HUMAN VACCINES & IMMUNOTHERAPEUTICS
2020, VOL. 16, NO. 12, 2944–2953
https://doi.org/10.1080/21645515.2020.1833579

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MINI-REVIEW



# Exploring the possible use of saponin adjuvants in COVID-19 vaccine

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**ABSTRACT**

There is an urgent need for a safe, efficacious, and cost-effective vaccine for the coronavirus disease 2019 (COVID-19) pandemic caused by novel coronavirus strain, severe acute respiratory syndrome-2 (SARS-CoV -2). The protective immunity of certain types of vaccines can be enhanced by the addition of adjuvants. Many diverse classes of compounds have been identified as adjuvants, including mineral salts, microbial products, emulsions, saponins, cytokines, polymers, microparticles, and liposomes. Several saponins have been shown to stimulate both the Th1-type immune response and the production of cytotoxic T lymphocytes against endogenous antigens, making them very useful for subunit vaccines, especially those for intracellular pathogens. In this review, we discuss the structural characteristics, mechanisms of action, structure–activity relationship of saponins, biological activities, and use of saponins in various viral vaccines and their applicability to a SARS-CoV-2 vaccine.

**ARTICLE HISTORY**
Received 16 July 2020
Revised 22 September 2020
Accepted 2 October 2020

**KEYWORDS**
Saponin adjuvant; immunostimulation; viral vaccines; COVID-19 vaccine

## Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by the novel coronavirus strain, severe acute respiratory syndrome CoV-2 (SARS-CoV-2), has created widespread global health concerns by rapidly spreading to more than 215 countries. To date, >30 million have been infected and >1 million are dead. Currently, there is no widely available specific treatment for this virus and the researchers face a challenge to develop a safe, efficacious, and cost-effective vaccine.[1,2] The protective immunity of vaccines can be enhanced by the addition of adjuvants. The addition of an adjuvant in the COVID-19 vaccine besides boosting immunogenicity would also reduce the requirement of vaccine protein per dosage. Many diverse classes of compounds have been used as adjuvants, including mineral salts, microbials products, emulsions, saponins, cytokines, polymers, microparticles, and liposomes. Based on their proposed mechanisms of action, vaccine adjuvants have been broadly divided in two different groups: (1) immunostimulants (e.g., saponins, Toll-like receptor (TLR) agonists, cytokines) and (2) delivery agents (e.g., emulsions, microparticles, mineral salts).[3] Immunostimulants activate the antigen-presenting cells (APCs) and promote the secretion of various cytokines. On the contrary, the delivery agents help to preserve the conformation of antigens (Ag) for proper presentation to the APCs and to provide a slow release for continuing immune stimulation. For instance, TLR agonists and other immunostimulatory substances enhance immune cell recruitment and cytokine secretion, whereas emulsions and mineral salts produce a depot effect at the injection site, resulting in a prolonged release of the antigen and continued stimulation of immune cells.[4] Saponins, the steroid or triterpenoid glycosides found in wild or cultivated plants, lower marine animals, and some bacteria, have immunostimulatory properties. Several saponins play critical role in stimulating both the Th1 immune response and the production of cytotoxic T lymphocytes against the exogenous antigens, thus have high potential to be used as ideal adjuvants in subunit vaccines and vaccines for the intracellular pathogens, as well as with therapeutic cancer vaccines.

Saponins are a diverse group of naturally occurring active compounds widely found in the plant kingdom and are active constituents in over 100 families of fungi, including endophytic terrestrial and marine fungi.[5] Structurally, saponins contain a triterpene or steroid aglycone called sapogenin, with one or more sugar chains attached to it. Steroidal saponins are present mainly in monocotyledons, while triterpenoid saponins occur in dicotyledon plants. Saponins exhibit emulsifying and foaming properties due to the presence of hydrophobic aglycone and hydrophilic sugar chains in their structure (amphiphilic nature).[6] Saponin foaming capacity is attributed to the combination of a hydrophobic (fat-soluble) sapogenin with a hydrophilic (water-soluble) sugar base.[7]

There is one saponin adjuvanted licensed vaccine approved by the FDA in the year 2017. It is the recombinant zoster vaccine (RZV, Shingrix, GlaxoSmithKline) containing AS01B which is a saponin-based adjuvant. It is a subunit vaccine that contains recombinant varicella herpes zoster virus glycoprotein E. The AS01 adjuvant system, consists of two immunostimulants, monophosphoryl lipid A (MPL) and QS-21 saponin. The QS-21 saponin is purified from the bark of the *Quillaja saponaria* Molina tree. It induces antigen-specific antibody as well as cell-mediated immune response. The MPL signals through Toll-like receptor-4 (TLR4), which results in the activation of APCs and the production of cytokines and interferons (IFNs).[8] This adjuvant system has been used in recently

