Received: 5 March 2022 | Revised: 20 August 2022 | Accepted: 3 September 2022

DOI: 10.1002/ptr.7624

REVIEW

WILEY

# Immunostimulant plant proteins: Potential candidates as vaccine adjuvants

Jilan A. Nazeam[1] | Abdel Nasser B. Singab[2]

[1]Pharmacognosy Department, Faculty of Pharmacy, October 6 University, Giza, Egypt

[2]Pharmacognosy Department, Faculty of Pharmacy, Ain-Shams University, Cairo, Egypt

**Correspondence**
Jilan A. Nazeam, Pharmacognosy Department, Faculty of Pharmacy, October 6 University, Egypt.
Email: jilannazeam@o6u.edu.eg

Abdel-Naser B. Singab, Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University, Egypt.
Email: dean@pharma.asu.edu.eg

## Abstract

The COVID-19 pandemic is shaking up global scientific structures toward addressing antibiotic resistance threats and indicates an urgent need to develop more cost-effective vaccines. Vaccine adjuvants play a crucial role in boosting immunogenicity and improving vaccine efficacy. The toxicity and adversity of most adjuvant formulations are the major human immunization problems, especially in routine pediatric and immunocompromised patients. The present review focused on preclinical studies of immunoadjuvant plant proteins in use with antiparasitic, antifungal, and antiviral vaccines. Moreover, this report outlines the current perspective of immunostimulant plant protein candidates that can be used by researchers in developing new generations of vaccine-adjuvants. Future clinical studies are required to substantiate the plant proteins' safety and applicability as a vaccine adjuvant in pharmaceutical manufacturing.

**KEYWORDS**
adjuvants, immunostimulants, lectins, plant protein, vaccines

## 1 | INTRODUCTION

Vaccines represent one of the greatest public health advances of the 20th century, the vaccinations make a remarkable difference in reducing morbidity and mortality worldwide (Schijns et al., 2020). According to the world health organization (WHO), it is estimated that it prevents between two and three million deaths annually https://www.who.int/immunization/global_vaccine_action_plan/GVAP_doc_2011_2020/en/. The rational vaccine construction is shaped by antigen selection, type of vaccine adjuvants, delivery route, linkers, and histidine tags (Coffman, Sher, & Seder, 2010). Vaccine adjuvant is an important vaccine component that could enhance overall vaccine immunogenicity and decline the likelihood of initiating immune tolerance (Saylor, Gillam, Lohneis, & Zhang, 2020).

Effective vaccines induce adaptive immunity in response to future infections or to treat an already diagnosed disease (Schijns et al., 2021). Many infectious diseases are prevented by vaccination such as diphtheria, haemophilus influenza type b, hepatitis B, human papillomavirus (HPV), measles, mumps, pertussis, pneumococcal diseases, poliovirus, rotavirus, rubella, tetanus, and tuberculosis (Rappuoli, Pizza, Del Giudice, & De Gregorio, 2014). Moreover, the therapeutic vaccines propose paradigm shifts in oncology healthcare and target breast, colorectal, lung, pancreatic, and prostate cancers (Boukhebza, Bellon, Limacher, & Inchauspé, 2012). Besides, peptide cancer vaccines (PCVs) are designed to target tumor-specific antigens and tumor-associated antigens (Tsung & Norton, 2016). The PCVs possess numerous advantages since they lack significant toxicity associated with chemotherapy and radiotherapy (Sotillo et al., 2015).

Despite these vaccines' success and recent advances in vaccinology, there are many diseases for which the development of safe and effective vaccine remains elusive, such as AIDS, arboviral disease, bird flu, chickenpox, Ebola, herpes, Zika, malaria, and hepatitis C other than different cancer types. Regulatory/economic/ethical factors associated with clinical trials and the unique characters of the individual pathogens could be attributed to the unavailability of vaccines for these emerging pathogens and intractable diseases (Tannock, Kim, & Xue, 2020). In addition, traditional design methods to produce a functionally protective vaccine are still a simple reason that hinders production. Hence, it is important to consider new technologies for the development and design of vaccine formulas (Vrba, Kirk, Brisse, Liang, & Ly, 2020).

 © 2022 John Wiley & Sons Ltd. 

Plant-based immunoadjuvants have the potential to optimize immune responses, indeed they can be considered a promising adjuvant candidate for the development of novel vaccines (Lakshmi, Kumar, Pawar, Sudheesh, & Pawar, 2018).

The present review illuminates the critical role of adjuvants in vaccination strategies, as well as the recent contribution of plant protein immunomodulators to preclinical immunization protocols, with an emphasis on promising plant proteins that could impact future vaccine applications.

## 2 | ADJUVANTS

Since the early 1920s, vaccine adjuvants have been used as chemical substances that strengthen and maintain immune responses to antigens (Gupta & Chaphalkar, 2015a, 2015b; Schijns et al., 2020). Although high-purity vaccines showed a wide therapeutic safety index, an impaired immunogenic potential has been recorded. Consequently, there is a growing demand for using immune potentiators in vaccination protocols (Fendler et al., 2022; Rüthrich, Giesen, Mellinghoff, Rieger, & von Lilienfeld-Toal, 2022).

Numerous vaccine types induce artificial adaptive immunity such as live attenuated, inactivated "killed," subunit peptide or polysaccharide, RNA vaccines, DNA vaccines, recombinant viral vector vaccines, and bacterial vector vaccines. Though live attenuated vaccines mimic the closest form of natural infection, they usually induce mild or moderate adverse effects, however, they are still highly effective vaccines. The potential for virulence reversion highlights a severe threat that emphasizes the importance of rational vaccine strain composition (Christensen, 2016).

In contrast to attenuated live vaccines, dead subunit vaccines (inactivated "killed" subunit peptide or polysaccharide), present weaker efficiency and shorter duration of immunity. Therefore, adjuvant booster immunizations are required (Gupta & Chaphalkar, 2015a, 2015b). Alternatively, DNA and recombinant viral vector vaccines significantly promoted antigen-specific cellular and humoral immune responses without adjuvants. Despite the advantages of the recombinant viral vector vaccine, few vaccines are licensed (Moingeon, Haensler, & Lindberg, 2001). Moreover, the immunogenicity of DNA vaccines in humans still needs to lot of effort to certify their immunological efficiency and safety (Hobernik & Bros, 2018).

The mRNA is a very large molecule, prone to degradation by nucleases, and intrinsically unstable. Hence the stability of mRNA vaccines is considered the main challenge that impedes successful translation into drugs. However, mRNA chemical modification partially solved such a hurdle, but intracellular delivery of mRNA still represents a major demand (Wadhwa, Aljabbari, Lokras, Foged, & Thakur, 2020). This limitation of DNA/RNA-based vaccines highlights the need for novel vaccine adjuvant constructs.

Adjuvants play a significant role in immune responses and they are mentioned below:

- *Boosting the immunogenicity of weak antigens*: to elicit adequate antibody responses (Wang & Xu, 2020).
- *Dose-sparing*: decrease antigen doses, which in consequence lower vaccine production costs and makes it affordable worldwide. The adjuvant use can reduce the incidents of any global vaccination shortages. The BCG tuberculosis vaccine was an example of a worldwide demand-deficient incidence in 2015 when 180 million doses were required, and only 107 million doses were available (Nicholls, Madera, & Hancock, 2010).
- *Enhancing the onset and duration of the immune response*: facilitating phagocytosis and antigen detection by immune cells, which prolongs the immunological memory (Schijns et al., 2021).
- *Modulation of antibody specificity, avidity, and isotope distribution*: adjuvants expand B cell diversity release and improve the durability and quality of antibody responses to destroy pathogens (Reed, Orr, & Fox, 2013).
- *Initiation of mucosal immune responses*: although most pathogens enter through mucosal channels such as the nose, mouth, or genital tract, vaccination is primarily applied to parenteral modes that stimulate the development of distinct isotype antibodies. Adjuvant oral immunization allows access to the intestine, which is the body's largest immune organ (Smith et al., 2020).
- *Improving vaccine efficacy for weak responders*: immunostimulant adjuvants are able to activate both innate and adaptive immunity. An attenuated microorganism may cause life-threatening health risks to people with a compromised immune system such as children, the elderly, and those patients who have a weak immune system (Reyna-Margarita et al., 2019). Therefore, the administration of non-traditional vaccines, along with immunostimulant adjuvants, might help in enhancing its efficacy in weak responders.

## 3 | ADJUVANT TYPES AND CHALLENGES

The immune potentiator adjuvants can activate innate immunity directly as cytokines or indirectly through pattern-recognition receptors (PRRs) (Ong, Lian, Kawasaki, & Kawai, 2021), while the use of mucosal adjuvants can induce local mucosal immunity (Savelkoul, Ferro, Strioga, & Schijns, 2015). The recent approach to optimize vaccine immune responses is the use of different adjuvant combinations that could trigger different signaling pathways (Lee & Nguyen, 2015).

Adjuvants can be primarily classified into two main types based on their particle size (Makwana et al., 2018) (a) particulate (liposomes, w/o emulsions, aluminum salts, nanoparticles, and microparticles) and (b) nonparticulate (protein, polysaccharide, saponin, lipid, bacterial toxins, and cytokines) (Gupta & Chaphalkar, 2015b; Schijns et al., 2020, 2021). Further classification based on mechanism of action, can be divided into delivery systems adjuvants, immunostimulatory, mucosal adjuvants, and adjuvants combination (Apostólico et al., 2016).

The delivery systems cover a wide range of materials such as mineral salts (alum), emulsions, and microparticles (virus-like particles) (Pashine, Valiante, & Ulmer, 2005). Until now aluminum hydroxide and aluminum phosphate (alum) are the most commonly used adjuvants in human vaccinations, though calcium phosphate and oil emulsions have also been used (Gupta & Siber, 1995). Alum has a fair

safety record, but comparative studies have indicated that it is a poor adjuvant for cell-mediated immunity and a weak adjuvant of antibody production to protein subunits. Furthermore, its (IgE) antibody response has been linked to some allergic reactions (Singh & O'Hagan, 2002).

Emulsion adjuvants have a long history of use, the water-in-oil (w/o) emulsion is classified into two forms; complete Freund's adjuvant (CFA) (mineral oil, emulsifier, and killed bacteria) and incomplete Freund's adjuvant, which has the same composition as CFA, but lacks the bacteria (Valverde et al., 2017). The virus-like particle adjuvant is formed from capsid or envelope structural viral proteins that mimic intact virus size and shape. While liposomes adjuvant is characterized by their biodegradability and biocompatibility. However, manufacturing costs and the generation of a weak immune response are still the major limitations of their application (Ali, Singh, & Datusalia, 2021).

Only a few adjuvants, such as aluminum salts, virosomes, MF59TM, AS01TM, AS03TM, AS04TM, and CpG, have been licensed for use in human vaccinations (Schijns et al., 2020). The incorporation of an adjuvant into a new or licensed vaccine is still a challenge that could take many years (Apostólico et al., 2016). Indeed, several factors contribute to the relatively slow development of new adjuvanted vaccines, where the assessment of adjuvant safety is critical. This necessitates large phase III studies with adequate sample sizes, which can take several years (Del Giudice, Rappuoli, & Didierlaurent, 2018).

Immunoadjuvants are a subcategory of immunomodulators that can increase the effectiveness of vaccines (Smith et al., 2020). Cytokines, which come in the form of interleukins (ILs), interferons, chemokines, and other soluble extracellular proteins or glycoproteins, are essential for both innate and acquired immunity. These cytokines maintain physiological stability in all nucleated cells (Mbawuike, Wyde, & Anderson, 1990). Based on the induction of cytokines signal to the bloodstream, several neurochemicals, neuroendocrine, and neuroimmune substances can be delivered (Jakobsen, Saeland, Gizurarson, Schulz, & Jónsdóttir, 1999; Morel & Turner, 2010).

