Chapter 5

# Plant Phenolic Compounds as Immunomodulatory Agents

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66112

**Abstract**

Immunology is a source of continuous discoveries; Immunology was and still is a source of continuous discoveries. Immunomodulation encompasses all therapeutic interventions aimed at modifying the immune response. Immunostimulation is desirable to prevent infection in states of immunodeficiency and to fight infections and cancer. On the other hand, immunosuppressive agents inhibit the activity of the immune system, and they are used to prevent the rejection of transplanted organs and tissues and to treat autoimmune diseases or diseases that are most likely of autoimmune origin (e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, etc.), or other nonautoimmune inflammatory diseases (e.g., allergic asthma). The discovery of immunomodulatory agents from medicinal plants devoid of toxic side effects, with enhanced bioavailability and that can be used for a long duration, is of great actuality. Research on natural immunomodulators provides a therapeutic solution that addresses a multitude of disorders. Plant phenolic compounds already proved beneficial effects in cardiovascular diseases, diabetes, and cancer, exerting mainly antioxidant and anti-inflammatory effects. The concepts of "immunomodulatory," "anti-inflammatory," and "antioxidant" are often strongly related, and a review of phenolic compound action on immune system should be analyzed in a context, revealing their mechanism of action on effector cells and also on the system as a whole.

**Keywords:** immunomodulation, immunostimulation, immunosuppression, phenolic compounds, bioavailability

## 1. Introduction

Immune response is one of the most complex mechanisms of the living body, involving the strong cooperation of a large variety of cell types for defending against any potential dangerous agent. Perturbation of this well-adapted process results in a cascade of disorders

and even the occurrence of chronic diseases, making the regulation of the immune system a key factor in maintaining a healthy equilibrium of the body. The discovery of immunomodulatory agents from medicinal plants devoid of toxic side effects, with enhanced bioavailability and that can be used for a long duration, is of great actuality.

In terms of molecular weight, phytocompounds are classified in high-molecular compounds such are peptides, polysaccharides, and low-molecular compounds—terpenes, alkaloids, and also phenolics. Plant phenolic compounds already proved beneficial effects in cardiovascular diseases, diabetes, and cancer, exerting mainly antioxidant and anti-inflammatory effects. Most of the plant-derived phenolics influence the nonspecific immune response mainly by enhancing phagocytosis and proliferation of macrophages and neutrophils. The concepts of "immunomodulatory," "anti-inflammatory," and "antioxidant" are often strongly related, and a review of phenolic compounds action on immune system should be analyzed in a context, revealing their mechanism of action on effector cells and also on the system as a whole.

## 2. Overview on the immune system

Immune response is controlled both by direct interaction of different types of cells (lymphoid cells: B and T lymphocytes, T helper (Th) cells, natural killer (NK) cells; myeloid cells: neutrophils, basophils, monocytes, macrophages) and by-products of synthesis they secrete (immunoglobulins, cytokines: interleukines, colony-stimulating factors, growth factors, interferons, etc).

Although innate and adaptive immunities work complementarily to provide an overall protection to the human body, they appeared at different times in evolution. Basic mechanisms of the innate immunity are found both in vertebrates and invertebrates and even in plants, while adaptive immune response is specific to vertebrates [1].

Innate immunity has no capacity for immunological memory and employs an antigen-independent defense mechanism that provides host defense immediately or within hours after exposure to pathogens. Cells involved in this response comprise phagocytic cells (neutrophils, monocytes, and macrophages), cells secreting inflammatory mediators (basophils, mast cells, and eosinophils), and natural killer (NK) cells. Pathogen-associated molecules (called pathogen-associated immunostimulants) stimulate two types of innate immune responses—inflammatory responses and phagocytosis by cells such as neutrophils and macrophages [1], processes regulated by soluble mediators known as cytokines. The mechanism is complex, and a precise delimitation of immunity, inflammation, and oxidation cannot be set. Innate immunity can also stimulate adaptive immune response with the help of a group of specialized cells known as antigen-presenting cells (APCs) such are dendritic cells (DCs). APCs display the processed antigen to lymphocytes and collaborate with them to elicit the immune response. Unlike innate immunity, the adaptive immune response involves antigen-specific antibodies, and a certain time interval is required for the maximal response to be achieved after exposure to the antigen.

http://dx.doi.org/10.5772/66112

Adaptive responses are mainly conducted by T cells, facilitated by APCs in cell-mediated immunity and B cells in antibody-mediated immunity. The T lymphocyte group represents 60–80% of total lymphocytes and has a very high lifetime and is mainly involved in eradication of intracellular pathogens by activating macrophages and by killing virally infected cells. These lymphocytes recognize the primary structure of an antigen, a mechanism different from that of B lymphocytes and plasma cells, which recognize the antigen by the spatial structure. T helper (Th) lymphocytes represent 2/3 of total lymphocytes and are of special importance because they secrete interleukins, messenger molecules that facilitate the communications between immune system cells. Depending on the type of cytokines that they secrete, Th1 cells producing interleukin-2, IFN-$\gamma$, and TNF-$\alpha$ and triggering inflammatory reactions and Th2 cells producing interleukins 3, 4, and 5, the main stimulator of immunoglobulin A and E synthesis, are distinguished [2].