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developed RTS, S/AS01 malaria vaccine, Mosquirix (phase 3 trial completed in 2019, vaccine approved in three pilot countries of South Africa, viz., Ghana, Malawi and Kenya, WHO), polyprotein HIV-1 candidate vaccine[9] and tuberculosis (Mtb72F/AS02 candidate) vaccine.[10] These vaccines have been administered to the susceptible population and the safety and efficacy evaluation of AS01 adjuvant is underway.[8]

Recently, saponin-based microemulsion adjuvant has also been studied in the vaccine for COVID-19. SARS-CoV-2 S1-Fc vaccine candidate with saponin microemulsion adjuvant developed high titers of S1 (recombinant protein)-specific neutralizing antibodies in cynomolgus monkeys.[11] The AS01 adjuvant system when co-administered with recombinant SARS-CoV S protein, induced high titers of antigen-specific serum antibodies and protected from viral infection.[12] Keeping in view the robust immune response induced by saponin adjuvants, it becomes important that saponin-based adjuvants be further explored for use in a subunit vaccine against COVID-19. Nevertheless, in the current situation, a proven safe and efficacious adjuvant should be used in the vaccine for SARS-CoV-2 to get rapid approval of the regulatory agencies. Therefore, saponin might not be preferred over other adjuvants, but definitely represents a viable alternative for SARS-CoV-2 subunit vaccine for long-term future use. In this review, we discuss the structural characteristics, mechanisms of action, structure–activity relationship of saponins, biological activities, use of saponins in various antiviral vaccines, and the possible use in a vaccine for SARS-CoV-2.

## Structural characteristics of saponins

Saponins belong to a class of compounds that contain a rigid skeleton of at least four hydrocarbon rings to which sugar chains are attached in groups of one or two (normally no more than 10 units) (Figure 1).[13,14] They may be classified as triterpenoid (C30) or steroid (C27) based on the number of carbon atoms present in the core (aglycone).[15] There are 11 major saponin classes and saponins containing the oleanane skeleton are the most common in the plant kingdom.[16,17] Saponins with the carbohydrate or oligosaccharide chains attached at position C-3 are monodesmosidic, while carbohydrate chains attached at two positions C-3 and C-26 or C-28 are bidesmosidic. Numerous forms of saponins can be derived from a variety of aglycones, carbohydrates, and different attachment positions. Both steroidal and triterpene saponins may contain other functional groups: – OH, – COOH, – CH3, which further add to their diversity.[18]

## Mechanism of action of adjuvants

Several specific groups of compounds, including mineral salts, microbial products, emulsions, saponins, cytokines, polymers, microparticles, and liposomes, have been classified as adjuvants.[19] The vaccine adjuvants have been narrowly divided into delivery systems and immunostimulatory adjuvants based on their suggested modes of action.[4] Nevertheless, recent progress in immunobiological research has revealed several mechanisms (Figure 2).[20]

## Depot effect

This is the most widely recognized mechanism of adjuvant action where the antigens are entrapped and then released slowly at the injection site. This slow delivery of antigens can enhance the continuous stimulation of the immune system to produce high antibody titers.[21] Depot formation was first observed with the alum adjuvants[22] and the antigens were detected for 2–3 weeks inside the granulomas produced by these alum compounds.[23] Various other adjuvants, such as water-in-oil emulsions [e.g., Complete Freund's Adjuvant (CFA)] and biodegradable micro- and nano-particles, have also been shown to act via the depot effect to produce prolonged and sustained high antibody titers.[24,25]

## Up-regulation of cytokines and chemokines

Recent studies of adjuvant mechanisms have focused on the recruitment of innate immune cells at the injection site. It has been shown that certain adjuvants establish a local pro-inflammatory environment necessary to recruit the immune

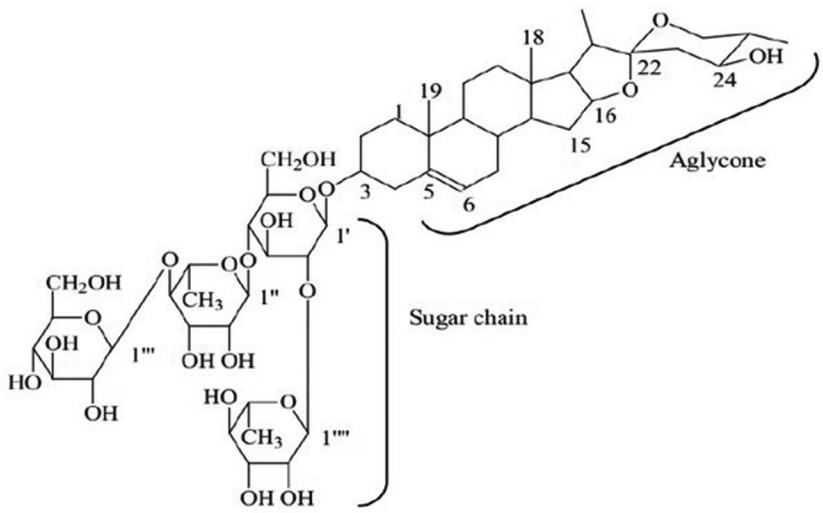


Figure 1. Chemical structure of steroid saponin.[13]