Different cytokines were reported as adjuvants that could induce antigen-specific serum/mucosal antibody and cell-mediated immunity. The most notable cytokine adjuvants are granulocyte/macrophage colony-stimulating factor (GM-CSF) (Faries, Hsueh, Ye, Hoban, & Morton, 2009), IFN (Le Bon et al., 2001), IL-1 (Staats & Ennis, 1999), IL-2 (Shah & Abraham, 1992), IL-6 (Kishimoto, 2006), IL-12 (Bermúdez-Humarán et al., 2005), IL-15 (Yang & Lundqvist, 2020), IL-18 (Mountforda & A., 2000), and chemokines. Moreover, activating major histocompatibility complex (MHC), costimulatory signals, or related intracellular signaling is also considered a different adjuvant-reported mechanism (Mohan, Verma, & Nageswara Rao, 2013).

Compatibility of adjuvant-vaccine components is critical and evaluation tests of immunogenicity/safety for the formulation are essential (Apostólico et al., 2016). The demonstration of the added value of adjuvant action over plain antigen necessitates the generation of additional evidence to validate the use of an adjuvant, thus increasing the time required for vaccine development (Del Giudice et al., 2018). The selection of adjuvants is determined by balancing the need for adjuvanticity and an acceptable low level of side effects (Gupta & Siber, 1995). The design of potential and less toxic adjuvants with optimal matching for specific antigens has typically been an empirical fact (Awate, Babiuk, & Mutwiri, 2013).

A better understanding of adjuvant mechanisms likely has sped up their development. New technologies, such as systems vaccinology, are being used earlier in the development of novel vaccine adjuvant formulations in the hopes of speeding up their development and introduction into clinical practice (O'Hagan, Friedland, Hanon, & Didierlaurent, 2017). Moreover, non-biodegradability, instability, and large-scale production costs are the main factors affecting adjuvant development (Wallis, Shenton, & Carlisle, 2019). Advancement in these areas could lead to the availability of economically feasible, potent vaccines that could not only aid in managing clinical autoimmune-based manifestations but also created new platform against viral pathogens and diseases for which no interventions currently exist.

## 4 | CURRENT HYPOTHESIS MECHANISM OF ADJUVANTS

Immunomodulatory peptides act directly on immune system-specific cells, however, to date, the mechanisms behind these interactions are unclear. Even the currently available adjuvants included in the licensed, function of vaccines' remains underdeveloped. However, advances that have occurred during the past decade are beginning to yield deeper insights into the mechanism of action of adjuvants and are revitalizing the process of adjuvant discovery and development (Pulendran, Arunachalam, & O'Hagan, 2021).

Although the major focus of adjuvant discovery during the past decade has been to target the Toll-like receptor (TLR) pathway, the dendritic cells (DCs) have long been considered the primary cellular targets of vaccine adjuvants (Pulendran & Ahmed, 2011). Recent reports evidenced that stimulation of TLR ligands, PRRs, and NLRs could be targeted by adjuvants to induce an immunostimulant effect (Ho, Huis In't Veld, Raaijmakers, & Adema, 2018). Additionally, there is emerging evidence that adjuvants that are already in use in the clinic may activate immune responses via tissue damage pathway, where the release of a plethora of damage-associated molecular patterns, such as ATP or uric acid, or fragments of DNA or RNA or high mobility group box 1, could activate DCs to stimulate adaptive immunity (De Lorenzo, Ferrari, Cervone, & Okun, 2018). The adjuvants' main molecular targets for currently licensed vaccines are represented in Figure 1.

Peptide adjuvant with either arginine in the N- or C-terminal regions, tryptophan chain, phosphoserine residues, or glutamine units, could recognize by immunological cells' opioid receptors. Such interaction with $\delta$-, $\mu$-, or $\kappa$-type opioid receptors can regulate the peripheral immune system (Haque, Chand, & Kapila, 2008). Additionally, the presence of arginine at the C-terminal is one of the structural characteristics of ACE inhibitory peptides that also may be related to the immunomodulatory activity. Besides, the membrane-bound receptors

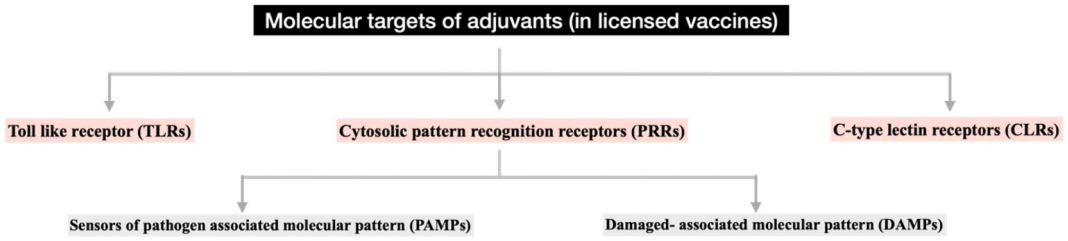


**FIGURE 1** Molecular targets of adjuvants in currently licensed vaccines

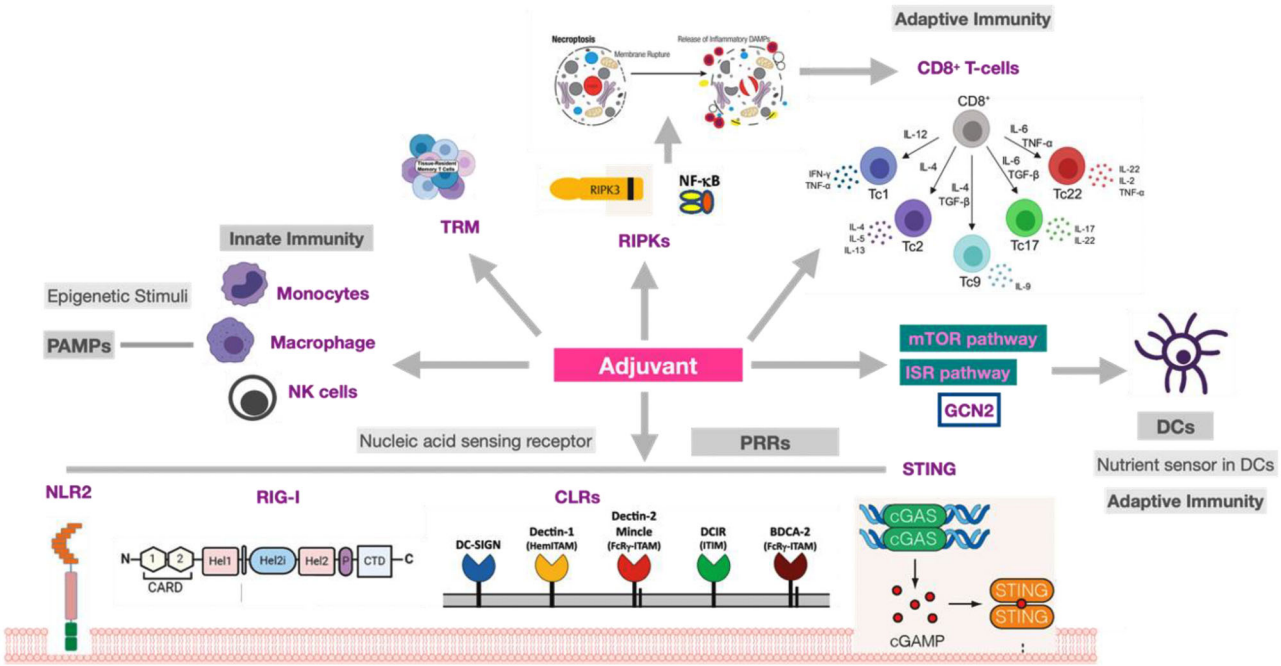


**FIGURE 2** Summary of different adjuvants' mechanisms of action according to Pulendran et al. (2021). Abbreviations: pattern recognition receptors (PRRs), tissue-resident memory t cells (TRM), nucleotide-binding oligomerization domain (noD)-like receptors (nlRs), retinoic acid-inducible gene I (RIG-I), cGAS-stimulator of interferon genes (STING), C-type lectin receptors (CLRs), receptor-interacting serine/threonine protein kinase 3 (RIPK3), nF-κB-dependent inflammation, nuclear factor kappa B (NF-κB), damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs)

of T cells could recognize the N- or C-terminal of peptides and stimulate charge-changing lymphokines (Santiago-López, Hernández-Mendoza, Vallejo-Cordoba, Mata-Haro, & González-Córdova, 2016). The peptides could also activate TLRs—which are typically expressed on DCs. They can sense highly conserved pathogen-associated molecular patterns (Hartmann & Meisel, 2007) in microbes—resulting in activation of DCs, which consequently stimulates antigen-specific T and B cell responses (Kitts & Weiler, 2003; Udenigwe & Aluko, 2012).

In addition, the coordinated action of the RIPK1-dependent cell death pathway and NF-κB-dependent inflammation synergize to promote enhanced cross-priming of $CD8^+$ T cells (Yatim et al., 2015). Taken together, these findings suggest that an adjuvant that targeted lymph node macrophages transiently induces RIPK3-mediated pathways, along with cell death, and stimulates $CD8^+$ T cell response. Summary for different types of adjuvants mechanism according to Pulendran et al., 2021 shown in Figure 2.

## 5 | PLANTS AND IMMUNOSTIMULATION

Plants are bio-factories of diverse active compounds that can significantly contribute to vaccine immunomodulation (Bhuiyan, Howlader, Raihan, & Hasan, 2020). Many studies revealed the efficacy of herbal medicine to induce the release of different cytokines (Burns, Zhao, Taylor, & Spelman, 2010; Woods et al., 2017). The plant phenolics that bind to the carrier protein (purified antigen) and plant proteins (e.g., lectins based on binding to carbohydrate cellular receptors, stimulating cell signaling, and triggering the immunological response) play an important role as a natural vaccine adjuvant (Gupta & Chaphalkar, 2015a, 2015b).

Several plant-based vaccine adjuvants have been purified from *Boswellia serata*, *Picrorhiza kurroa* (Khajuria et al., 2007), and *Emblica officinalis* (Gupta & Chaphalkar, 2015b). Furthermore, medicinal plants including *Asparagus racemosus*, *Emblica officinalis*, *Withania somnifera*,

*Panax notoginseng*, and *Tinospora cordifolia* have shown significant immunostimulatory activity at the humoral level (Gupta & Chaphalkar, 2015b). *Aristolochia longa*, *Datura stramonium*, *Marrubium vulgare*, *Sinapis nigra*, *Delphynium staphysagria*, *Lepidium sativum*, *Ammi visnaga*, and *Tetraclinis articulata* extracts markedly alter the proliferation of immune cells (Daoudi, Aarab, & Abdel-Sattar, 2013; Gupta & Chaphalkar, 2015b).

*Polypodium leucotomos* stimulates interleukin IL-1α, IL-1β, and TNFα release in vitro (Bernd et al., 1995). The immune-modulating effects of astragalus root and elderberry fruit extracts were examined in bone marrow-derived murine DCs. The ELISA and RT-PCR tests indicated that both extracts enhanced IFN-ß production, increased endocytosis in immature DCs, upregulated toll-like receptor 3, and enhanced the IL-12, IL-6, IL-1b, and TNFα cytokines release (Aldahlawi, 2016). Also, induction, maturation, and differentiation of DCs were reported by *Mucuna pruriens* extract (Kurokawa et al., 2011). The *Abelmoschus esculentus* increased the expression of IL-12, interferon IFNγ, and stimulated DCs by modulating the expression of class II-MHC and costimulatory $CD^{+}80/86$ molecules (Aldahlawi, 2016; Sheu & Lai, 2012).

Garlic is one of the most used flavoring plants in cooking, the plant and its formulations have been extensively used as an immunomodulator in vitro and in vivo. Modulation of cytokine profile, in addition to direct stimulation of immune cells, has been reported as the main mechanism of action (Moutia, Habti, & Badou, 2018). Furthermore, the biological studies of *Aloe vera* have shown its ability to improve the production of TNFα and IL-6 levels in human peripheral blood macrophages (Ali et al., 2021).