In antibody-mediated immunity, activation of B lymphocytes conducts to plasma cells synthesizing immunoglobulins or memory B cells leading to immunological memory.

## 3. Overview on phytophenols

Phytophenols are secondary metabolites based on a common carbon skeleton structure—the C6–C3 phenylpropanoid unit [3]. Among this group, several classes are described:

**Flavonoids** are natural phenolic substances of C6–C3–C6 type, derivatives of 2-phenylbenzopyran (flavan) or 3-phenylbenzopyran (isoflavan). Flavonoids are present in plant organs as glycoside or aglycone. Flavonoid aglycones are based on 2-phenylbenzo-$\gamma$-pyrone core (2-phenylchromane) grafted with hydroxyl, methoxyl, dimethylallyl, etc. groups.

Most of the compounds are hydroxylated on A ring, at C5 and C7 positions. On the ring B are grafted 1–3 phenolic groups at C4′, 3′, and 5′. Depending on the degree of oxidation and substituent type of segment, several classes are classified:

– Flavones—double bond between C2 and C3 (e.g., apigenin, luteolin)

– Flavonols—flavones 3-hydroxylated (e.g., kaempferol, galangin, quercetin, miricetin)

– Flavanones—flavones 2,3-dihydrogenated (e.g., naringenin, hesperetin)

– Flavanonols—flavonols 2,3-dihydrogenated (e.g., taxifolin, dihidrokaempferol) [4]

Also, other varieties of flavonoids are:

– Biflavonoids are dimer of flavonoids. The monomers are linked in positions 6 and 8, which are highly reactive. The links established can be C–C (amentoflavone, bilobetol, ginkgetol) or C–O–C (hinokiflavona). The hydroxyl groups may be free or, most often, methylated.

– Isoflavones are 3-phenylbenzo-$\gamma$-pyrone derivatives (3-phenylchromone), specific for Fabaceae family (species of this family contain a specialized enzyme responsible for converting 2-phe-

nylchromane to 3-phenylchromane). Isoflavones are usually found in free state (daidzein, genistein) and very rare as heterosides (mainly O-heterosides) (daidzein, puerarin) [4].

– Chalcones (1,3-diaryl-2-propen-1-ones) are $\alpha,\beta$-unsaturated ketones, comprised of two aromatic rings, that function as precursors in the synthesis of flavonoids and isoflavonoids (phloretin, arbutin).

In regard to **phenolic acids,** two classes can be distinguished: derivatives of benzoic acid (C6–C1 structure) and derivatives of cinnamic acid (C6–C3 structure). Hydroxybenzoic acids include gallic, *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids having C6–C1 structure [5]. The hydroxycinnamic acids are more common than are the hydroxybenzoic acids and consist mainly of *p*-coumaric, caffeic, ferulic, and sinapic acids [6] and also of esters of caffeic acid with quinic acid (chlorogenic acid), tartaric acid (cichoric acid), and 2-hydroxydihydrocaffeic acid (rosmarinic acid).

**Lignans** are compounds resulted from the condensation of two to five molecules of phenylpropane derivatives (C6–C3). Dietary lignans are metabolized by the intestinal microflora to enterodiol and enterolactone, compounds associated to many positive effects for human health [6]. This class comprises valuable antineoplastic agents—podophyllotoxin, matairesinol, and immunostimulants—syringaresinol, arctigenin, etc.

**Tannins**, both procyanidins and hydrolysable tannins, are so named for their use in the tanning of leather or hides based on their ability to bind and precipitate proteins. There are a vast number of processes for which plants employ tannins, ranging from herbivore protection to hormone regulation. Procyanidins and hydrolysable tannins differ in their core polyphenol structure and have differing functions both in the plant and on mammalian cells [7]. Hydrolysable tannins contain a sugar core surrounded by phenolic groups such as gallic acid residues. These residues can be subsequently modified by further addition of phenolic groups, oxidation reactions, or other polyphenols [8, 9], thereby generating increasingly complex polyphenols. The procyanidins are produced by assemblage into oligomers; up to 28-mers of procyanidins have been recorded [10]. These oligomers are formed via combinations of the monomer subunits epicatechin or catechin and are often modified by the addition of gallic acid residues. In grapes, for example, about 20% of the residues are galloylated [10].

**Stilbenoids** contain two phenyl moieties connected by a two-carbon methylene bridge (C6–C2–C6) [11], the most known representative compound of this class being resveratrol.