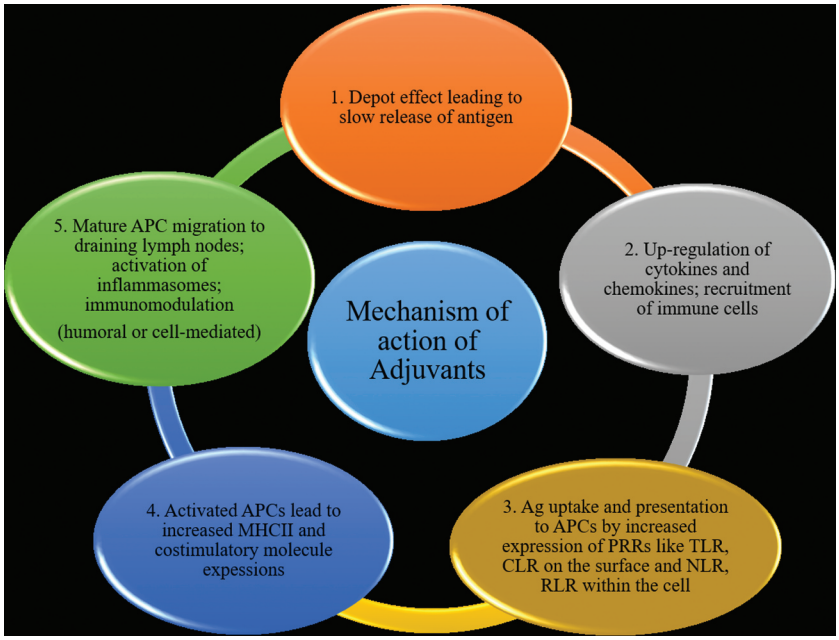


**Figure 2.** Mechanism of action of adjuvants.

**Table 1.** Activity exhibited by licensed adjuvants.

| Adjuvants | Activity | Reference |
|---|---|---|
| Alum | • Promote Th2 type immune responses.<br>• Robust antibody production and B-cell differentiation. | 28 |
| MF59 | • Mediate recruitment of immune cells at the injection site.<br>• Enhance the release of chemo-attractant like CCL2, CCL3 and CXCL8. | 29<br>30 |
| AS01 | • Promotes both antigen-specific antibodies and CD4 + T cells.<br>• CD4 + T cells express many cytokines such as IL-2, TNF-α, and IFNγ. | 8 |
| AS03 | • Enhanced recruitment of neutrophils, eosinophils, and monocytes at the site of injection.<br>• Antigen trafficking and draining in the lymph node. | 31 |
| AS04 | • Induces transient local NFkB activity and upregulate cytokine production.<br>• Up-regulates the pro-inflammatory genes and protein expression (IL-1, IL-6, IL-12, IL-18, and TNF-a). | 32–34 |
| CpG-1018 | • Targets TLR9, stimulate CD4+ and CD8 + T cells, Th1 helper T cells. | 35 |

cells.[26] At the injection site, adjuvant core response genes expressed by the immune cells are strongly upregulated and modulated by alum, CpG-ODN, and MF59 adjuvants. Activation of these core response genes promotes upregulation of cytokine and chemokine synthesis.[27] The activity exhibited by some of the common adjuvants have been shown in Table 1.

## Antigen cross-presentation

Some adjuvants such as alum, oil-based emulsions, and microparticles work by "targeting" antigens to the APCs, resulting in increased MHC antigen presentation.[36,37] Alum plays an important role by enhancing the antigen internalization by the APCs and limiting the rate of internalized antigen degradation.[38] QS-21, a purified saponin adjuvant from *Quillaja saponaria*, can activate both Th1 and $CD8^+$ T cells to produce a robust antibody and cell-mediated immune response.[39] One study showed that QS-21 can stimulate the production of IL-1β and IL-18 via activation of NLRP3 inflammasome in murine dendritic cells (DCs).[40] However, in the presence of QS-21, NLRP3-deficient mice showed higher levels of Th1 and Th2 antigen-specific T-cell responses, and increased IgG1 and IgG2c, suggesting a more complex regulatory role for NLRP3. Duewell and coworkers[41] demonstrated that subcutaneous administration of saponin-based adjuvant vaccines in mice resulted in mobilization and activation of immune cells in vaccine site-draining lymph nodes. This group also demonstrated an efficient antigen uptake by dendritic cells (DCs), DC maturation induction, and *in vivo* production of IL-12.