## 6 | PLANT IMMUNOMODULATOR PROTEINS

According to the food and agriculture organization of the United Nations, the latest forecast for world cereal production in 2021 stands at 2.793 million tonnes, 0.8% higher year on year (Canton, 2021). Grains are important sources of diet proteins, and for some populations, they are the major protein sources (Duranti, 2006). Up to 20% of legumes' dry weight represents their protein, therefore it may be a potential source of peptides with varying biological activities (Clemente, MacKenzie, Johnson, & Domoney, 2004). Nevertheless, the immunomodulatory activity of peptides derived from plant proteins that have been not fully explored (Santiago-López et al., 2016).

The composition, sequence, hydrophobicity, and length of plant protein amino acids are all vital parameters that influence the immunomodulatory activity of a hydrolysate (Chalamaiah, Yu, & Wu, 2018). Short peptides have immense potential for vaccine adjuvant development as they play a significant immunomodulatory role by stimulating the natural killer cells (Dong & Kobinger, 2013).

Various plant proteins and peptides have been used in a vaccination strategy against influenza virus, *Neospora lysate*, *Trypanosoma cruzi*, *leishmania*, and *Paracoccidioides brasiliensi* (Figure 3). There are many reports that the immune enhancer vegetable proteins like the peptides hydrolysate of rice, soybean, and pea can enhance lymphocyte proliferation and improve macrophage phagocytosis (Anderson, 1997). The chickpea peptides with an active sequence Met-Ile-Thr-Leu-Ala-Ile-Phe-Val-Asn-Lys-Phe-Gly-Arg that derived from microbial proteases (Dominguez-Vega, Kotkowska, Garcia,

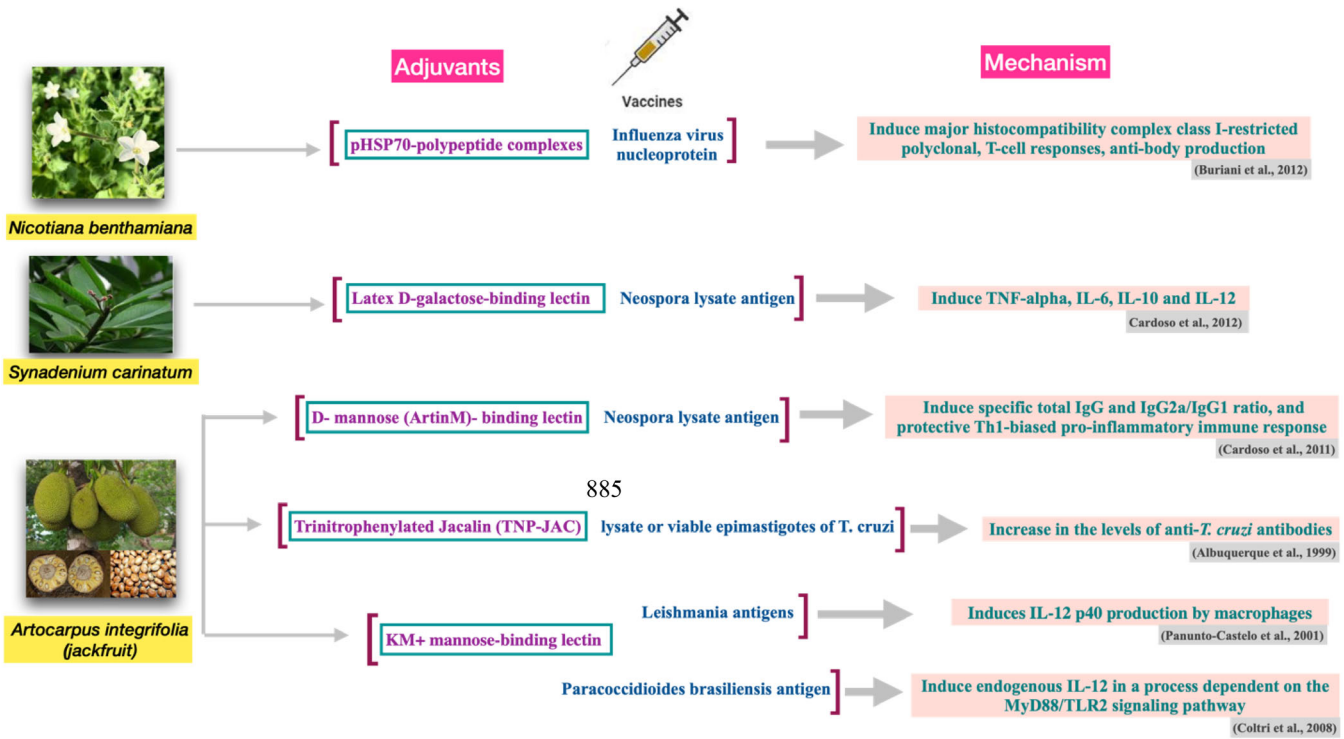


**FIGURE 3** Finding of preclinical studies for some plant proteins as vaccine adjuvants

Crego, & Marina, 2011) and a bioactive peptide derived from rice albumin with a sequence, Gly-Tyr-Pro-Met-Tyr-Pro-LeuPro-Arg stimulated the immune system through phagocytosis (Takahashi, Moriguchi, Yoshikawa, & Sasaki, 1994).

Soy protein hydrolysate has been found to induce IgG and IgA, and enhance B-cell differentiation and antibody production (Kiewiet, Faas, & de Vos, 2018). The soybean tridecapeptide isolated by a trypsin digest, common bean peptides (Vital, De Mejia, Dia, & Loarca-Piña, 2014), and lupine protein hydrolysate (Cruz-Chamorro et al., 2019), are reported as stimulants to phagocytosis of human neutrophils (Tsuruki et al., 2003). Furthermore, in a clinical examination of a small group of volunteers, an increase in granulocytes, NK, $CD11b^{+}$, $CD56^{+}$, and a change in leukocyte count was also observed (Yimit, Hoxur, Amat, Uchikawa, & Yamaguchi, 2012).

Gluten is the main protein of wheat as it accounts for about 80% of their total protein content. Peptides derived from hydrolysates of gluten activate NK cells in humans and act as immunostimulants (Horiguchi, Horiguchi, & Suzuki, 2005). Also, the peptides showed modulation of lymphocytes, monocytes, and granulocytes (Sawaki, Takaoka, Sakuraba, & Suzuki, 2004). Furthermore, the ex vivo immunomodulatory effect of alcalase-generated wheat gluten protein hydrolysates showed an increase in IL-10/IFNγ ratio in treated cultured human peripheral blood mononuclear cells (Cruz-Chamorro et al., 2020). Several reports screened the plant proteins' immunostimulatory effects in vitro are summarized and shown in Table 1, along with the in vivo models in Table 2.

The major drawback of plant peptide production using conventional chemical synthesis was the high costs involved, which ultimately hinders the scale-up. Over the last years, bioactive and immunogenic peptides have been economically synthesized in mass production using a genetic fusion of the corresponding nucleotide sequence of a carrier protein, followed by stable nuclear plastid transformation, or transient expression using bacterial or viral vectors (Lico, Santi, Twyman, Pezzotti, & Avesani, 2012). This process can lower the cost per vaccine and allow for an abundance of more doses, which is particularly important during a pandemic and epidemic situation. Hence, mass production represents the advantageous use of plant peptides, and the peptides can be quickly and cheaply synthesized (Patel et al., 2012). The clinical assessment of immunomodulatory peptides and understanding of their molecular structure permits the establishment of their structure–activity relationships, which is important for developing next-generation of adjuvants (Azmi, Fuaad, Skwarczynski, & Toth, 2014).

## 7 | PLANT LECTINS

Various plant lectins have been proposed as potential antigen-delivery agents (Massa & Franconi, 2012). The reported immunomodulatory effect of various lectins has prompted the screening for their adjuvant potential in pharmaceutical applications (Sander, Corigliano, & Clemente, 2019). The characteristic features of plant lectins rely on their interaction with the mucosal epithelium and their translocation across the gut (Lavelle, Grant, Pusztai, Pfüller, & O'Hagan, 2001). Hence, they are considered a targeting agent to induce potent systemic and mucosal antibody responses, that could be exploited in vaccine formulations to enhance the efficacy of orally administered vaccines (Lavelle, Grant, Pfuller, & O'Hagan, 2004).

The aromatic amino acids found in the lectin-sheet protein structures interact with the chiral ligand center, resulting in the binding processes between the lectins and their ligands (Teixeira et al., 2006). In terms of the plant lectins' action mechanisms, it is reported to interact with glycosylated TLR on macrophages and/or DCs. Indeed, several plant lectins act as TLR agonists, polyclonal cellular activators, enhance T lymphocytes proliferation, and are involved also in the amplification of IL-12 and IFNγ production (Unitt & Hornigold, 2011; Venkatalakshmi, Vadivel, & Brindha, 2016). According to Daoudi, Abdel-Satter, & Aarab, 2014 lectins of *Datura stramonium*, *Lepidium sativum*, and *Delphinium staphisagria* seeds possess a proliferation stimulatory effect on immune cells (Bousfiha et al., 2016).

Lectins have been reported to be an effective immunoadjuvant candidate (Sander et al., 2019). It is used against coccidial infections, as the lectins from wheat germ agglutinin (WGA), *Phaseolus vulgaris* (PHA), *Viscum album* (mistletoe lectin 1; ML-1), *Ulex europaeus* (UEA-1), and *Lycopersicum esculentum* (tomato lectin; LEA), enhanced the production of specific ovalbumin antigen (OVA), and serum IgA and IgG antibodies when assessed as adjuvants with immunization protocols (Lavelle, Grant, Pusztai, Pfüller, & O'hagan, D. T., 2000). Additionally, vaccine formulations containing *U. europaeus* UEA-1 conjugated to kill *Helicobacter pylori* and *Campylobacter jejuni* showed induction of immune responses in orally immunized mice (Chionh, Wee, Every, Ng, & Sutton, 2009; Daoudi et al., 2014). According to Lavelle et al. (2004) mistletoe lectins (ribosome-inactivating proteins (RIPs); (ML) I, II, and II (MLI, MLII, and MLIII)) are potent immunogens when administered through the nasotracheal route and could be used as a platform for the generation of effective mucosal adjuvants. In addition, *Proteus vulgaris* lectin possesses potent lymphocyte stimulation effect (Daoudi et al., 2014).

ArtinM, a mannosyl-binding lectin derived from the seeds of *Artocarpus heterophyllus*; activates innate immune cells and in consequence T helper type 1 (Th1) is induced (Da Silva, Oliveira-Brito, de Oliveira Thomaz, & Roque-Barreira, 2020). The ArtinM's interactions with TLR2 N-glycans on the surface of DCs, neutrophils, mast cells, and macrophages are primarily responsible for its potent immunomodulatory activity (Sander et al., 2019). This activity is distinguished by the induction of IL-12, and the ability to provide in vivo protection against intracellular pathogens, such as *Leishmania* spp and *Paracoccidioides brasiliensis* (Souza, Carvalho, Ruas, Ricci-azevedo, & Roque-barreira, 2013).