## 4. Aspects related to the structure-activity relation of phytophenols

It was showed that there are differences in immunomodulation exerted by flavonoid glycosides and their corresponding aglycones. While quercetin is able to activate concomitantly lymphocytes and secretion of IFN-c, the similar flavonoid rutin (quercetin-3-rutinoside) significantly stimulates the secretion of IFN-c, but do not elevate the proliferation of human peripheral blood mononuclear cells (PBMC), indicating the sugar moiety as the key point for different responses [12].

The importance of the sugar at position 3 for the selective immunosuppression by astilbin (taxifolin 3-rhamnoside) was highlighted by Guo et al. Most of the flavonoid glycosides have glucose attached to aglycones and are usually hydrolyzed by glucosidase. In the case of astilbin, the sugar attached to aglycone is rhamnose, which is likely difficult to hydrolyze, and it is suggested that this phytocompound may show a different metabolic route from other flavonoids, owing to the type and position of sugar attached [13, 14].

Hydroxylations of flavonoids at positions 5 and 7, together with the double bond at C2–C3 and the position of the B ring at 2, appear to be associated to the highest inhibition of pro-inflammatory cytokine expression [15]. Luteolin and apigenin contain hydroxyl groups in their backbone, and it was suggested that these may be involved in immunomodulatory activities since luteolin, which contains hydroxyl groups both at the 3′ and 4′ positions in ring B, exhibits stronger immunomodulatory properties than apigenin that has only a 4′ hydroxyl group in ring B [16]. For chalcone class, trimethoxy chalcones at the A ring with fluoro, chloro, and bromo substitution in the B ring, like 2′-hydroxy-3-bromo-6′-methoxychalcone, 2′-methoxy-3,4-dichlorochalcone, flavokawain A, or flavokawain B, are considered better inhibitors of NF-κB [17]. The number and position of methoxy group seems to be correlated to immunomodulatory capacity as in the case of coumarins. For instance, two methoxy groups (isopimpinellin) are correlated to lymphocyte activation, while one methoxy group (xanthotoxin) conducts to IFN secretion; bergapten (5-methoxypsoralen) is a better IFN-γ activator than xanthotoxin (8-methoxypsoralen) [12].

Due to the fact that the immune response is very complex, some studies were focused on anti-oxidative immune-mediated mechanisms, and it was shown that the most important feature is the presence of a C–2,3 double bond in combination with a 4-oxo group as it is proved by the higher antioxidant activity of luteolin comparing to apigenin [16]. Souza et al. [18] showed that flavonoid aglycones have high-antioxidant inhibitory activities, while C-glycosylated flavonoids have no significant effect even at the highest concentration tested (50 μmol/L).

Another factor that appears to be important for the influence on the immune response, in particular stimulation of T-cell cytokine production by polyphenols, is the size of the polyphenol molecule [19, 20]. Schepetkin et al. [19] showed that molecular subunits of oenothein B with smaller molecular weights do not have the same leukocyte immunomodulatory capacity, and also procyanidin oligomers, but not monomers, are able to stimulate innate lymphocytes [7]. Also, the chain length of flavanol fractions has a significant effect on cytokine release from both unstimulated and LPS-stimulated PBMCs. Long-chain flavanol fraction and short-chain flavanol fraction, in the absence of LPS, stimulated the production of GM-CSF and increase expression of the B-cell markers CD69 and CD83. The oligomers are potent stimulators of both the innate immune system and early events in adaptive immunity [21].

## 5. Aspects related to phytophenol bioavailability

Research regarding bioavailability of phytophenols is essential for the establishment of dietary management of diseases [22]. Increased intake of flavonoids with higher in vitro activity is not a guaranty for a strong pharmacological effect in vivo because low absorption and rapid elimination

cause a limited bioavailability. In most of the published data regarding this issue, the concentration of polyphenols in blood and urine after ingestion of phenols rich food was measured as an indicator of their absorption [23], but there are complex reactions of metabolization hindering the biological activity of the parent compounds [24]. Many of these phytophenols with high activity in vitro are not object of industrial investment because of their oral bioavailability below 30% [25].

The absorption of some, but not all, dietary polyphenols occurs in the small intestine. Before the absorption, these compounds must be hydrolyzed by intestinal enzymes. It is believed that the phenolic compounds are absorbed by a passive diffusion mechanism (aglycones) or by carriers present in the intestine [26]. Polyphenols that are not absorbed in the small intestine reach the colon, where they undergo substantial structural modifications by colonic microflora that hydrolyzes glycosides into aglycones and degrades them to simple phenolic acids [27]. Once absorbed, and prior to the passage into the bloodstream, the polyphenol-derived aglycones undergo other structural modifications due to the conjugation process [23]. Glucuronidation and sulfation conjugation reactions are described to have a significant impact on the bioactivity of polyphenols. In particular, the low oral bioavailability of some phenolic substances could be explained by glucuronidation [28]. The low absorption profile of curcumin was demonstrated in human and rat models [29], and it was attributed not only to the poor solubility of this compound but also to the glucuronidation or sulfation processes.

