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**REVIEW ARTICLE**

# Plant Phenolics as Pathogen-Carrier Immunogenicity Modulator Haptens

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**Abstract:** ***Background*:** Pathogens use multiple mechanisms to disrupt cell functioning in their host and allow pathogenesis. These mechanisms involve communication between the pathogen and the host cell through protein-protein interactions.

***Methods*:** Protein-protein interactions chains referred to as signal transduction pathways are the processes by which a chemical or physical signal transmits through a cell as series of molecular events so the pathogen needs to intercept these molecular pathways at few positions to induce pathogenesis such as pathogen viability, infection or hypersensitivity.

***Results*:** The pathogen nodes of interception are not necessarily the most immunogenic; so that novel immunogenicity-improvement strategies need to be developed thought a chemical conjugation of the pathogen-carrier nodes to develop an efficient immune response in order to block pathogenesis. On the other hand, if pathogen-carriers are immunogens; toleration ought to be induced by this conjugation avoiding hypersensitivity. Thus, this paper addresses the biological plausibility of plant-phenolics as pathogen-carrier immunogenicity modulator haptens.

***Conclusion*:** The plant-phenolic compounds have in their structure functional groups such as hydroxyl, carbonyl, carboxyl, ester, or ether, capable of reacting with the amino or carbonyl groups of the amino acids of a pathogen-carrier to form conjugates. Besides, the varied carbon structures these phenolic compounds have; it is possible to alter the pathogen-carrier related factors that determine the immunogenicity: 1) Structural complexity, 2) Molecular size, 3) Structural heterogeneity, 4) Accessibility to antigenic determinants or epitopes, 5) Optical configuration, 6) Physical state, or 7) Molecular rigidity.

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## 1. INTRODUCTION

The immune system is responsible for protecting the body against foreign substances. It involves the cooperation and interaction of different types of cells and proteins in various organs and tissues of the body [1]. To study the immune system, it is useful to divide it into two types of responses: 1) Innate and 2) Adaptive. The innate immune response represents the first protective barrier of the organism that also functions as a regulator of the adaptive immune response. If the mechanisms of the innate immune response fail, the adaptive immune response takes action whose objectives are to develop a specific humoral response that consists of the production of soluble proteins known as antibodies capable of specifically recognizing the foreign agent with the consequent neutralization or elimination thereof [2]. An immunization scheme consisting of the programmed administration of a substance called antigen, can induce a protective mechanism with the objective of developing an adaptive immune response against the antigen.

This process takes advantage of the ability of the adaptive immune response to develop memory towards the antigen [3, 4]. The implications of this immunization in a health setting, are for instance either avoiding pathogen infection

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through its neutralization by specific antibodies (consequence of immune activation by a pathogen antigen very immunogenic); or avoiding a deleterious hypersensitivity through anergy induction (consequence of immune inactivation by a pathogen antigen-conjugated with a small molecule that reduces its immunogenicity). The ability to induce a specific humoral or cellular immune response (immunogenicity) of the antigen is an intrinsic characteristic of the same antigen dependent on several factors as shown in Table **1**. Thus, not all antigens are capable of being administered in the same concentration, periodicity or way, to produce a detectable immune response [5]. Furthermore, depending on the type of antigen used, there are advantages and disadvantages such as a risk of pathogenicity, low or high immunogenicity, or a response against epitopes without relevance [6]. It is therefore necessary to develop specific immunization schemes for different antigens, as well as to consider the simultaneous supply of adjuvants to reinforce or inhibit the immune response against the antigen [2]. Thus, this paper addresses the biological plausibility of antigen conjugates to inhibit pathogenesis, for instance, pathogen infection or hypersensitivity by means of said conjugates that forms to immunogen or toleragen.