*Allium sativum* agglutinins (ASAs, mannose-binding lectins) showed in vitro potent immunomodulatory effects (Clement et al., 2010). Besides, garlic lectins (ASA I and ASA II) were co-administered with weak antigen ovalbumin (OVA) in BALB/c mice at a 30 μg dose (50 days duration, intranasal). The adjuvanticity character was indicated by the induction of anti-OVA IgG, also anti-lectin IgG response increased by threefold and 2.4-fold for ASA I and ASA II,

**TABLE 1** In vitro immunostimulant assays of plant proteins

| Source | Protein | Concentration | Finding/mechanism | References |
|---|---|---|---|---|
| *Momordica charantia* *Phytolacca americana* | *Momordica Charantia* inhibitor (MCI) protein pokeweed antiviral protein (PAP-S) | 0.1–1 μg/mL range<br>MCI; 5 μg/mL ($1.6 \times 10^{-7}$ M)<br>PAP-S; 10 μg/mL ($1.6 \times 10^{-7}$ M) | 50% inhibition of macrophage protein synthesis<br>Inhibition of both ConA and PHA responsiveness | Spreafico et al. (1983) |
| *Triticum aestivum* (Wheat) | Wheat gluten protein hydrolysates (WGPHs) | 0.5 mg/mL | Potent anti-proliferative effect<br>Decreased IFNγ, IL-17, IL-10, increased ratios of IL-4/IFNγ, IL-4/IL-17, and IL-10/IFNγ, IL-10 mRNA | Cruz-Chamorro et al. (2020) |
| *Pseudostellaria heterophylla* | *P. heterophylla* peptide (PPH <1,000 Da) | 100 $\mu g/mL^{-1}$ | SI was 1.53 for 48 h/promoted TNFα, IFNγ, IL-10<br>Activated spleen lymphocytes via the $Ca^{2+}$/CaN/NFATc1/IFN-signaling pathway | Yang et al. (2019) |
| | YG-9 (YGPSSYGYG) $C_{44}H_{55}N_9O_{15}$ | 50 μg/mL | Proliferation index = 1.19<br>Promoted pinocytosis activity, TNFα<br>Activated RAW264.7 cells via the TLRs/NF-$_k$B/TNFα signaling pathway | Yang et al. (2020a) |
| | RGPPP<br>Proline—molecular weight 522.29 Da<br>47.0% β-sheet, 10.6% β-turn, and 42.4% random coil | Spleen lymphocyte proliferation rate = 1.27 at 100 μg/mL.<br>RP-5 activates RAW264.7 cells to secrete NO, ROS, and TNFα. A signaling pathway, TLR2/NF-κB, revealed | Promoted proliferation of spleen lymphocytes<br>Arginine in the N- or C-terminal regions, phosphoserine, glutamine, or tryptophan, recognized by opioid receptors on the immunological cells and resulted in immunomodulation<br>The stimulation index = 1.27 at 100 μg/mL, subsequently dropped to 1.04 at 400 μg/mL. Proliferation index = 1.52 in spleen lymphocytes | Yang et al. (2020b) |
| *Oryza sativa* (Rice protein) | Tyr-Gly-Ile-Tyr-Pro-Arg (YGIYPR) protein hydrolysate | 12.5 μg/mL | Enhanced macrophage proliferation SI value = 1.324 | Xu et al. (2019) |
| *Berberis hispanica* | Protein fractions<br>F1 = 75 kDa,<br>F2; 20 KDa<br>F3; 5 KDa | 1 mg/mL | Stimulated the thymocyte and splenocyte proliferation<br>Enhanced complement activity<br>Promoted allogenic mixed lymphocyte | El Youbi et al. (2012) |
| *Allium sativum* (Garlic) | Three protein components of 13 kD (QR-1, QR-2, and QR-3 in the ratio 7:28:1) | 1 μg/mL | Mitogenic activity towards human peripheral blood lymphocytes, murine splenocytes, and thymocytes QR-2 > QR-1, QR-3.<br>Mannose-binding activities | Clement, Pramod, and Venkatesh (2010) |
| *Allium sativum* (Aged garlic) | Three major proteins (12–14 kDa) QA-1, QA-2, and QA-3 | 4 μg/mL | QA-2 showed the highest mitogenic activity<br>QA-3 exhibits mitogenic activity, mannose-binding activity | Chandrashekar and Venkatesh (2009) |
| *Viscum album* var. (*coloratum*) | Korean mistletoe lectin (KML) | 2–20 ng/mL | Enhanced macrophage responses<br>Induced cytokines (IL-3, IL-23, and TNFα), phagocytic uptake<br>Up-regulation of functional activation of adhesion molecules | Lee et al. (2007) |
| *Anoectochilus formosanus* | Immunomodulatory protein (IPAF) | 8 μg/mL | Induced the cell proliferation in mouse splenic B lymphocytes | Kuan et al. (2011) |

**TABLE 2** In vivo immunostimulatory screening of plant proteins

| Source | Extract | Tests | Animal | Dose/day | Duration | Induction | Finding | References |
|---|---|---|---|---|---|---|---|---|
| *Seeds of Momordica charantia*, Leaves of *Phytolacca americana* | *Momordica Charantia* inhibitor (MCI) protein Pokeweed antiviral protein (PAP-S) | Spleen hemolytic plaque-forming cells (PFC) | $CD2F_1$ | MCI (4.3 mg/kg) PAP-S (3.2 mg/kg) | 5 days | $4 \times 10^8$ sheep erythrocytes (SRBC) i.p. on day 0, (PFC were counted on day 4) Type III pneumococcal polysaccharide was injected i.p. at 0.5 μg (PFC specific for S III were counted on day 5) | For 200 μg/kg—2 days before an antigen, producing over 90% reductions from control values For 400 μg/kg—6 days before SRBC were associated with over 90% inhibition of PFC counts | Spreafico et al. (1983) |
| | | Skin grafting | C57B1/6 mice | | Grafts were inspected daily starting on day 7 | Skin grafting from male C3H donors | 400 μg/kg given 2 days before transplant significantly delayed graft rejection by approximately 3 days | |
| *Cocos nucifera* L (Coconut) | Protein extract | Hematological test | Swiss albino mice | Orally 120 g/35 mL | 30 days protein intake | Cyclophosphamide 20 mg/kg/day for 10 days | Increased levels of RBC, WBC, platelet counts | Vigila and Baskaran (2008) |
| *Glycine max* (Soy) | Soya protein isolate (SPI) | Measurement of serum total and soya protein-specific antibodies | Sprague–Dawley rats | 20% alcohol–SPI (age before 28 days) | For 70, 190, or 310 days | – | Increased serum total IgA and IgM, induced the production of SPI-specific IgA, IgG, IgM, and IgE antibodies | Cornish et al. (2011) |
| *Anoectochilus formosanus* | Protein (IPAF) | Production of anti-IPAF monoclonal antibody | BALB/c mice | 50 μg | 30 days | I.P. injection IPAF + aluminum potassium sulfate (10% w/v). | Induced B cells, CD69, MHC class II & IgM production | Kuan et al. (2011) |

respectively. Besides, the intradermal administration of ASA I and ASA II had shown the same fold increase (four and twofold anti-lectin IgG response) for only 14 days. Moreover, the spleen and thymus stimulation index were determined and the results indicate the potential use of garlic lectins as a mucosal and oral adjuvant (Padiyappa et al., 2022; Smart, Ryan, Holdworth, & Preston, 1978).

*Viscum album coloratum* lectins (KML-C) at a dose of 50 ng/mouse induce humoral and cellular immune responses against an immunogenic protein antigen (keyhole limpet hemocyanin; KLH). The assay revealed that KML-C augmented KLH-specific antibody titers of IgG1, IgG2a, IgG2b, specific Th1, IL-2, IFN, as well as Th-2 type cytokine IL-4 (Yoon et al., 2001). In BALB/c mice, the purified Chinese mistletoe lectin ACML-55 showed activation of innate lymphocytes, the increased cell number of NK, and γδT cells, and enhanced both antigen-specific activation and proliferation of $CD4^+$ and $CD8^+$ T cells (Ma et al., 2008). Korean mistletoe lectin (KML) enhanced macrophage responses, phagocytosis, and induce cytokines (IL-3, IL-23, and TNFα) (Lee et al., 2007). Furthermore, the *Viscum album* (mistletoe 1; ML-1) lectin combined with *herpes simplex* virus vaccine antigen glycoprotein D2 (gD2), and administrated by a nasotracheal route, the results indicated an induction in mucosal and systemic responses (Leavy, Mcneela, & Mills, 2002).

*Narcissus tazetta* lectin (26 kDa) (NTL)-induced cytokines gene expression in vivo, where after 10-day consecutive peritoneal injections of 5 mg NTL/kg/day, the expression levels of IL-1β, TNFα, IFNγ, and TGF-β were markedly increased, and the levels of IL-2–IL-4 were up-regulated in splenocytes. While at the onset of 12–24 h, the TGF-β was expressed in both macrophages and splenocytes (Ooi, Liu, Ooi, Ng, & Fung, 2002).

Tomato lectin (LEA) delivered intranasally elicited a potent systemic and mucosal antibody response, while, wheat germ agglutinin (WGA) and *Ulex europaeus* lectin 1 (UEA-I) when combined with ovalbumin (OVA), a specific serum IgG response to OVA was induced (Lavelle et al., 2001). Additionally, soybean, wheat germ, and peanut agglutinin lectins are reported as extracellular TLRs agonist and induce cytokine gene expression (Sander et al., 2019; Venkatalakshmi et al., 2016).

It worthy noted that lectins are the most common plant immunomodulatory proteins and plant heat-shock proteins (hsps) have been considered less attention as a biological modifier agent, although the bacteria Hsps have been used as an adjuvant in current vaccine production (Zininga, Ramatsui, & Shonhai, 2018).

Heat-shock proteins or stress-induced proteins (Hsps) play an important cytoprotective role in cells exposed to stressful conditions. Hsps induction are a common phenomenon in bacteria, plants, and human beings (Al-Whaibi, 2011). Plant Hsps vary in size from 15 to 30 kDa and are grouped into five classes according to their molecular weight (small Hsps [sHsps], Hsp60, Hsp70, Hsp90, and Hsp100). The transcription is controlled by the heat stress transcription factors (Hsfs) regulatory gene, and encoded by three classes of cytosolic sHSPs (classes CI, CII, and CIII) and three classes of sHSPs targeted to intracellular organelles (Miroshnichenko et al., 2005). Future screening of the immunomodulator potential of plant Hsps could open up a new area for further exploration in this regard.

## 8 | PLANT PEPTIDE AS A DELIVERY VEHICLE FOR RECOMBINANT PROTEINS

Biopharming is the use of a host living system for the manufacture of biological drugs that are non-natively produced using plant cells as bio-factories allow the economical production of candidate vaccines at large scales that are unavailable with the current in vitro synthesis. The biopharming technologies involve the integration of the desired genes encoding the vaccine antigen protein into the genome of plant tissues (Laere et al., 2016).

Many challenges facing the upstream processes include the selection of antigen and plant expression host, consistency of dosage, and manufacturing of vaccines according to good manufacturing practice procedures. The use of plant peptides offers a simple and flexible way to deal with much of this complexity by acting as a delivery vehicle for recombinant proteins. Moreover, peptides display more drug-like properties and therefore attract increasing interest from the pharmaceutical industry as a vaccine delivery agent (Purcell, McCluskey, & Rossjohn, 2007).

Schwestka et al., 2020 fused the N-terminal sequence of the maize storage prolamine protein (γ-zein) as delivery vehicles for bioencapsulate recombinant proteins. The resultant protein bodies (PBs) showed an internalization into the intestinal epithelial cells at a higher rate than synthetic polystyrene beads. In addition, the PBs showed a pivotal role in the initiation of a humoral response with a potential immunostimulatory effect, resulting in an induced secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) chemoattractant molecules. The GM-CSF is involved in the differentiation of granulocytes, macrophages, and also in the proliferation of neutrophils, macrophages, and DCs (Hamilton, 2002). Furthermore, GM-CSF increased antigen-specific antibody production (Okada et al., 1997), promotes IL-6 secretion, and accordingly IL-6 levels were also elevated (Evans, Shultz, Dranoff, Fuller, & Kamdar, 1998).

The use of plant protein as a delivery vehicle could open new avenues as an adjuvant for the next-generation of vaccines, including the design of functionalized multicomponent PBs with defined structures and uptake kinetics for the pharmaceutical industry (Schillberg, Raven, Spiegel, Rasche, & Buntru, 2019).