These conjugation reactions significantly reduce the polyphenol antioxidant activity, since both sulfation and glucuronidation occur at the reducing hydroxyl groups in the phenolic structure. It was already shown that these groups are mainly responsible for the antioxidant and immunomodulatory properties of polyphenols [30]. Nevertheless, conjugation reactions might enhance certain specific bioactivities. For example, Koga [31] described that the plasma metabolites of catechin have an inhibitory effect on monocyte adhesion to interleukin-1 in beta-stimulated human aortic endothelial cells, while catechin had no effect [32]. Lignans, for example, need to be biotransformed by gut microflora to be biologically active [26].

Manach et al. [33] suggested that among the most well-absorbed phytophenols in humans are gallic acid and isoflavones, catechins, flavanones, and quercetin glucosides, while the least well-absorbed are proanthocyanidins, the galloylated tea catechins, and the anthocyanins.

The efficiency of absorption of phenolic acids is markedly reduced when they are present in the esterified form rather than in the free forms as it was observed in patients with colonic ablation where caffeic acid was better absorbed than chlorogenic acid [34]. Moreover, it was shown that the occurrence of ferulic acid and antioxidant activity in plasma is increased following intake of food matrix with ferulic acid bound to arabinoxylans compared with results after intake of free ferulic acid, proving that the action of gut microbiota may lead to improved bioavailability [35].

## 6. Interaction of phytophenols with the immune system

### 6.1. Dendritic cells

Dendritic cells (DCs), as essential component of the innate immune system, are the most potent antigen-presenting cells (APCs), allowing the critical decision between immune activation and

http://dx.doi.org/10.5772/66112

tolerance. Aberrant activation of DCs can cause detrimental immune responses; thus, agents effectively modulating their functions are of great clinical value. Several plant phenolic compounds proved their ability to influence DC function, especially in a suppressive way. Because Th1 cells are either functionally immunogenic or provide protection against invading pathogens, the inhibition of DC-mediated Th1 polarization may constitute an associated immunosuppressive mechanism [36].

Similar modes of action were established for daidzein (isoflavone) [37], silibinin (flavonolignan) [38], fisetin (flavonol) [39], apigenin [36], and baicalin (flavone glycoside) [40] in LPS-stimulated DCs, all compounds exhibiting immunosuppressive activity by inhibiting cell maturation and activation. They significantly and dose-dependently inhibit the expression levels of maturation-associated cell surface markers including CD40, costimulatory molecules (CD80, CD86), and major histocompatibility complex class II (I-A(b)) molecule. An impaired induction of the T helper type 1 immune response and a normal cell-mediated immune response induced by the abovementioned compounds were noticed as it was previously found in the case of curcumin [41]. This well-known phytophenol is also a potential therapeutic adjuvant for DC-related acute and chronic diseases being highly efficient at Ag capture, via mannose receptor-mediated endocytosis [41]. The suppressive effect on DCs was also showed for another phenolic compound belonging to ellagitannins class, oenothein B; it was associated with the induction of apoptosis without the activation of caspase-3/7, 8, and 9; and this was supported by the morphological features indicating significant nuclear condensation [42].

### 6.2. Lymphocytes

Stimulation of cell-mediated immune response is one of the most studied effects of plant phenolic compounds, the experiments being carried out both in vitro and in vivo on different species: humans, fish, bovine, etc. In this respect, it was showed that oenothein B, a polyphenol isolated from *Epilobium angustifolium* and other plant sources, is known to activate myeloid cells and stimulate innate lymphocytes, including bovine and human γδ T cells and NK cells, resulting in either increased CD25 or CD69 expression [43]. Moreover, it enhances IFNγ production by both bovine and human NK cells and T cells [44]. Low concentrations of dihydroquercetin (0.025 and 0.0125%) as food supplements are able to increase the immune status—high phagocytic and respiratory bust activities of gilthead sea bream [45].

Stimulation of both humoral and cell-mediated seroresponse was observed (increases of the antibody titers, lymphocyte, and macrophage cells) also in chicks, after administration of an 80% aqueous methanol extract from the leaves of *Jatropha curcas* L. (Euphorbiaceae) and a biflavone di-C-glucoside, 6,6″-di-C-beta-D-glucopyranoside-methylene-(8,8″)-biapigenin) (0.25 mg/kg body wt) [46].

In healthy well-nourished humans, it was showed that consumption within the usual daily intake range of orange juice and its major polyphenol hesperidin (daily 500 mL of orange juice or an isocaloric control beverage with hesperidin (292 mg in a capsule) for 3 weeks) do not induce immunomodulation of cell immune function [47].

Differences between cell-mediated immune response modulations of different compounds belonging to the same class were found in the case of isoflavones.