The importance of using natural compounds, as pathogen-carrier immunogenicity modulator haptens, relays on their key mechanisms of immune response recently reported by researchers; *via* interaction with Mitogen-Activated Protein Kinases (MAPKs), Toll-Like Receptors (TLR), Nuclear Factor-κB (NF-κB), and iNOS (inducible nitric oxide synthase) or COX (cyclooxygenase)-2 gene expression [7-10]. Consequently, some plant phenolics may induce, polarize or suppress immune responses. Such as innate or adaptive; towards Th1 or Th2; or induce anergy; respectively.

## 2. OVERVIEW ON THE IMMUNE RESPONSE

As previously stated to study the immune system, it is useful to divide it into two types of responses: 1) Innate and 2) Adaptive; among the types of cells found in the innate immune response are epithelial cells, phagocytes, dendritic cells and Natural Killer cells (NK) in addition to proteins like those of the complement system. In the case of the adaptive immune response, cells such as B-lymphocytes and T-lymphocytes in addition to proteins such as immunoglobulin or antibodies [1].

The innate immune response represents the first barrier of protection of the organism. For example, after acute exposure to a foreign substance, epithelial barriers block the entry of the foreign substance; however, if said first line of defense fails the foreign substance can be contained through phagocytosis by phagocytes or dendritic cells. Alternatively, in the case that the foreign agent is a pathogen, the natural killer cells or the proteins of the complement system take action, consequently leading to the elimination of the foreign agent [11]. Therefore, the response time to contain this potentially dangerous situation must be fast in terms of minutes or hours. On the other hand, if the mechanisms of containment of the innate immune response fail in the containment process, the adaptive immune response takes action whose containment mechanisms are highly specific at the expense of the response time that usually takes days [12]. For example, after a chronic exposure to the foreign agent or in case of evasion of the innate immune response, professional antigen-presenting cells ingest and process the antigen. Subsequently a process called antigen presentation, selection and clonal expansion occurs, which involves the active participation of cellular differentiation of both B-lymphocytes and T-lymphocytes, which recognize small fragments of the foreign antigen as shown in Fig. (**1**) [1, 11, 12]. Therefore, it is possible for the adaptive immune response to develop a specific humoral response. This consists of the production of soluble proteins (antibodies) capable of specifically recognizing the foreign agent with the consequent neutralization or elimination thereof [2]. Even though, these antibodies when produced in high quantities might lead to pathogenesis such as hypersensitivity.

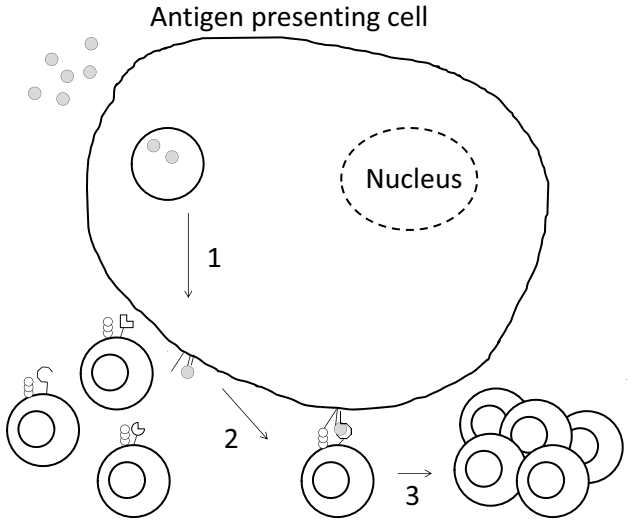



**Fig. (1).** Differentiation of the adaptive immune response. 1 An antigen presenting cell ingests and processes the foreign agent (grey dot) with the consequent antigen presentation of fragments thereof in membrane receptors; 2 Subsequently, a process called selection occurs where a lymphocyte capable of recognizing the antigen will be selected and; 3 finally, clonal expansion occurs in which the size of the original clone increases, so that it is possible for the adaptive immune response to develop a specific humoral response that consists of the production of specific antibodies against the antigen.

