nitrotyrosine, which can lead to enzyme inactivation.<sup>[77]</sup> It can also kill *Escherichia coli* cells directly.<sup>[78]</sup> However, NO is an important cytotoxic effector molecule in defense against tumor cells, various protozoa, fungi, helminths, and mycobacteria.<sup>[79]</sup>

## ADVERSE EFFECT OF OXIDATIVE STRESS IN THE IMMUNE SYSTEM

Cellular components of immune system are rich in polyunsaturated fatty acids, and these are highly susceptible to oxidative attack resulting in highly damaging lipid peroxidation. The peroxidation products are highly cytotoxic and, in turn, affect the cell-mediated immune response. Chronic inflammatory disorders such as rheumatoid arthritis, asthma, psoriasis and inflammatory bowel disease result in the production of several cytokines. These can recruit activated immune cells to the site involved thereby amplify and perpetuate the inflammatory process.<sup>[80]</sup> Hence, it is evident that oxidative stress may influence the immune system either by hyperexcitation to cause autoimmune disorders or suppress it, resulting in higher susceptibility to infections.

## ANTIOXIDANTS AS PROTECTIVE AGENTS

In recent years, antioxidants have gained much importance as prophylactic and therapeutic agents in curing human ailments. Antioxidants may function as immune modulators and can be used along with the mainstream therapy in certain diseases. Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: SOD, glutathione peroxidase, glutathione reductase,

thioredoxin, thiols and disulfide bonding are buffering systems in every cell. Other non-enzymatic antioxidants include carotenoids, flavonoids and related polyphenols,  $\alpha$ -lipoic acid and glutathione, vitamins C and E.<sup>[81]</sup>

The natural foods, spices and medicinal plants are rich sources of antioxidants. Compounds with potent antioxidant activity include carotenoids, curcumin, flavonoids, epigallocatechin-3-O-gallate, lycopene, ellagic acid, coenzyme  $Q_{10}$ , indole-3-carbinol, genistein, quercetin, caffeine, orientin, vicenin, glabridin, glycyrrhizin, emblicanin, punigluconin, pedunculagin, 2-hydroxy-4-methoxy benzoic acid, dehydrozingerone, picroliv, withaferin, yakuchinone, gingerol, chlorogenic acid, vanillin (food flavoring agent), and chlorophyllin (a water-soluble analogue of chlorophyll).<sup>[71]</sup>

## CONCLUSION

There are a number of epidemiological studies and experimental evidence to establish the relationship between the levels antioxidants/phytochemicals and immunomodulation in animals as well as human beings. Many indigenous plants which have been investigated for their immunomodulatory potential concurrently exhibited significant antioxidant activities. Therefore, targeting oxidative stress or boosting the endogenous levels of antioxidants is likely to have a beneficial outcome in the management of diseases and in developing immunity.

## ACKNOWLEDGMENTS

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