Daidzein potentiates proliferation of mixed splenocyte cultures activated with ConA or LPS and the secretion of interleukins 2 and 3, while genistein have no influence, although a significant cooperation between these compounds may occur [48]. Contradictory findings regarding genistein (25, 250, 1250 ppm) were presented by Guo et al. [49], which showed that exposure to genistein increases the number of splenic B cells (L), macrophages (L and M), T cells (H), T helper cells (L and H), and cytotoxic T cells (M and H). It was suggested that genistein may modulate the immune system by functioning as either an estrogen agonist or antagonist. The differential effects of genistein on thymocytes in F(1) male and female mice indicate that genistein immunomodulation might be related to its effect on thymus [50].

*6.2.1. B cells*

Several studies show that epigallocatechin gallate (EGCG) enhances the mitogenic activity of B lymphocytes but not T lymphocytes. Gallic acid and tannic acid induced some enhancement, but rutin, pyrogallol, and caffeine did not, indicating that the galloyl group on EGCG was responsible for enhancement [51].

Cumella et al. [52] found that quercetin, but not taxifolin (dihydroquercetin), inhibited mitogen-stimulated immunoglobulin secretion of IgG, IgM, and IgA isotypes in vitro with an IC50 of approximately 30 mM for each isotype.

*6.2.2. T cells*

These cells express TCR on their surface to recognize specific antigens processed by APCs, such as dendritic cells, macrophages, and fibroblasts. Activated T cells differentiate into either cytotoxic T cells (CD8+ cells) or Th cells (CD4+). Cytotoxic T cells participate in the destruction of infected cells by secreting perforin, granzyme, and granulysin. Th cells have no direct killing activity in the infected cells but direct other immune cells to act against pathogen-infected cells, mainly by secreting several cytokines. After infection with a certain pathogen, the immune system must select the best defense mechanism, which involves the differentiation of Th cells into Th1 (to promote the bactericidal activities of macrophages) and Th2 cells (to activate or recruit IgE-producing B cells, mast cells, and eosinophils).

*Th balance*

Intake of representative polyphenols (flavones, flavone-3-ols, catechins, antocyanidins, flavanones, procyanidins, and resveratrol) can improve a skewed Th1/Th2 balance and suppress antigen-specific IgE antibody formation [53]. This was suggested as one mechanism of action of quercetin contributing to its anti-inflammatory and immunomodulating properties having potential of being utilized in several types of allergic reactions. Quercetin is able to inhibit IL-6 and IL-8 better than cromolyn (antiallergic drug disodium cromoglycate) [54], and it ameliorates experimental autoimmune encephalomyelitis, which is associated with Th1-mediated immune responses [55].

A preventive effect on IgE synthesis mediated by Th2 cells was suggested for cocoa. On the other hand, cocoa intake modifies the functionality of gut-associated lymphoid tissue by means of modulating IgA secretion and intestinal microbiota [56].

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In allergic diseases, besides the influence on Th2 activation, regulatory T cells represent another possible target for polyphenols activity [57].

Jaceosidin, a flavone isolated from *Artemisia vestita*, exerts an immunosuppressive effect both in vitro and in vivo through inhibiting T-cell proliferation and activation, which is closely associated with its potent downregulation of the IFN-γ/STAT1/T-bet signaling pathway [58]. Naringenin also alleviates symptoms of contact hypersensitivity by its inhibitory effects on the activation and proliferation of T cells. In vitro, naringenin reduces CD69 (the protein level) and cytokines such as IL-2, TNF-alpha, and IFNγ (the mRNA level) expressions, which highly expressed by activated T cells and induces T-cell apoptosis by upregulation of Bax, Bad, PARP, cleaved caspase-3 and downregulation of phosphorylated Akt, Bcl-2 [59].

Kawamoto et al. showed that 6-gingerol suppresses the expression of Th1 cytokines even in strong Th1-polarizing conditions in vitro and also the expression of Th2 cytokines due not to enhancement of Th1 cytokine production but to inhibition of the general pathway for cytokine expression. Another phenolic phytocompounds that contribute to Th1 polarization of the immune response are procyanidin C1 [60] and proanthocyanidin 1 [39].

An immune shift from Th1 to Th2 is suggested for tea polyphenols taking into consideration increased serum concentrations of anti-inflammatory cytokine, such as IL-4. A T lymphocyte transformation test (LTT) demonstrated that dietary tea polyphenols promote the proliferation and activation of T lymphocytes, reflected by elevation of CD4+/CD8+ ratio, inhibition of pro-inflammatory IL-1, and IFNγ expression caused by oxidative stress [61]. Also, umbelliprenin (UMB) and methyl galbanate (MG), terpenoid coumarins isolated from *Ferula szowitsiana*, reduced remarkably PHA-induced splenocyte proliferation and both preferentially induced T(H)2 IL-4 and suppressed T(H)1 IFNγ secretion [62]. Auraptene, a citrus fruit-derived coumarin, has been reported to exert valuable pharmacological properties, including suppression of cell cycle progression, which contributes to inhibiting T-cell proliferation and cell division. Administration of auraptene decreases the CD3/CD28-activated T lymphocyte secreting T helper (Th)1 cytokines at lower levels (10 and 20 μM), and it could decrease Th2 cytokine IL-4 at a higher level (40 μM) [63]. The dose administrated is essential also in the case of curcumin, which at 2.5 μg/ml inhibits ConA, PHA, and PMA-stimulated human spleen lymphocyte proliferation at 77, 23, and 48%, respectively, over controls, reaching 100% inhibition in higher dose (5 μg/ml) [64].