## 3. OVERVIEW ON THE ADAPTIVE IMMUNE RESPONSE AND IMMUNOGENICITY

To describe the adaptive immune response also known as humoral, it is necessary to define the following concepts:

1) Antigen: It is an own or foreign substance, of high molecular weight (usually greater than 10,000 Da) that in the organism may induce an immune response like the adaptive immune response; that is, it leads to the formation of antibodies [13].

2) Antibody: It is a protein, also known as immunoglobulin, synthesized by plasma cells. It is soluble in the blood and other body tissues of vertebrates whose purpose is to identify and neutralize poten-

tially harmful agents. It is important to state that an exacerbate antibody production might lead to pathogenesis such as hypersensitivity [1, 11].

3) Hapten: It is a chemical substance of small molecular weight (usually less than 10,000 Da), which does not itself induce an immune response such as the formation of antibodies; but, by binding to a carrier protein it stimulates an immune response like the adaptive immune response [13, 14].

4) Immunogen: It is an antigen capable of generating an innate or adaptive immune response. Not all antigens are immunogenic [15].

5) Immunogenicity: An antigen can induce an innate or adaptive immune response [5, 15].

6) Antigenic determinant: It is the part of the antigen (epitope), which induces the immune response [12].

7) Tolerogen: It is an antigen capable of suppressing immune response, or producing immune tolerance. In contrast to immunogen that induces an immune response, a tolerogen evokes immune tolerance.

Next, Table **1** shows the main factors that determine the immunogenicity of an antigen.

**Table 1. Factors that determine the immunogenicity of an antigen.**

| | |
|---|---|
| 1 | Structural complexity of the antigen. |
| 2 | Molecular size between 6-10 kDa. |
| 3 | Structural heterogeneity. |
| 4 | Accessibility to antigenic determinants or epitopes. |
| 5 | Optical configuration (The levorotatory configuration is more immunogenic than the dextrorotatory configuration). |
| 6 | Physical state (Solid molecules are more immunogenic than soluble molecules). |
| 7 | Molecular rigidity (Aromatic amino acids provide rigidity to proteins causing immunogenicity). |

A specific adaptive immune response refers to the production of antibodies against an antigen. Antibodies mediate the specificity of this type of response. After first exposure to the antigen; the organism produces a primary antibody response during a period of about a week, first antigenic processing and presentation occur, then clonal selection, and finally clonal expansion takes place leading to the production of antibodies mainly of Ig M isotype. Once neutralized the antigen, the circulating concentration of antibodies decreases but memory plasma cells persist. Given a second antigen exposure, the adaptive immune response is faster and of greater intensity with the consequent production of antibodies more related to the antigen mainly of Ig G isotype. After antigen neutralization, the circulating concentration of antibodies decreases since antigen stimuli vanishes by the antibody neutralization but memory plasma cells persist because of the immune system ontogeny [16, 17].

## 4. OVERVIEW ON THE REASONS FOR PATHOGEN-CARRIER-HAPTEN CONJUGATION

Pathogens use multiple mechanisms to disrupt the cellular machinery of the host and allow pathogenesis (*e.g.* pathogen infection or hypersensitivity). These mechanisms include the alteration of endocytosis, exocytosis, replication, transcription, expression and degradation of proteins; as well as antigen processing, presentation, selection and clonal expansion. These mechanisms involve communication between the pathogen and the host cell through protein-protein interactions between the nodes of the pathogen and the host *i.e.* the proteins of the pathogen and the cellular proteins of the host [18]. The communication through protein-protein interactions is the universal cellular language; chains of interactions referred to as signal transduction pathways are the processes by which a chemical or physical signal transmits through a cell as series of molecular events, most commonly protein phosphorylation catalyzed by protein kinases, which ultimately results in cellular response to the environment. Signal-transduction pathways follow a broadly similar course that can be viewed as a molecular circuit [19]; so that, the pathogen simply needs to intercept these molecular circuits at few positions to induce pathogenesis such as allowing pathogen viability, infection or hypersensitivity as shown in Fig. (**2**). Pathogens, both bacterial and viral, tend to interact with hubs (proteins with many interacting partners) and bottlenecks (proteins that are central to many paths in the network) in the human signal-transduction pathway network; perhaps because these proteins control critical processes in the host cell [20]. The pathogen points of interception (pathogen nodes) are not necessarily the most immunogenic; so that, novel immunogenicity-improvement strategies need to be developed through a chemical modification of the pathogen nodes (antigen) to develop an efficient immune response [21] to block pathogen viability or pathogen infection. On the other hand, in the case that pathogen nodes are immunogens; toleration might be induced by this protein modification avoiding hypersensitivity.