## 9 | HURDLES AND SOLUTIONS IN THE PRODUCTION OF NEW PROTEIN ADJUVANTS

The key hurdle in formulating plant-based adjuvant vaccines is the choosing of formulation components, to prevent vaccine denaturation due to hydrophobic interactions, as well as preventing chemical degradation during storage (Wakankar & Borchardt, 2006). The excipients may interact with adjuvants or antigen proteins which could alter immunogenicity positively or negatively. Also, during the formulation process, the antigen release and quality control assays could prove to be enormous challenges. Where partial antigen release, inefficient entrapment, and/or degradation after nanoparticle encapsulation

leads to high production cost due to wasted antigens (Jain, O'Hagan, & Singh, 2011).

Moreover, interactions between therapeutic protein products and the container closure polyethylene glycol (PEG) chains may negatively affect product quality and immunogenicity. Where the pegylation of vaccine products has been found to diminish the immunogenicity via inducing product solubility, inhibiting product aggregation, and in consequence immunogenicity diminished (Harris, Martin, & Modi, 2001). Moreover, the PEG itself may cause a loss of product efficacy and adverse safety risk (Liu et al., 2011). The glass and air interfaces of prefilled syringes can denature therapeutic protein products and chemical modification could also be observed by leached materials from the container closure system (Fradkin, Carpenter, & Randolph, 2011).

To overcome such challenges excipients and adjuvant-antigen protein interactions should be carefully evaluated, especially in terms of protein-excipient adducts formation. Moreover, to confirm and maintain product quality, appropriate in-use product shelf-life stability studies should be performed, and a risk assessment must be conducted. Besides, multiple analytical techniques need to be applied to assess the ability of the container closure system to interact and/or degrade protein products (Pulendran et al., 2021), Food and Drug Administration, and Center for Drug Evaluation and Research, 2014.

In using plant protein adjuvants, antibodies can develop not only to the desired antigen protein but also to adjuvant and any other foreign protein components that are potentially present in the product. Such proteins may contain regions of homology to endogenous human proteins. Clinical trials face a challenge in evaluating the capacity of the adjuvant protein to break tolerance and induce antibody responses to the homologous human factor (Pulendran et al., 2021).

The unknown mechanistic hypothesis is one of the challenges that face new development of adjuvant protein, and the use of systems vaccinology could help to answer different questions and aid in clarifying different aspects that could be hindered to demonstrate the clinical trials. The information from vaccinology could indicate (i) mechanistic insights, (ii) rational design of optimal formulations for vaccine delivery, (iii) underlying mechanisms by which formulations work, and (iv) underlying mechanisms of adverse reactions (Petitdemange et al., 2019). Moreover, novel adjuvants can be rapidly tested in small phase I (phase 0) human trials, the results obtained from such phase 0/I studies will create the mechanistic hypothesis about adjuvant antigen formulation (Wagar et al., 2021).

The polymorphisms (the presence of two variant forms of a specific DNA sequence) that could be resulted from the mismatching between the sequence of the patient endogenous protein and the therapeutic product protein are considered a risk factor for the development of immune response (Viel et al., 2009). Additionally, glycosylation may strongly modulate vaccine product immunogenicity by enhancing product solubility, minimizing protein aggregation, and diminishing immunogenicity as well as by shielding immunogenic protein epitopes from the immune system (Cole, Steckbeck, Rowles, Desrosiers, & Montelaro, 2004).

Hence, careful consideration should be given to the primary sequences of adjuvant plant proteins, antigen proteins, and especially of protein product counterparts of endogenous proteins, because of potential polymorphisms across human populations. For proteins that are normally glycosylated, it is recommended to use appropriate manufacturing methods that glycosylate the protein product in a non-immunogenic manner.

## 10 | REGULATORY AND COMPLIANCE REQUIREMENTS

For the acceptance of new vaccines/adjuvants, safety and a lack of universality appear to be the major critical factors, the current attitude regarding the risk–benefit of vaccination puts a large emphasis on safety. The cautious approach taken by manufacturers and authorities at the labor of new vaccines often leads to an impasse and explains why aluminum is still the predominant adjuvant used despite its limitations as an immunostimulator, with poor biodegradability and limited use in terms of delivery routes. However, the picture is different for therapeutic vaccines, such as tumor vaccines that are usually administered to seriously ill patients, a higher level of risk is considered acceptable (Goldenthal, Cavagnaro, Alving, & Vogel, 1993).

European union (EU) regulatory developments for the assessment of adjuvants include different stages, (i) development stages where laboratory studies are performed, (ii) development stages/preclinical evaluation, (iii) small-scale clinical trial, (iv) large-scale clinical studies, and (v) batch-to-batch consistency, where after the product is given marketing approval it monitored to provide ongoing evaluation of the product using the same assays of preclinical and clinical studies (Harandi, Medaglini, & Shattock, 2010).

*Preclinical safety studies* are also required not only for adjuvant safety, the qualitative and quantitative composition of the adjuvant, physical characteristics, and manufacturing process parameters but also for the adjuvant–antigen compatibility, dose ratio, repeat dose toxicity, characterization of immune response, proof of consistent antigen adjuvant adsorption, demonstration that no significant desorption during the shelf-life period, biochemical purity and pyrogenicity, systemic toxicity, reproduction toxicity, and carcinogenicity (Aguilar & Rodriguez, 2007).

*Clinical studies* include the final evaluation of a developed vaccine formulation that requires controlled studies of the adjuvant–antigen combination, which should deliver the following information: (i) justification for adjuvant inclusion, (ii) enhancement of immune response without undue increase in systemic reactions, and (iii) risk–benefit assessment on a case-by-case basis and for a modified product this should be at least as favorable as for the existing product (Sesardic, Rijpkema, & Patel, 2007).

*Before licensure*, the vaccine indication (therapeutic and prophylactic), the disease profile, route of administration, number of doses, and the population targeted to receive the vaccine should be established as the basis for safety assessment.

*Post-licensure safety evaluation* should provide safety information in specific populations that were not included in the pre-registration studies. Moreover, the adverse effects that increase in these populations relative to what was seen in clinical trials should be demonstrated (Da Silva, Di Pasquale, Yarzabal, & Garçon, 2015).

Among the biggest regulatory hurdles is the required population size that needs to be tested to prove the efficacy and particularly safety of a new adjuvant or vaccine. Market withdrawal could happen if a new vaccine is associated with hazardous adverse reactions, which challenged the public perception of safety and have an impact on the regulatory field (Aguilar & Rodriguez, 2007).

## 11 | CONCLUSION AND FUTURE PROSPECTIVE

Currently, the use of different types of adjuvants to optimize the vaccine immune response has received less attention. When preliminary trials with a "standard" adjuvant do not reveal the expected response, researchers typically seek another type of antigen rather than looking for an appropriate adjuvant that may produce the preferred immune response (Patel et al., 2012).

Adjuvants cannot receive FDA approval as standalone products, but as part of a registered vaccine adjuvant–antigen combination. Hence, only a few adjuvants are approved by regulatory authorities. Using plant protein as a safer immunomodulator adjuvant candidate could overcome the cost hinder of developing novel adjuvant formulations (Massa & Franconi, 2012).

Future advances in new adjuvants are more likely to be driven by better knowledge of the action mechanisms. Technological progress are expected to cut vaccine production costs, as well as novel prophylactic/therapeutic vaccine-adjuvant discoveries will be available (Singh & O'Hagan, 2002). A graphical presentation of plants that possess immunomodulation and can be considered as a promising biosource candidate for future novel adjuvants is represented in Figure 4 and its corresponding mechanisms are demonstrated in Figure 5.

Improved purification and high-throughput screening procedures can enhance the yield of proteins and reduce decades of research from chemical elucidation to commercial development. Drug carrier technology increases immunomodulator delivery of polypeptides and proteins, enhances pharmacokinetics, and introduces the optimum absorption rates that may allow effective interactions with the immune system components.


**FIGURE 4** Graphical presentation for plants-derived immunomodulator proteins as promising natural vaccine adjuvant agents

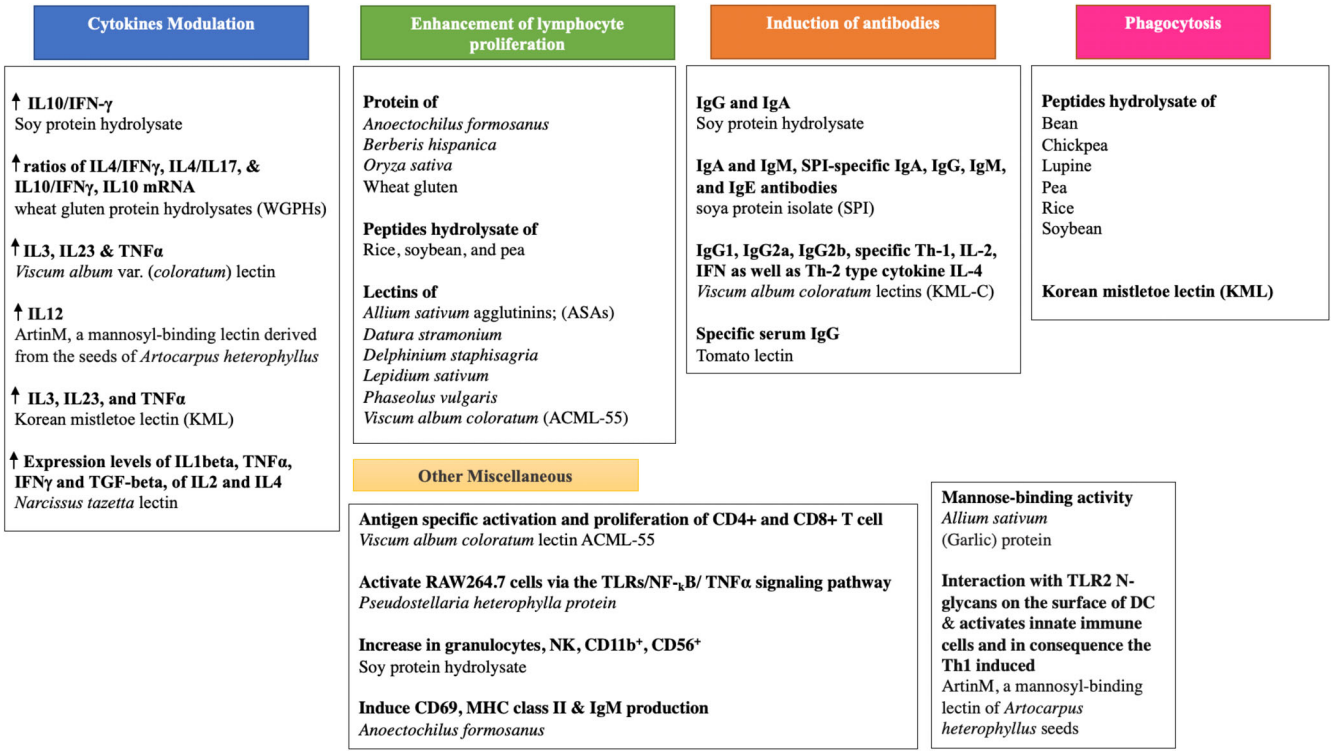


**FIGURE 5** Summary of immunomodulator plant proteins mechanisms of action according to previous reports

It is worthy to mention that there is the possibility that some of the studies in the present review, may not be performed according to the recent best practices/bioactive plant preparations/ pharmacological research (Izzo et al., 2020). To the best of our knowledge, there are no clinical reports that investigated the immunomodulatory effect of plant proteins, and further high-quality studies are needed to firmly establish the clinical efficacy of the plant proteins hydrolysates (Izzo, Hoon-Kim, Radhakrishnan, & Williamson, 2016; Kiewiet et al., 2018).

## ACKNOWLEDGMENT

We thank Dr. Walaa A. AL-Shareef (assistant professor of microbiology and immunology department, Faculty of Pharmacy, October 6 University, Giza, Egypt) who provided insight and expertise in editing the manuscript.

## CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

## DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

## ORCID

*Jilan A. Nazeam* https://orcid.org/0000-0003-1149-8222

## REFERENCES

Aguilar, J. C., & Rodriguez, E. G. (2007). Vaccine adjuvants revisited. *Vaccine*, *25*(19), 3752–3762.

Aldahlawi, A. M. (2016). Modulation of dendritic cell immune functions by plant components. *Journal of Microscopy and Ultrastructure*, *4*(2), 55–62.

Ali, S. A., Singh, G., & Datusalia, A. K. (2021). Potential therapeutic applications of phytoconstituents as immunomodulators: Pre-clinical and clinical evidences. *Phytotherapy Research*, *35*(7), 3702–3731.

Al-Whaibi, M. H. (2011). Plant heat-shock proteins: A mini review. *Journal of King Saud University-Science*, *23*(2), 139–150.

Anderson, P. M. (1997). Delivery systems for immunomodulatory proteins and peptides. *BioDrugs*, *7*(1), 51–65.

Apostólico, J. D. S., Alves, V., Lunardelli, S., Coirada, F. C., Boscardin, S. B., & Rosa, D. S. (2016). Adjuvants: Classification, modus operandi, and licensing. *Journal of Immunology Research*, *2016*(6), 1–16.

Awate, S., Babiuk, L. A., & Mutwiri, G. (2013). Mechanisms of action of adjuvants. *Frontiers in Immunology*, *4*, 1–10.

Azmi, F., Fuaad, A. A. H. A., Skwarczynski, M., & Toth, I. (2014). Recent progress in adjuvant discovery for peptide-based subunit vaccines. *Human Vaccines & Immunotherapeutics*, *10*(3), 778–796.

Bermúdez-Humarán, L. G., Cortes-Perez, N. G., Lefèvre, F., Corthier, G., Gruss, A., & Langella, P. (2005). A novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type 16-induced tumors. *The Journal of Immunology*, *175*(11), 7297–7302.

Bernd, A., Ramirez-Bosca, A., Huber, H., Thaci, D., Sewell, A., & Holzmann, H. (1995). *In vitro* studies on the immunomodulating effects of *Polypodium leucotomos* extract on human leukocyte fractions. *Arzneimittel-Forschung*, *45*(8), 901–904.

Bhuiyan, F. R., Howlader, S., Raihan, T., & Hasan, M. (2020). Plants metabolites: Possibility of natural therapeutics against the COVID-19 pandemic. *Frontiers in Medicine*, *7*, 1–26.

Boukhebza, H., Bellon, N., Limacher, J. M., & Inchauspé, G. (2012). Therapeutic vaccination to treat chronic infectious diseases: Current clinical developments using MVA-based vaccines. *Human Vaccines & Immunotherapeutics*, *8*(12), 1746–1757.

Bousfiha, A., Mejrhit, N., Azdad, O., El Kabbaoui, M., Chda, A., Tazi, A., … Aarab, L. (2016). Immunomodulating properties of protein fractions isolated from *Delphinium staphysagria* seeds. *Journal of Medicinal Plant Research*, *10*(3), 29–34.

Burns, B. J. J., Zhao, L., Taylor, E. W., & Spelman, K. (2010). The influence of traditional herbal formulas on cytokine activity. *Toxicology*, *278*(1), 140–159.

Canton, H. (2021). Food and agriculture organization of the United Nations—FAO. In *The Europa directory of international organizations 2021* (pp. 297–305). Oxfordshire: Milton Park, Abingdon-on-Thames.

Chalamaiah, M., Yu, W., & Wu, J. (2018). Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review. *Food Chemistry*, *245*, 205–222.

Chandrashekar, P. M., & Venkatesh, Y. P. (2009). Identification of the protein components displaying immunomodulatory activity in aged garlic extract. *Journal of Ethnopharmacology*, *124*(3), 384–390.

Cornish, S. M., Wood, C. M., L'Abbé, M. R., Gilani, G. S., Cooke, G. M., Curran, I. H., & Xiao, C. W. (2011). Sex-and age-specific immunomodulatory effects of dietary soya protein isolate and isoflavones in rats. *British Journal of Nutrition*, *106*(5), 683–687.

Chionh, Y.-T., Wee, J. L. K., Every, A. L., Ng, G. Z., & Sutton, P. (2009). M-cell targeting of whole killed bacteria induces protective immunity against gastrointestinal pathogens. *Infection and Immunity*, *77*(7), 2962–2970.

Christensen, D. (2016). Vaccine adjuvants: Why and how. *Human Vaccines & Immunotherapeutics*, *12*(10), 2709–2711.

Clement, F., Pramod, S. N., & Venkatesh, Y. P. (2010). Identity of the immunomodulatory proteins from garlic (Allium sativum) with the major garlic lectins or agglutinins. *International Immunopharmacology*, *10*(3), 316–324.

Clemente, A., MacKenzie, D. A., Johnson, I. T., & Domoney, C. (2004). Investigation of legume seed protease inhibitors as potential anticarcinogenic proteins. *Publication-European Association for Animal Production*, *110*, 137–142.

Coffman, R. L., Sher, A., & Seder, R. A. (2010). Vaccine adjuvants: Putting innate immunity to work. *Immunity*, *33*(4), 492–503.

Cole, K. S., Steckbeck, J. D., Rowles, J. L., Desrosiers, R. C., & Montelaro, R. C. (2004). Removal of N-linked glycosylation sites in the V1 region of simian immunodeficiency virus gp120 results in redirection of B-cell responses to V3. *Journal of Virology*, *78*(3), 1525–1539.

Cruz-Chamorro, I., Álvarez-Sánchez, N., del Carmen Millán-Linares, M., del Mar Yust, M., Pedroche, J., Millán, F., … Carrillo-Vico, A. (2019). Lupine protein hydrolysates decrease the inflammatory response and improve the oxidative status in human peripheral lymphocytes. *Food Research International*, *126*, 108585.

Cruz-Chamorro, I., Álvarez-Sánchez, N., Santos-Sánchez, G., Pedroche, J., Fernández-Pachón, M. S., Millán, F., … Carrillo-Vico, A. (2020). Immunomodulatory and antioxidant properties of wheat gluten protein hydrolysates in human peripheral blood mononuclear cells. *Nutrients*, *12*(6), 1–12.

Da Silva, F. T., Di Pasquale, A., Yarzabal, J. P., & Garçon, N. (2015). Safety assessment of adjuvanted vaccines: Methodological considerations. *Human Vaccines & Immunotherapeutics*, *11*(7), 1814–1824.

Da Silva, T. A., Oliveira-Brito, P. K. M., de Oliveira Thomaz, S. M., & Roque-Barreira, M. C. (2020). ArtinM: Purification and evaluation of biological activities. In *Lectin purification and analysis* (pp. 349–358). Humana, New York: Springer.

Daoudi, A., Aarab, L., & Abdel-Sattar, E. (2013). Screening of immunomodulatory activity of total and protein extracts of some Moroccan medicinal plants. *Toxicology and Industrial Health*, *29*(3), 245–253.

Daoudi, A., Abdel-Satter, E., & Aarab, L. (2014). The relationship between lectin compounds and immunomodulatory activities of protein extracted from plants. *Journal of Plant Studies*, *3*(1), 56.

De Lorenzo, G., Ferrari, S., Cervone, F., & Okun, E. (2018). Extracellular DAMPs in plants and mammals: Immunity, tissue damage and repair. *Trends in Immunology*, *39*(11), 937–950.

Del Giudice, G., Rappuoli, R., & Didierlaurent, A. M. (2018). Correlates of adjuvanticity: A review on adjuvants in licensed vaccines. *Seminars in Immunology*, *39*, 14–21.

Dominguez-Vega, E., Kotkowska, O., Garcia, M. C., Crego, A. L., & Marina, M. L. (2011). Fast determination of the functional peptide soymetide in different soybean derived foods by capillary-high performance liquid chromatography. *Journal of Chromatography A*, *1218*(30), 4928–4933.

Dong, J. C., & Kobinger, G. P. (2013). Hypothesis driven development of new adjuvants: Short peptides as immunomodulators. *Human Vaccines & Immunotherapeutics*, *9*(4), 808–811.

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia*, *77*(2), 67–82.

El Youbi, A. E. L. H., Ouahidi, I., & Aarab, L. (2012). In vitro immunomodulation effects of the aqueous and protein extracts of *Berberis hispanica* Boiss and Reut. (Family Berberidaceae). *Journal of Medicinal Plants Research*, *6*(25), 4239–4246.

Evans, R., Shultz, L. D., Dranoff, G., Fuller, J. A., & Kamdar, S. J. (1998). CSF-1 regulation of I6 gene expression by murine macrophages: A pivotal role for GM-CSF. *Journal of Leukocyte Biology*, *64*(6), 810–816.

Faries, M. B., Hsueh, E. C., Ye, X., Hoban, M., & Morton, D. L. (2009). Effect of granulocyte/macrophage Colony-stimulating factor on vaccination with an allogeneic whole-cell melanoma vaccine. *Clinical Cancer Research*, *15*(22), 7029–7035.

Fendler, A., de Vries, E. G., Geurtsvan Kessel, C. H., Haanen, J. B., Wörmann, B., Turajlic, S., & von Lilienfeld-Toal, M. (2022). COVID-19 vaccines in patients with cancer: Immunogenicity, efficacy and safety. *Nature Reviews. Clinical Oncology*, *19*, 1–17.

Fradkin, A. H., Carpenter, J. F., & Randolph, T. W. (2011). Glass particles as an adjuvant: A model for adverse immunogenicity of therapeutic proteins. *Journal of Pharmaceutical Sciences*, *11*, 4953–4964.

Goldenthal, K. L., Cavagnaro, J. A., Alving, C. R., & Vogel, F. R. (1993). National cooperative vaccine development working group. Safety evaluation of vaccine adjuvants. *AIDS Research and Human Retroviruses*, *9*, S45–S49.

Gupta, A., & Chaphalkar, S. R. (2015a). Vaccine adjuvants: The current necessity of life. *Shiraz E Medical Journal*, *16*(7), e28061.

Gupta, A., & Chaphalkar, S. R. (2015b). Use of flow cytometry to measure the immunostimulatory activity of aqueous extract of *Jasminum auriculatum*. *International Journal of Current Advanced Research*, *4*(5), 87–91.

Gupta, R. K., & Siber, G. R. (1995). Adjuvants for human vaccines-current status, problems and future prospects. *Vaccine*, *13*(14), 1263–1276.

Hamilton, J. A. (2002). GM-CSF in inflammation and autoimmunity. *Trends in Immunology*, *23*(8), 403–408.

Haque, E., Chand, R., & Kapila, S. (2008). Biofunctional properties of bioactive peptides of milk origin. *Food Reviews International*, *25*(1), 28–43.

Harandi, A. M., Medaglini, D., & Shattock, R. J. (2010). Vaccine adjuvants: A priority for vaccine research. *Vaccine*, *28*(12), 2363–2366.

Harris, J. M., Martin, N. E., & Modi, M. (2001). Pegylation: A novel process for modifying pharmacokinetics. *Clinical Pharmacokinetics*, *40*(7), 539–551.

Hartmann, R., & Meisel, H. (2007). Food-derived peptides with biological activity: From research to food applications. *Current Opinion in Biotechnology*, *l18*(2), 163–169.

Ho, N. I., Huis In't Veld, L. G. M., Raaijmakers, T. K., & Adema, G. J. (2018). Adjuvants enhancing cross-presentation by dendritic cells: The key to more effective vaccines? *Frontiers in Immunology*, *9*, 2874.

Hobernik, D., & Bros, M. (2018). DNA vaccines—How far from clinical use? *International Journal of Molecular Sciences*, *19*(11), 3605.