The mechanism of decreasing the activity of effector Th1 cells proposed for cirsilineol (a trimethoxyflavone isolated from *Artemisia vestita*) is the selective inhibition of IFNγ signaling, mediated through downregulating STAT1 activation and T-bet expression in colonic lamina propria CD4(+) T cells. Therefore, it is strongly suggested that cirsilineol might be potentially useful for treating T-cell-mediated human inflammatory bowel diseases [65].

*Treg cells*

Besides the cytotoxic T cells and Th cells mentioned above, there are the regulatory T (Treg) cells, which are critical in maintaining immune tolerance and suppressing autoimmunity.

Green tea and its active ingredient, epigallocatechin-3-gallate (EGCG), have been shown to improve symptoms and reduce the pathology in some animal models of autoimmune diseases. Mice treated with EGCG had significantly increased Treg frequencies and numbers in the spleen and lymph nodes and had inhibited T-cell response [66, 67] and dose-dependently attenuated the disease's severity [68].

### 6.3. Macrophages

Macrophages are the main cells responsible for the innate immunity, and their activation by lipopolysaccharide (LPS) from Gram-negative bacteria or IFNγ from host immune cells is important for controlling infections. Activation of mononuclear cells and increase of the phagocytic response are induced by several phytophenols, mainly via influencing of MAPK and nuclear factor κB (NF-κB) signaling pathways: daidzein at high doses (20 and 40 mg/kg) [69], coumarin (1,2-benzopyrone) [70], procyanidin A1 [39], procyanidin C1 (max dose 62.5 μg/ml) and procyanidin dimer B2 [60], kaempferitrin from *Justicia spicigera* extracts at 25 μM [71], biflavone isolated from 80% aqueous methanol extract of *Jatropha curcas* L-di-C-glucoside,6,6″-di-C-beta-D-glucopyranoside-methylene-(8,8″)-biapigenin (0.25 mg/kg body wt to 1-day-old specific-pathogen-free (SPF) chicks) [46], oenothein B [19], morin hydrate (5, 10, and 15 μM) [72], geraniin, and isocorilagin (up to 12.5 lg/ml) [73].

As macrophages are stimulated to secrete a battery of inflammatory mediators and cytokines, regulation of their activity ensures an appropriate immune response. Inappropriate or prolonged macrophage activation is largely responsible for various inflammatory states. There are also phytophenols that inhibit the secretion of various pro-inflammatory molecules from macrophages or their migration: grape polyphenols [74], cianidol [75], EGCG [76], fisetin [77], quercetin, kaempferol, daidzein, genistein [78], xanthohumol [79], etc.

Orange juice and hesperidin, a flavanone glycoside contained in the juice, showed different immune responses, suggesting that hesperidin displays a suppressive effect on inflammation generated by LPS, while the juice seems to enhance the functions of macrophages associated with antimicrobial activity [80].

### 6.4. Neutrophils

These cells provide rapid response and nonspecific protective effect against invading pathogens, and the exposure of antigen by APCs is not required to activate these cells [38]. Most of the effects exerted by phytophenols on neutrophils are based on inhibition of superoxide anion production: biflavonoids like procyanidin, fukugetin, amentoflavone, and podocarpusflavone isolated from *Garcinia brasiliensis* showed potent inhibitory effects on the oxidative burst of human neutrophils, inhibiting reactive oxygen species (ROS) production by 50% at 1 μmol $L^{-1}$ [81], catechol (1–10 μM) [82], broussochalcone A—a prenylated chalcone [83], and viscolin [84].

The effects of flavonoids on human neutrophils are complex and suggest several sites of action depending upon the flavonoid's subcellular distribution and pathway of stimulation [85].

http://dx.doi.org/10.5772/66112

### 6.5. Modulation of soluble factor secretion

#### *6.5.1. Immunoglobulins*

Humoral immunity is mostly quantified by serum levels of specific immunoglobulins. A stimulatory effect on IgM- and IgG-mediated humoral immune response was observed in the case of green tea, a well-known rich source of polyphenols [86]. Serum IgM and IgG levels are also significantly increased, whereas specific IgA and IgE are not changed after ellagic acid (a natural phenolic compound found in fruits and nuts) treatment [87].

IgG response is increased after treatment with pomegranate extract rich in polyphenols (16.9% gallic acid equivalent (GAE) per day in calves) [88] and red wine (Negroamaro) pre-treatment of lymphomonocytes [57]. Immunoglobulin synthesis is induced also by cianidol [75], its O-methyl-derivative [89] and daidzein [69].