If a non-immunogenic pathogen node (carrier) conjugates with a small molecule to induce immunogenicity toward the native pathogen protein, through immunization schemes, as shown in Fig. (**3**) to form an immunogen; the small synthetic molecule mimics some critical epitopic structures to provide certain biochemical characteristics to create an immune response to the larger native pathogen protein *i.e.* the carrier [22]. In this case, the synthetic small molecule due to its molecular size has low immunogenicity on its own (it is a hapten) minimizing the potential for antibody production against it. In this example, the T-lymphocyte will have specific binding domains on the native pathogen protein but will not recognize the hapten alone. In a kind of synergism, the B and T cells cooperate to induce a native pathogen protein response after such an immune response has taken place. Then, when challenged the host only with the carrier, it will respond by producing carrier-specific antibodies from memory cells formed after the initial immunization. In this context, the hapten biochemical characteristics should provide molecular rigidity and structural complexity such as those provided by aromatic amino acids in order to improve immunogenicity; so that, plant-phenolics as pathogen-carrier immunogenicity improvement haptens may represent an ideal candidate to

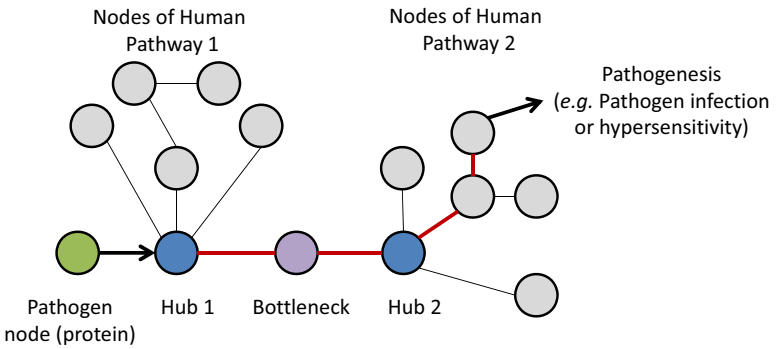



**Fig. (2).** Pathogen molecular interception (red) in the human signal-transduction pathway network leading to pathogenesis (*e.g.* Pathogen infection or hypersensitivity). Pathogens, both bacterial and viral, tend to interact with hubs (proteins with many interacting partners) and bottlenecks (proteins that are central to many paths in the network) in the human signal-transduction pathway network; perhaps because these proteins control critical processes in the host cell.

this goal [23-25]. On the other hand, plant-phenolics may disrupt immunogenicity when the carrier causes hypersensitivity. This immunogenic pathogen node (carrier) conjugates with a small molecule to either induce anergy or reduce immunogenicity toward the native pathogen protein through immunization schemes as shown in Fig. (**3**) to form a toleragen. Although plant-based chemical compounds pose different biological activities [26, 27], some plant phenolics exhibit anergic properties itself by means of not fully understood mechanisms [28-30]. However, the mechanisms of action to reduce the immunogenicity of the carrier bases in the structural modification thereof [31].

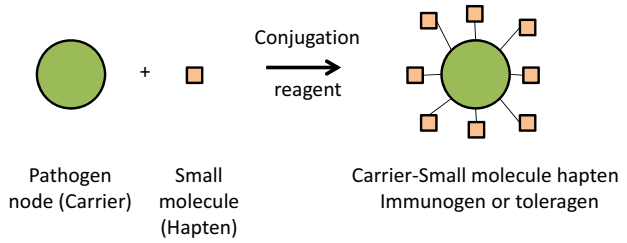



**Fig. (3).** A pathogen node (carrier) conjugates with a small molecule either to induce immunogenicity or to avoid immunogenicity toward the native pathogen protein, in order to disrupt pathogenesis (*e.g.* Pathogen infection or hypersensitivity).