Horiguchi, N., Horiguchi, H., & Suzuki, Y. (2005). Effect of wheat gluten hydrolysate on the immune system in healthy human subjects. *Bioscience, Biotechnology, and Biochemistry*, *69*(12), 2445–2449.

Izzo, A. A., Hoon-Kim, S., Radhakrishnan, R., & Williamson, E. M. (2016). A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies. *Phytotherapy Research*, *30*(5), 691–700.

Izzo, A. A., Teixeira, M., Alexander, S. P., Cirino, G., Docherty, J. R., George, C. H., … Sobey, C. G. (2020). A practical guide for transparent reporting of research on natural products in the British Journal of Pharmacology: Reproducibility of natural product research. *British Journal of Pharmacology*, *177*(10), 2169–2178.

Jain, S., O'Hagan, D. T., & Singh, M. (2011). The long-term potential of biodegradable poly(lactide-co-glycolide) microparticles as the next-generation vaccine adjuvant. *Expert Review of Vaccines*, *10*, 1731–1742.

Jakobsen, H., Saeland, E., Gizurarson, S., Schulz, D., & Jónsdóttir, I. (1999). Intranasal immunization with pneumococcal polysaccharide conjugate vaccines protects mice against invasive pneumococcal infections. *Infection and Immunity*, *67*(8), 4128–4133.

Khajuria, A., Gupta, A., Malik, F., Singh, S., Singh, J., Gupta, B. D., … Ella, K. (2007). A new vaccine adjuvant (BOS 2000) a potent enhancer mixed Th1/Th2 immune responses in mice immunized with HBsAg. *Vaccine*, *25*(23), 4586–4594.

Kiewiet, M. B. G., Faas, M. M., & de Vos, P. (2018). Immunomodulatory protein hydrolysates and their application. *Nutrients*, *10*(7), 904.

Kishimoto, T. (2006). Interleukin-6: Discovery of a pleiotropic cytokine. *Arthritis Research and Therapy*, *8*(2), 1–6.

Kitts, D. D., & Weiler, K. (2003). Bioactive proteins and peptides from foodsources. Applications of bioprocesses used in isolation and recovery. *Current Pharmaceutical Design*, *9*(16), 1309–1323.

Kuan, Y. C., Wu, T. J., Kuo, C. Y., Hsu, J. C., Chang, W. Y., & Sheu, F. (2011). Molecular cloning of a new immunomodulatory protein from *anoectochilus formosanus* which induces B cell IgM secretion through a T-independent mechanism. *PLoS One*, *6*(6), e21004.

Kurokawa, K., Ishii, T., An, W., Kanazawa, Y., Ozawa, M., Ichiyanagi, T., … Nakaya, K. (2011). A heat-stable extract from mucuna stimulates the differentiation of bone marrow cells into dendritic cells and induces apoptosis in cancer cells. *Nutrition and Cancer*, *63*(1), 100–108.

Laere, E., Ling, A. P. K., Wong, Y. P., Koh, R. Y., Mohd Lila, M. A., & Hussein, S. (2016). Plant-based vaccines: Production and challenges. *Journal of Botany*, *10*(1), 100–111.

Lakshmi, P. K., Kumar, S., Pawar, S., Sudheesh, M. S., & Pawar, R. S. (2018). Plant-based adjuvant in vaccine immunogenicity: A review. *Current Traditional Medicine*, *4*(3), 215–236.

Lavelle, E. C., Grant, G., Pfuller, U., & O'Hagan, D. T. (2004). Immunological implications of the use of plant lectins for drug and vaccine targeting to the gastrointestinal tract. *Journal of Drug Targeting*, *12*(2), 89–95.

Lavelle, E. C., Grant, G., Pusztai, A., Pfüller, U., & O'Hagan, D. T. (2000). Mucosal immunogenicity of plant lectins in mice. *Immunology*, *99*(1), 30–37.

Lavelle, E. C., Grant, G., Pusztai, A., Pfüller, U., & O'Hagan, D. T. (2001). The identification of plant lectins with mucosal adjuvant activity. *Immunology*, *102*(1), 77–86.

Le Bon, A., Schiavoni, G., D'Agostino, G., Gresser, I., Belardelli, F., & Tough, D. F. (2001). Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells *in vivo*. *Immunity*, *14*(4), 461–470.

Leavy, O., Mcneela, E., & Mills, K. H. G. (2002). Mistletoe lectins enhance immune responses to intranasally co-administered herpes simplex virus glycoprotein D2. *Immunology*, *107*(2), 268–274.

Lee, J. Y., Kim, J. Y., Lee, Y. G., Byeon, S. E., Kim, B. H., Rhee, M. H., … Cho, J. Y. (2007). *In vitro* immunoregulatory effects of Korean mistletoe lectin on functional activation of monocytic and macrophage-like cells. *Biological and Pharmaceutical Bulletin*, *30*(11), 2043–2051.

Lee, S., & Nguyen, M. T. (2015). Recent advances of vaccine adjuvants for infectious diseases. *Immune Network*, *15*(2), 51–57.

Lico, C., Santi, L., Twyman, R. M., Pezzotti, M., & Avesani, L. (2012). The use of plants for the production of therapeutic human peptides. *Plant Cell Reports*, *31*(3), 439–451.

Liu, Y., Reidler, H., Pan, J., Milunic, D., Qin, D., Chen, D., … Yin, R. (2011). A double antigen bridging immunogenicity ELISA for the detection of antibodies to polyethylene glycol polymers. *Journal of Pharmacological and Toxicological Methods*, *64*(3), 238–245.

Ma, Y. H., Cheng, W. Z., Gong, F., Ma, A. L., Yu, Q. W., Zhang, J. Y., … Zhang, D. Q. (2008). Active Chinese mistletoe lectin-55 enhances colon cancer surveillance through regulating innate and adaptive immune responses. *World Journal of Gastroenterology*, *14*(34), 5274–5281.

Makwana, P., Kalyani, I., Desai, D., Patel, D., Sakhare, P., & Muglikar, D. (2018). Role of adjuvants in vaccine preparation: A review. *International Journal of Current Microbiology and Applied Sciences*, *7*(11), 972–988.

Massa, S., & Franconi, R. (2012). Plant genes and plant proteins as adjuvants in cancer vaccination. *Medicinal and Aromatic Plant Science and Biotechnology*, *6*, 1–9.

Mbawuike, I. N., Wyde, P. R., & Anderson, P. M. (1990). Enhancement of the protective efficacy of inactivated influenza A virus vaccine in aged mice by IL-2 liposomes. *Vaccine*, *8*(4), 347–352.

Miroshnichenko, S., Tripp, J., Nieden, U. Z., Neumann, D., Conrad, U., & Manteuffel, R. (2005). Immunomodulation of function of small heat shock proteins prevents their assembly into heat stress granules and results in cell death at sublethal temperatures. *The Plant Journal*, *41*(2), 269–281.

Mohan, T., Verma, P., & Nageswara Rao, D. (2013). Novel adjuvants & delivery vehicles for vaccines development: A road ahead. *Indian Journal of Medical Research*, *138*, 779–795.

Moingeon, P., Haensler, J., & Lindberg, A. (2001). Towards the rational design of Th1 adjuvants. *Vaccine*, *19*(31), 4363–4372.

Morel, P. A., & Turner, M. S. (2010). Designing the optimal vaccine: The importance of cytokines and dendritic cell. *Open Vaccine Journal*, *3*(1), 7–17.

Mountforda, M. S. A. (2000). IL-18 potentiates the adjuvant properties of IL-12 in the induction of a strong Th1 type immune response against a recombinant antigen. *Vaccine*, *18*(19), 2002–2008.

Moutia, M., Habti, N., & Badou, A. (2018). *In vitro* and *In vivo* immunomodulator activities of *Allium sativum* L. *Evidence-based Complementary and Alternative Medicine*, *2018*, 1–10.

Nicholls, E. F., Madera, L., & Hancock, R. E. W. (2010). Immunomodulators as adjuvants for vaccines and antimicrobial therapy. *Annals of the New York Academy of Sciences*, *1213*(1), 46–61.

O'Hagan, D. T., Friedland, L. R., Hanon, E., & Didierlaurent, A. M. (2017). Towards an evidence based approach for the development of adjuvanted vaccines. *Current Opinion in Immunology*, *47*, 93–102.

Okada, E., Sasaki, S., Ishii, N., Aoki, I., Yasuda, T., Nishioka, K., … Okuda, K. (1997). Intranasal immunization of a DNA vaccine with IL-12- and granulocyte-macrophage colony-stimulating factor (GM-CSF)- expressing plasmids in liposomes induces strong mucosal and cell- mediated immune responses against HIV-1 antigens. *Journal of Immunology*, *159*(7), 3638–3647.

Ong, G. H., Lian, B. S. X., Kawasaki, T., & Kawai, T. (2021). Exploration of pattern recognition receptor agonists as candidate adjuvants. *Frontiers in Cellular and Infection Microbiology*, *11*, 1–17.

Ooi, L. S., Liu, F., Ooi, V. E., Ng, T. B., & Fung, M. C. (2002). Gene expression of immunomodulatory cytokines induced by *Narcissus tazetta* lectin in the mouse. *Biochemistry and Cell Biology*, *80*(2), 271–277.

Padiyappa, S. D., Avalappa, H., Somegowda, M., Sridhara, S., Venkatesh, Y. P., Prabhakar, B. T., … El-sabrout, A. M. (2022). Immunoadjuvant and humoral immune responses of Garlic (*Allium sativum*

L.) lectins upon systemic and mucosal administration in BALB/c Mice. *Molecules*, *27*(4), 1375.

Pashine, A., Valiante, N. M., & Ulmer, J. B. (2005). Targeting the innate immune response with improved vaccine adjuvants. *Nature Medicine*, *11*(4S), S63–S68.

Patel, A., Dong, J. C., Trost, B., Richardson, J. S., Tohme, S., Babiuk, S., … Kobinger, G. P. (2012). Pentamers not found in the universal proteome can enhance antigen specific immune responses and adjuvant vaccines. *PLoS One*, *7*(8), 1–13.

Petitdemange, C., Kasturi, S. P., Kozlowski, P. A., Nabi, R., Quarnstrom, C. F., Reddy, P. B. J., … Kovalenkov, Y. O. (2019). Vaccine induction of antibodies and tissue-resident $CD8^{+}$ T cells enhances protection against mucosal SHIV-infection in young macaques. *JCI Insight*, *4*(4), e126047.

Pulendran, B., & Ahmed, R. (2011). Immunological mechanisms of vaccination. *Nature Immunology*, *12*(6), 509–517.

Pulendran, B., Prabhu, S. A., & O'Hagan, D. T. (2021). Emerging concepts in the science of vaccine adjuvants. *Nature Reviews Drug Discovery*, *20*(6), 454–475.

Purcell, A. W., McCluskey, J., & Rossjohn, J. (2007). More than one reason to rethink the use of peptides in vaccine design. *Nature Reviews Drug Discovery*, *6*(5), 404–414.

Rappuoli, R., Pizza, M., Del Giudice, G., & De Gregorio, E. (2014). Vaccines, new opportunities for a new society. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(34), 12288–12293.

Reed, S. G., Orr, M. T., & Fox, C. B. (2013). Key roles of adjuvants in modern vaccines. *Nature Medicine*, *19*(12), 1597–1608.

Reyna-Margarita, H.-R., Irais, C.-M., Mario-Alberto, R.-G., Agustina, R.-M., Luis-Benjamín, S.-G., & David, P.-E. (2019). Plant Phenolics and lectins as vaccine adjuvants. *Current Pharmaceutical Biotechnology*, *20*(15), 1236–1243.

Rüthrich, M. M., Giesen, N., Mellinghoff, S. C., Rieger, C. T., & von Lilienfeld-Toal, M. (2022). Cellular immune response after vaccination in patients with cancer—Review on past and present experiences. *Vaccine*, *10*(2), 182.