Humoral immunity measured by anticomplement activity showed an increase in inhibition of the complement system after the addition of morin (natural flavonoid that is the primary bioactive constituent of the family Moraceae) hydrate (significant effect at 15 μM concentration) [72].

#### *6.5.2. Interleukins*

##### *6.5.2.1. IL-2*

Catechin, epigallocatechin gallate (EGCG), epicatechin (EC), luteolin, chrysin, quercetin, and galangin increase IL-2 secretion, while EGC, apigenin, and fisetin inhibit the secretion. There was no obvious structure-activity relationship with regard to the chemical composition of the flavonoids and their cell biological effects [90]. Contradictory results were obtained by Xiao et al. [91], which reported the inhibitory action of chrysin on splenic mononuclear cell secretion of interleukin-2, after oral administration of the phytocompound from day 1 to day 16 (50 mg/kg once daily), while, for therapeutic treatment, rats received chrysin from day 7 to day 16 at the same dose once daily).

Inhibitory effects on IL-2 production have also equol (4′,7-isoflavandiol) [14], quercetin by IL-2R alpha-dependent mechanism [55] and curcumin, which inhibits IL-2 synthesis in ConA, PHA, and PMA stimulated SP-L in a concentration-dependent manner with an ED50 measured at 3.5 μg/ml. Exogenous IL-2-stimulated SP-L proliferation is also inhibited by curcumin in a concentration-dependent manner with an ED50 of 2 μg/ml [64]. 8-Methoxypsoralen (140 μM) induces a dose-dependent decrease in IL-2 receptor expression on PHA-stimulated lymphocytes, explaining the mechanism by which this compound impairs lymphocyte function, since IL-2 receptors play a central role in lymphocyte proliferation and immune reactivity [92, 93].

##### *6.5.2.2. IL-12*

IL-12 is the most important factor driving Th 1 immune responses. An interesting dynamic was showed in the case of the orange juice and its main component, hesperidin. In non-LPS-

stimulated macrophages, IL-12 level was increased by orange juice by 143% and hesperidin by 72%. For LPS-stimulated macrophages, the orange juice treatment did not alter IL-12 level, while hesperidin treatment decreased IL-12 level by 29%, suggesting that hesperidin displays a suppressive effect on inflammation generated by LPS [80]. Curcumin exhibits impaired IL-12 expression in DCs [41]; quercetin blocks IL-12-dependent JAK-STAT signaling in Th cells [55]; ellagic acid reduces IL-12 production both ex vivo and in vivo treatment [87]; chrysin [91], licochalcone E [94], xanthohumol, shows the strongest inhibitory effect on IL-12 production in LPS-stimulated xanthohumol 4′-O-beta-D-glucopyranoside (XNG) being less effective, followed by isoxanthohumol and 8-prenylnaringenin while (2S)-5-methoxy-8-prenylnaringenin 7-O-beta-D-glucopyranoside have no effect [95]. macrophages, xanthohumol 4′-O-beta-D-glucopyranoside (XNG) being less effective, followed by isoxanthohumol and 8-prenylnaringenin, while (2S)-5-methoxy-8-prenylnaringenin 7-O-beta-D-glucopyranoside [94] and licochalcone E have no effect [95].

*6.5.2.3. IL-1β*

Inhibitory effect on IL-1b secretion has apigenin [96] and flavokawain A in the LPS-stimulated cells [97]; curcumin in DCs [41]; curculigoside in B16F10-induced metastatic tumor progression in experimental animals [98]; ellagic acid in ex vivo and in vivo experiments [87]; chrysin, in splenic mononuclear cells [91]; equol (4′,7-isoflavandiol) [14]; and kurarinone and kuraridin in RAW264.7 macrophages [99]. There are also phenolic phytocompounds, which promote pro-inflammatory IL-1b secretion: oenothein B [43], polyphenols contained in red wine (Negroamaro) [57], and 1% dietary EGCG [67].

*6.5.2.4. IL-4*

Quercetin [54], 6-gingerol [100], and ellagic acid [87] suppress interleukin IL-4 production, one of the key cytokines secreted by Th2 cells.

*6.5.2.5. IL-6*

Suppression of LPS-induced expression of pro-inflammatory cytokine IL-6 is induced by licorice flavonoids [101]; 1% dietary EGCG [67]; flavokawain A [97]; curcumin [41]; quercetin attenuates TLR7-induced expression, effect of mediated by HO-1 [102]; curculigoside [98]; syringic acid or vanillic acid [103]; licochalcone A [104]; chrysin [91]; apigenin, through modulating multiple intracellular signaling pathways in macrophages and prevents LPS-induced IL-6 production by reducing the mRNA stability via inhibiting ERK1/2 activation [96]; and luteolin at transcriptional level [13].

*6.5.2.6. IL-17*

It is well known that IL-17 is an essential factor involved in autoimmune diseases, and some synthetic inhibitors are already in clinical testing. As regards phytophenols, grape seed proanthocyanidin extract (GSPE) shows promising results, attenuating clinical symptoms in a model of collagen-induced arthritis in mice [105].