## 5. OVERVIEW ON PLANT-PHENOLICS AS PATHOGEN-CARRIER IMMUNOGENICITY MODULATOR HAPTENS

Plant metabolites classify in two types: 1) Primary metabolites: As carbohydrates, lipids, proteins and nucleic acids, essential molecules for the proper cellular function; and, 2) Secondary metabolites: All those compounds that are dispensable for the proper cellular function; however, these secondary metabolites provide advantages for survival in the environment. For example, they may have insecticidal properties against pests or attract pollinating insects [2].

Among the most abundant secondary metabolites of plants are the phenolics, which characterize by containing in their structure the base compounds: Gallic acid, Cinnamic acid or Catechin. The synthesis of these phenolics based compounds depends mainly on the pathways of Shikimic acid and the Phenylpropanoids outlined in Fig. (**4**) [23-25, 28-32].

The classification of plant-phenolics bases on the structural similarity they share with different organic molecules considered base units; a group of compounds classifies as hydroxybenzoic acids because in their structure it is possible to find a structure similar to that of benzoic acid. Another group of compounds classifies as stilbenes because in their structure it is possible to find a similarity to stilbene. And so on as shown in Table **2** [2].

These phenolic compounds have in their structure functional groups such as those shown in Fig. (**5**) (hydroxyl, carbonyl, carboxyl, ester, or ether) capable of reacting with the amino or carbonyl groups of the amino acids of a protein antigen (pathogen-carrier) to form conjugates. On the other hand, the varied carbon structure (linked to the functional groups mentioned above) that these phenolic compounds have; it is possible to alter the factors that determine the immunogenicity of an antigen: 1) Structural complexity, 2) Molecular size, 3) Structural heterogeneity, 4) Accessibility to antigenic determinants or epitopes, 5) Optical configuration, 6) Physical state, or 7) Molecular rigidity.

In a hypothetical example, the following conjugates are possible: antigen-caffeic acid conjugates to improve the immunogenicity of the pathogen-carrier (antigen), antigen-acetophenone derivative conjugates to reduce immunogenicity or induce anergy. An antigen-ferulic acid conjugate to polarize the response toward Th1, an antigen-quercetin conjugate to polarize the response toward Th2, or an antigen-lawsone conjugate to induce an innate response.

## 6. OVERVIEW ON IMMUNIZATIONS WITH CARRIER-PLANT-PHENOLICS CONJUGATES USED IN CURRENT IMMUNE STUDIES

Current immune studies with possible carrier conjugates applications on immunization schemes have reported that

**Table 2. Classes of plant-phenolics, carbon structure and examples.**

| Classes | Carbon Structure | Examples |
|---|---|---|
| Phenolics and benzoquinones. | $C_6$ | Catechol and 1,4-benzoquinone. |
| Hydroxybenzoic acids. | $C_6$-$(C_1)_n$ | Gallic acid and Vanillic acid. |
| Acethophenones and phenylacetic acids. | $C_6$-$C_2$ | Acetophenone. |
| Phenylpropanoids and coumarin derivatives. | $C_6$-$C_3$ | Caffeic acid, Cinnamic acid and Ferulic acid. |
| Napthoquinones. | $C_6$–$C_4$ | Alkannin, Juglone and Lawsone. |
| Xanthones. | $C_6$–$C_1$–$C_6$ | Mangostin. |
| Stilbenes and anthraquinones. | $C_6$–$C_2$–$C_6$ | Resveratrol and Aloe emodin. |
| Flavonoids. | $C_6$–$C_3$–$C_6$ | Quercetin. |
| Lignans. | $(C_6$–$C_3)_2$ | Enterodiol, Niranthin and Arctigenin. |