Sander, V. A., Corigliano, M. G., & Clemente, M. (2019). Promising plant-derived adjuvants in the development of coccidial vaccines. *Frontiers in Veterinary Science*, *6*, 20.

Santiago-López, L., Hernández-Mendoza, A., Vallejo-Cordoba, B., Mata-Haro, V., & González-Córdova, A. F. (2016). Food-derived immunomodulatory peptides. *Journal of the Science of Food and Agriculture*, *96*(11), 3631–3641.

Savelkoul, H. F. J., Ferro, V. A., Strioga, M. M., & Schijns, V. E. J. C. (2015). Choice and design of adjuvants for parenteral and mucosal vaccines. *Vaccine*, *3*(1), 148–171.

Sawaki, K., Takaoka, I., Sakuraba, K., & Suzuki, Y. (2004). Effects of distance running and subsequent intake of glutamine rich peptide on biomedical parameters of male Japanese athletes. *Nutrition Research*, *24*(1), 59–71.

Saylor, K., Gillam, F., Lohneis, T., & Zhang, C. (2020). Designs of antigen structure and composition for improved protein-based vaccine efficacy. *Frontiers in Immunology*, *11*, 283.

Schijns, V., Fernández-Tejada, A., Barjaktarović, Ž., Bouzalas, I., Brimnes, J., Chernysh, S., … Nativi, C. (2020). Modulation of immune responses using adjuvants to facilitate therapeutic vaccination. *Immunological Reviews*, *296*(1), 169–190.

Schijns, V., Majhen, D., Van Der Ley, P., Thakur, A., Summerfield, A., Berisio, R., … Zamyatina, A. (2021). Rational vaccine design in times of emerging diseases: The critical choices of immunological correlates of protection, vaccine antigen and immunomodulation. *Pharmaceutics*, *13*(4), 501.

Schillberg, S., Raven, N., Spiegel, H., Rasche, S., & Buntru, M. (2019). Critical analysis of the commercial potential of plants for the production of recombinant proteins. *Frontiers in Plant Science*, *10*, 720.

Schwestka, J., Tschofen, M., Vogt, S., Marcel, S., Grillari, J., Raith, M., … Stoger, E. (2020). Plant-derived protein bodies as delivery vehicles for recombinant proteins into mammalian cells. *Biotechnology and Bioengineering*, *117*(4), 1037–1047.

Sesardic, D., Rijpkema, S., & Patel, B. P. (2007). New adjuvants: EU regulatory developments. *Expert Review of Vaccines*, *6*(5), 849–861.

Shah, S., & Abraham, E. (1992). Intranasal immunization with liposomes containing IL-2 enhances bacterial polysaccharide antigen-specific pulmonary secretory antibody response. *Journal of Immunology*, *149*(11), 3719–3726.

Sheu, S.-C., & Lai, M.-H. (2012). Composition analysis and immunomodulatory effect of okra (*Abelmoschus esculentus* L.) extract. *Food Chemistry*, *134*(4), 1906–1911.

Singh, M., & O'Hagan, D. T. (2002). Recent advances in vaccine adjuvants. *Pharmaceutical Research*, *19*(6), 715–728.

Smart, R. C., Ryan, F. P., Holdworth, C. D., & Preston, F. E. (1978). Relationship between splenic size and splenic function. *Gut*, *19*(1), 56–59.

Smith, T. R. F., Patel, A., Ramos, S., Elwood, D., Zhu, X., Yan, J., … Broderick, K. E. (2020). Immunogenicity of a DNA vaccine candidate for COVID-19. *Nature Communications*, *11*(1), 1–13.

Spreafico, F., Malfiore, C., Moras, M. L., Marmonti, L., Filippeschi, S., Barbieri, L., … Stirpe, F. (1983). The immunomodulatory activity of the plant proteins *Momordica charantia* inhibitor and pokeweed antiviral protein. *International Journal of Immunopharmacology*, *5*(4), 335–343.

Sotillo, E., Barrett, D. M., Black, K. L., Bagashev, A., Oldridge, D., Wu, G., … Martinez, N. M. (2015). Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discovery*, *5*(12), 1282–1295.

Souza, M. A., Carvalho, F. C., Ruas, L. P., Ricci-azevedo, R., & Roque-barreira, M. C. (2013). The immunomodulatory effect of plant lectins: A review with emphasis on ArtinM properties. *Glycoconjugate Journal*, *30*(7), 641–657.

Staats, H. F., & Ennis, F. A. (1999). IL-1 is an effective adjuvant for mucosal and systemic immune responses when coadministered with protein immunogens. *Journal of Immunology*, *162*(10), 6141–6147.

Takahashi, M., Moriguchi, S., Yoshikawa, M., & Sasaki, R. (1994). Isolation and characterization of oryzatensin: A novel bioactive peptide with ileum-contracting and immunomodulating activities derived from rice albumin. *Biochemistry and Molecular Biology International*, *33*(6), 1151–1158.

Tannock, G. A., Kim, H., & Xue, L. (2020). Why are vaccines against many human viral diseases still unavailable; an historic perspective? *Journal of Medical Virology*, *92*(2), 129–138.

Teixeira, C. R., Cavassani, K. A., Gomes, R. B., Teixeira, M. J., Roque-Barreira, M. C., Cavada, B. S., … Barral-Netto, M. (2006). Potential of KM+ lectin in immunization against Leishmania amazonensis infection. *Vaccine*, *24*(15), 3001–3008.

Tsung, K., & Norton, J. A. (2016). In situ vaccine, immunological memory and cancer cure. *Human Vaccines & Immunotherapeutics*, *12*(1), 117–119.

Tsuruki, T., Kishi, K., Takahashi, M., Tanaka, M., Matsukawa, T., & Yoshikawa, M. (2003). Soymetide, an immunostimulating peptide derived from soybean beta-conglycinin, is an fMLP agonist. *FEBS Letters*, *540*(1–3), 206–210.

Udenigwe, C. C., & Aluko, R. E. (2012). Food protein-derived bioactive peptides: Production, processing and potential health benefits. *Journal of Food Science*, *71*(1), R11–R24.

Unitt, J., & Hornigold, D. (2011). Plant lectins are novel toll-like receptor agonists. *Biochemical Pharmacology*, *81*(11), 1324–1328.

US Department of Health and Human Services, Food and Drug Administration and Center for Drug Evaluation and Research, 2014. Guidance for industry immunogenicity assessment for therapeutic protein products.

Valverde, J. M., Rodríguez, K., Herrera, M., Segura, Á., Vargas, M., Villalta, M., … León, G. (2017). Comparison of the adjuvant activity of

emulsions with different physicochemical properties on the antibody response towards the venom of west African carpet viper (*Echis ocellatus*). *Toxicon*, *127*, 106–111.

Venkatalakshmi, P., Vadivel, V., & Brindha, P. (2016). Role of phytochemicals as immunomodulatory agents: A review. *International Journal of Green Pharmacy*, *10*(1), 1–18.

Vigila, A. G., & Baskaran, X. (2008). Immunomodulatory effect of coconut protein on cyclophosphamide induced immune suppressed swiss albino mice. *Ethnobotanical Leaflets*, *2008*(1), 160.

Viel, K. R., Ameri, A., Abshire, T. C., Iyer, R. V., Watts, R. G., Lutcher, C., … Kasper, C. K. (2009). Inhibitors of factor VIII in black patients with hemophilia. *New England Journal of Medicine*, *360*(16), 1618–1627.

Vital, D. A. L., De Mejia, E. G., Dia, V. P., & Loarca-Piña, G. (2014). Peptides in common bean fractions inhibit human colorectal cancer cells. *Food Chemistry*, *157*, 347–355.

Vrba, S. M., Kirk, N. M., Brisse, M. E., Liang, Y., & Ly, H. (2020). Development and applications of viral vectored vaccines to combat zoonotic and emerging public health threats. *Vaccine*, *8*(4), 680.

Wadhwa, A., Aljabbari, A., Lokras, A., Foged, C., & Thakur, A. (2020). Opportunities and challenges in the delivery of mRNA-based vaccines. *Pharmaceutics*, *12*(2), 102.

Wagar, L. E., Salahudeen, A., Constantz, C. M., Wendel, B. S., Lyons, M. M., Mallajosyula, V., … Jackson, K. J. (2021). Modeling human adaptive immune responses with tonsil organoids. *Nature Medicine*, *27*(1), 125–135.

Wakankar, A. A., & Borchardt, R. T. (2006). Formulation considerations for proteins susceptible to asparagine deamidation and aspartate isomerization. *Journal of Pharmaceutical Sciences*, *95*(11), 2321–2336.

Wallis, J., Shenton, D. P., & Carlisle, R. C. (2019). Novel approaches for the design, delivery and administration of vaccine technologies. *Clinical and Experimental Immunology*, *196*(2), 189–204.

Wang, Z. B., & Xu, J. (2020). Better adjuvants for better vaccines: Progress in adjuvant delivery systems, modifications, and adjuvant–antigen codelivery. *Vaccine*, *8*(1), 128.

Woods, N., Niwasabutra, K., Acevedo, R., Igoli, J., Altwaijry, N. A., Tusiimire, J., … Ferro, V. A. (2017). Natural vaccine adjuvants and Immunopotentiators derived from plants, fungi, marine organisms, and insects. In *Immunopotentiators in Modern Vaccines* (2nd ed., pp. 211–229). London: Academic Press.

Xu, Z., Mao, T. M., Huang, L., Yu, Z. C., Yin, B., Chen, M. L., & Cheng, Y. H. (2019). Purification and identification immunomodulatory peptide from rice protein hydrolysates. *Food and Agricultural Immunology*, *30*(1), 150–162.

Yang, Q., Cai, X., Huang, M., Jia, L., & Wang, S. (2019). Immunomodulatory effects of: *Pseudostellaria heterophylla* peptide on spleen lymphocytes via a Ca2+/CaN/NFATc1/IFN-γ pathway. *Food and Function*, *10*(6), 3466–3476.

Yang, Y., & Lundqvist, A. (2020). Immunomodulatory effects of IL-2 and IL-15; implications for cancer immunotherapy. *Cancers*, *12*(12), 3586.

Yang, Q., Cai, X., Huang, M., & Wang, S. (2020b). A specific peptide with immunomodulatory activity from *Pseudostellaria heterophylla* and the action mechanism. *Journal of Functional Foods*, *68*, 103887.

Yang, Q., Cai, X., Huang, M., Chen, X., Tian, Y., Chen, G., … Xiao, J. (2020a). Isolation, Identification, and Immunomodulatory effect of a Peptide from *Pseudostellaria heterophylla* Protein Hydrolysate. *Journal of Agricultural and Food Chemistry*, *68*(44), 12259–12270.

Yatim, N., Jusforgues-Saklani, H., Orozco, S., Schulz, O., Barreira da Silva, R., Sousa, C. R. E., … Albert, M. L. (2015). RIPK1 and NF-κB signaling in dying cells determines cross-priming of $CD8^+$ T cells. *Science*, *350*(6258), 328–334.

Yimit, D., Hoxur, P., Amat, N., Uchikawa, K., & Yamaguchi, N. (2012). Effects of soybean peptide on immune function, brain function, and neurochemistry in healthy volunteers. *Nutrition*, *28*(2), 154–159.

Yoon, T. J., Yoo, Y. C., Kang, T. B., Her, E., Kim, S. H., Kim, K., … Kim, J. B. (2001). Cellular and humoral adjuvant activity of lectins isolated from Korean mistletoe (*Viscum album colaratum*). *International Immunopharmacology*, *1*(5), 881–889.

Zininga, T., Ramatsui, L., & Shonhai, A. (2018). Heat shock proteins as immunomodulants. *Molecules*, *23*(11), 2846.

**How to cite this article:** Nazeam, J. A., & Singab, A. N. B. (2022). Immunostimulant plant proteins: Potential candidates as vaccine adjuvants. *Phytotherapy Research*, 1–16. https://doi.org/10.1002/ptr.7624