*6.5.3. TNF-α*

Based on the inhibitory effect on TNF-α secreted by LPS-stimulated cells, flavonoids were classified in four groups: strong (flavones, flavonols, chalcones), moderate (flavanones, naringenin, antocyanidin, pelargonidin), weak (genistein), and inactive (eriodictyol) [106]. Several phenolic phytocompounds successfully suppress the expression of pro-inflammatory cytokines such as TNF-α; flavokawain A [97]; curcumin [41]; quercetin [102]; curculigoside [98]; ellagic acid [87]; syringic and vanillic acids [103]; apigenin [96]; chrysin [91]; kurarinone and kuraridin [99]; luteolin [13]; equol (4′,7-isoflavandiol) [14]; and cardamonin, a chalcone derivative isolated from *Artemisia absinthium* L. [107]. An enhancement of TNF-α production is noticed for 1% dietary EGCG, while no effect was exhibited by lower concentrations of compound (0.15–0.3%) [67].

*6.5.4. IFNγ*

Chrysin inhibited the splenic mononuclear cell secretion of IFNγ [91]; quercetin is suggested to exert T-bet-dependent IFNγ suppression [55], ellagic acid [87], syringic and vanillic acid [103], equol (4′,7-isoflavandiol) [14]. On the other hand, other phytophenols enhance IFNγ level: curculigoside [98], oenothein B, by both bovine and human NK cells and T cells, alone and in combination with IL-18 [44], and a response not observed with other commonly studied polyphenols [43].

**6.6. Influence on transcription factors**

Nuclear factor κB (NF-κB) plays an important role in inflammatory processes, in autoimmune response, apoptosis, and cell proliferation, by regulating the genes involved in these processes. This factor is activated mainly under conditions of oxidative stress, under the action of various pathogenic stimuli (viruses and bacteria but also inflammatory cytokines). Because of its effects on vital biological processes, modulation of its activation pathway is of great therapeutic potential.

Curcumin inhibits PMA-stimulated NF-κB activation in lymphocytes by 24, 38, and 73%, respectively, at final concentrations of 2.5, 5, and 10 μg/ml, respectively [64], and fisetin also inhibits LPS-induced nuclear factor κB activation and JNK/Jun phosphorylation [77]. In LPS-stimulated macrophages, activation of NF-κB that is inhibited was reported for caffeic acid phenethyl ester [108]; licochalcone E, a constituent of licorice [94]; luteolin [13]; kurarinone and kuraridin [99]; astragalin (kaempferol-3-O-glucoside) [109]; naringin [110]; nodakenin, a coumarin isolated from the roots of *Angelica gigas* [111]; quercetin (100 ppm) [112]; carnosol (20 μM) [113]; and apigenin [36]. The main mechanism of inhibition consists in the degradation of inhibitor κB and nuclear translocation of NF-κB p65 subunit. These events are strongly linked with modulation of reactive oxygen species generation. A correlation between antioxidant and immune function was presented for equol (4′,7-isoflavandiol), an isoflavandiol metabolized from daidzein, which at an optimal concentration of 40 μmol/L exerts mainly antioxidant effects in chicken macrophages by increasing T-SOD, GSH levels but collateral immune enhancement by increasing expression of TLR4 and genes encoding cytokines [14].

It was found that both phenolic acids and other phenolic compounds found in free form in cereal grains are significant modulators of NF-κB activity, but only their combinatorial action

gives the desirable effect. Although ferulic and p-coumaric acids alone are effective modulators of NF-κB activity, a mixture of ferulic, caffeic, p-coumaric, and sinapic acids in low concentrations has significant synergistic, enhanced, and additive effects on NF-κB activity [35].

## 7. Conclusions

Considerable attention is currently focused on the development of natural medicines with less or no side effects, maximum efficacy, and low cost. Plant phenolic compounds proved to be competitive candidates for therapy in several disorders, and some of them undergone clinical trials (e.g., quercetin, curcumin). Modulation of immune system is a challenge, due to complex mechanisms involved and to route of administration, knowing that most of the plant-derived compounds are given orally in the form of medicines or even as functional foods.

Several aspects resulted from reviewing the literature: plant extracts are not always well characterized or standardized, making difficult to assign the immunomodulatory effect to a single compound; high concentrations of phenolic compounds are used for in vitro studies, and substances that have proven effective on laboratory scale are often ineffective in clinical trials, often due to bioavailability aspects (as these compounds are an important part of the human diet). Up to now, few human trials were carried out, most of them being focused on proving only the antioxidant or anti-inflammatory effect. This review reveals that phenolic compounds are a rich source of valuable potential therapeutic agents for immune system modulation, but further work needs to be carried out in order to establish therapeutical doses, precise mechanism of action, and optimal ways of administration.

## Acknowledgements

The work was supported by a grant of *UEFISCDI*, Romania, PN-II-PT-PCCA-2 no. 134/2012.

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