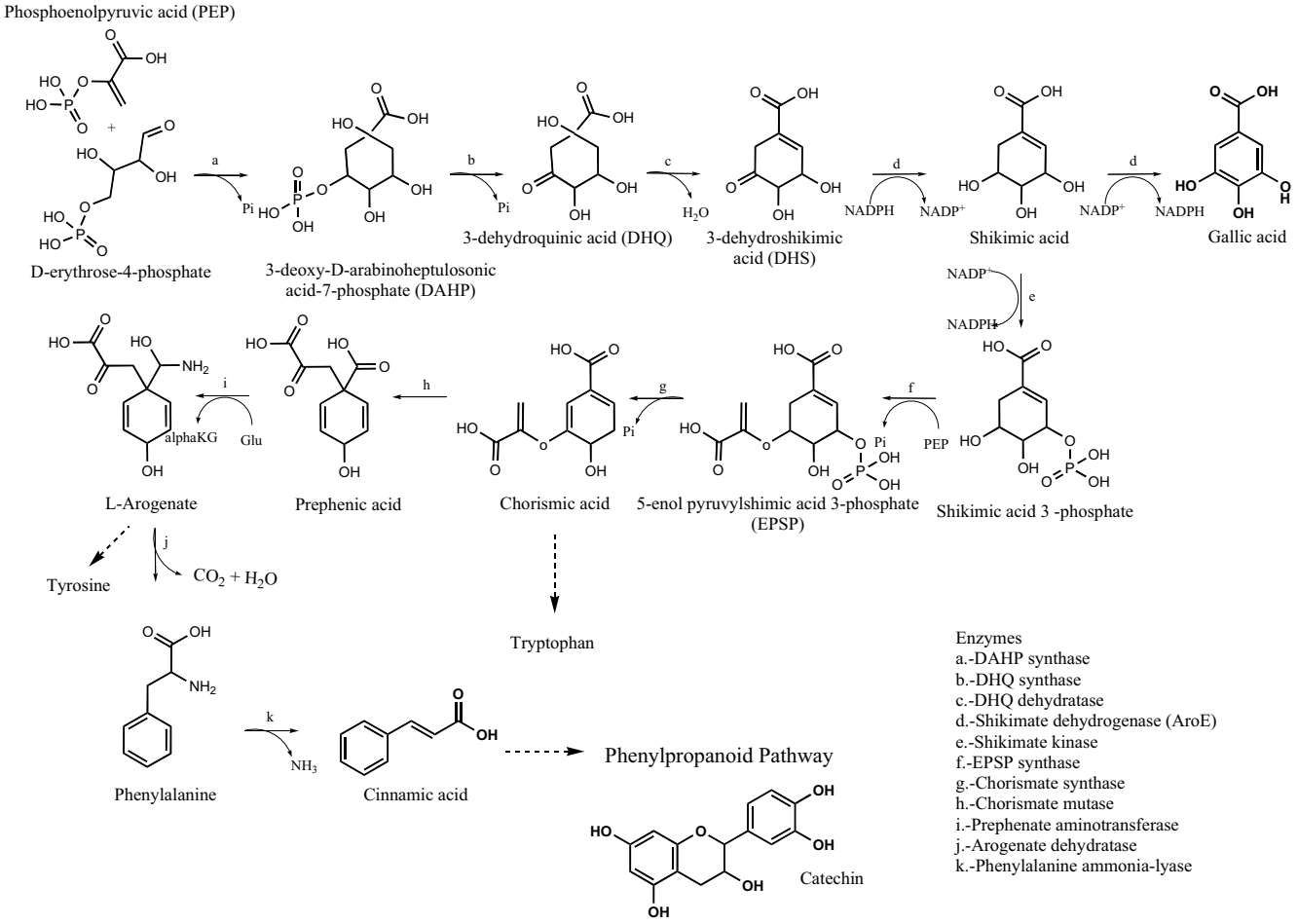



**Fig. (4).** Main chemical reactions of the Shikimic acid and Phenylpropanoid pathways, which give rise to the plant-phenolics base compounds.

phenolics and benzoquinones derivatives (4-(Hydroxymethyl)-catechol and benzoquinone) modulate immune pathways such as PI3K/Akt/NF-κB, and STAT1 signaling; respectively [33, 34]. Hydroxybenzoic acids such as gallic and vanillic acids promote differentiation of regulatory T-cells and enhance immune responses in murine models [35, 36]. Acethophenon derivatives exert anti-allergic properties by preserving the morphology of Mast Cells (MC) and attenuating the release of pro-inflammatory mediators in both cellular and animal model of IgE-mediated MC activation whereby inhibition of β-hexosaminidase and TNF-α were reported [37].

Studies involving phenylpropanoids and coumarin derivatives report caffeic acid as a safe mucosal adjuvant that augments antigen-specific mucosal and systemic immune responses in mice [38]; cinnamic acid enhances the immunity and upregulates TLR-4 expression by human monocytes

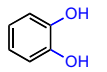
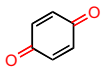
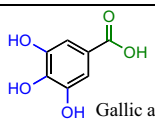
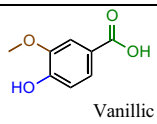
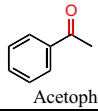
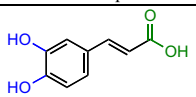
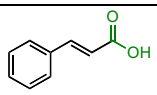
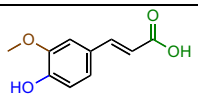
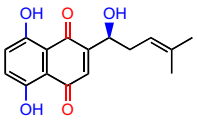
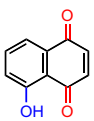
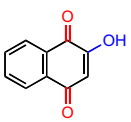
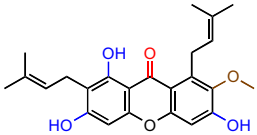
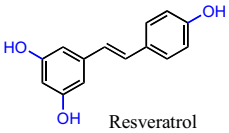
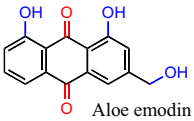
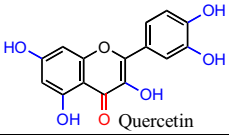
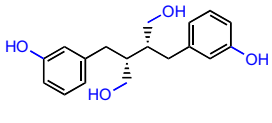
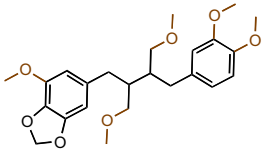
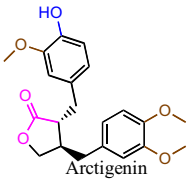
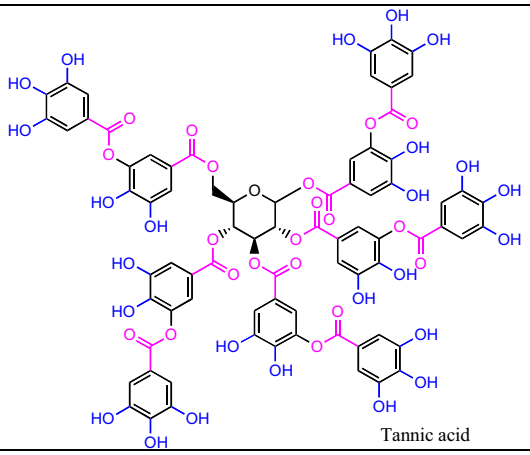
| | |
|---|---|
| Phenolics and benzoquinones |  Catechol  1,4-benzoquinone |
| Hydroxybenzoic acids |  Gallic acid  Vanillic acid |
| Acethophenones and phenylacetic acids |  Acetophenone |
| Phenylpropanoids and coumarin derivatives |  Caffeic acid  Cinnamic acid  Ferulic acid |
| Napthoquinones |  Alkannin  Juglone  Lawsone |
| Xanthones |  Mangostin |
| Stilbenes and anthraquinones |  Resveratrol  Aloe emodin |
| Flavonoids |  Quercetin |
| Lignans |  Enterodiol  Niranthin  Arctigenin |
| Tannins |  Tannic acid |

**Fig. (5).** Carbon structure and functional groups of plant-phenolics. Identification of the potential functional groups for conjugation, according to chemical reactivity in plant-phenolics used in current immune studies: Hydroxyl (Blue), Carbonyl (Red), Carboxyl (Green), Ester (Magenta), and Ether (Brown).

[39, 40]. Moreover, ferulic acid induces Th1 responses by modulating the function of dendritic cells and ameliorates Th2-mediated allergic airway inflammation in mice [41, 42]. Napthoquinones such as alkannin, juglone and lawsone have immunomodulatory effects and enhance antitumor immunity by modulating the innate response of human phagocytes [43-46].

Xanthones such as α-Mangostin (α-M) in murine peritoneal macrophages induces autophagy and then inhibits LPS-stimulated NLRP3 inflammasome activation, as well as interleukin-1β (IL-1β) production [47]. Stilbenes and anthraquinones such as aloe emodin and resveratrol exhibit enhancement of innate immune response, disease resistance, pro and/or anti-inflammatory cytokine gene transcription [48-49]. Resveratrol is one of the most studied phenolics and some reviews indicate that resveratrol exhibits enhancement of innate immune response, disease resistance, pro and/or anti-inflamatory cytokine gene transcription levels [49-51].

Flavonoids such as quercetin besides resveratrol is another of the most studied plant-phenolics that shows several activities on inflammation and immunity as well as adjuvant activity by enhancing Th2 immune response in ovalbumin immunized mice [52, 53]. Lignans such as enterodiol modulates the immune response by acting on nuclear factor-κB (NF-κB) signaling, niranthin favors a Th1 immune response in mice and arctigenin may regulate immune responses in activated macrophages and lymphocytes including TNF-α and Nitric Oxide (NO) production and lymphocyte proliferation [54-56].

Finally, tannins immune response analysis shows that, compared with the control group, non-infected Tannic Acid (TA)-treated mice displayed increased levels of interferon-γ (IFN-γ), Monocyte Chemoattractant Protein-1 (MCP-1), and interleukin-10 at 3 days post-infection and a further increase in IFN-γ and MCP-1 at 14 days post-infection; in a mice model of *Brucella* infection [57].

## CONCLUSION

Plant phenolic compounds are molecules with potential use as pathogen-carrier immunogenicity modulator haptens because these compounds have reported key mechanisms to induce, polarize or suppress immune responses when combined with an antigen. For instance, these compounds might induce the innate or adaptive immune response; polarize the immune response towards Th1 or Th2; or, induce anergy. Although not tested as immunomodulators alone without an antigen. Perhaps due to the small molecular size that classifies them as haptens, the functional groups they have in their structure, such as hydroxyl, carbonyl, carboxyl, ester, or ether, permits chemical conjugation with the amino or carbonyl groups of the amino acids of a pathogen-carrier to form conjugates. Consequently, due to the varied carbon structures, this hapten-residue may activate the key mechanisms of immune response previously reported; or, participate in the alteration of the pathogen-carrier factors that determine the immunogenicity such as: 1) Structural complexity, 2) Molecular size, 3) Structural heterogeneity, 4) Accessibility to antigenic determinants or epitopes, 5) Optical configuration, 6) Physical state, or 7) Molecular rigidity.

## LIMITATIONS

This manuscript is a review of current studies that present combinations of plant phenolics and antigens with effects on immune response. In addition, it focuses on the immune response and chemical plausibility for said hapten-antigen chemical conjugation providing a hypothesis to immune improvement response against the antigen. The chemical formula of the molecules presented in this manuscript provides graphical information to sustain biochemical conjugation. However, this manuscript concludes neither the pharmaceutical effect those plant-phenolics molecules may have; nor evidence to corroborate that all plant phenolics when joined to other molecules will modify the immune response.

## CONSENT FOR PUBLICATION

Not applicable.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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