## Activation of inflammasomes

Inflammasomes are innate immune system receptors and sensors that control caspase-1 activation and induce inflammation in response to infectious microbes and host protein-derived molecules.[42] Innate immune cells express pattern-recognition receptors (PRRs) to identify various pathogens. The family of PRRs includes TLRs, C-type lectin-like receptors (CLRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), and retinoic acid-inducible gene-1 (RIG-1)-like receptors (RLR). NLRP3 inflammasome activation induces caspase-1, which, in turn, cleaves inactive proforms of IL-1β, IL-18, and IL-33 into their active forms.[43] Alum is one of the most widely used vaccine adjuvants; however, it is a poor inducer of cell-mediated immunity.[44,45] *In vitro*, alum and other inflammatory activators, such as lipopolysaccharide (LPS), upregulate the expression pro-IL-1β and NLRP3.[46,47]

QS-21, a highly purified and soluble saponin adjuvant currently used in licensed and exploratory vaccines, induces caspase-1-dependent release of IL-1β and IL-18 in APCs, such as macrophages and DCs, when co-stimulated with the TLR4-agonist

monophosphoryl lipid A adjuvant.[48] QS-21 elicits a strong antibody and cell-mediated immune response and is also a potent Th1 and $CD8^+$ T cell activator.[49,50] It is an adjuvant candidate for many current trials of vaccines, such as HIV-1,[51,52] cancer,[53,54] hepatitis B,[55] malaria.,[56,57] tuberculosis[58] and Alzeihmer's disease.[59] Due to the promising immunopotentiator effects exhibited by QS-21, it should be studied and evaluated as an adjuvant in a COVID 19 subunit vaccine as the SARS-CoV-2 could reemerge every year and new vaccines may be needed to combat it.

## Mechanisms of action of saponin adjuvants

Saponins exert their adjuvant activity via the immunostimulatory effects and by activation of cytokine production, e.g., interferons and interleukins.[60,61] It was proposed that saponins interact with APCs, activate intracellular signaling, and, therefore, enhance the cytokines release.[62] Saponins promote the entry of antigens via the endogenous pathway of antigen presentation and by enhancing cytotoxic T-lymphocyte mediated immune responses.[63] Adjuvant activity of saponin compounds is mainly due to the presence of aldehyde group and sugar side chain[64] or acyl residues in the aglycone portion of saponin.[61] Several saponins, such as lablabosides and soyasaponins, lack acyl group in their chemical structure and produce immunostimulatory activity with the help of sugar moiety.[65]

The lipophilic acyl side chain present in saponins can favor strong cytotoxic T lymphocyte (CTL) activation by the exogenous antigens and can promote hemolysis. Deacylation of this lipophilic side chain decreases the toxic hemolytic effect and activates Th2 immune response with simultaneous inactivation of Th1 response.[39] Saponin contains an aldehyde group at its C-4 position and acylated fucopyranosyl residue at its C-28 position.[66,67] Arabinose present at the terminal of acyl chain strongly elicits Th1/Th2 immunity with the CTL production. Furthermore, deacylation of these fucosyl pyranose molecules can solely trigger Th2 immunity.[68] The ε-amino group of the T-cell receptor forms an imine with the aldehyde group of the saponin. Activated receptors then trigger signals via tyrosyl phosphorylation leading to stimulation of mitogen-activated protein (MAP) kinase along with changes noticed in $K^+$ and $Na^+$ transport channels. Consequently, activated T-cells are biased toward Th1 immunity with increased production of Th1 cytokines.[62]

Whereas the *Quillaja* saponins adjuvants such as QS-21 act on dendritic cells (DCs) in a non-receptor-mediated manner. The exogenous protein antigens (ag) and QS-21 enter DCs by endocytosis, where QS-21 disrupts the endosomal membrane, allowing early escape of the antigens for further processing inside the cell into peptides. Properly degraded antigens are loaded into MHCI by the vacuolar pathway, while antigens that need more processing are transported to the cytosol to be cleaved by the proteasome (cytosolic pathway). These peptides are then transported to the endoplasmic reticulum (ER), which after additional processing are loaded into MHCI. Peptides derived from either the vacuolar or cytosolic pathways after binding to MHCI are presented on the DC surface to naive CD8 + T cells in a process called cross-presentation to yield CTLs.[69]

## Structure-activity relationship of saponins

### Aldehyde

The mechanism of saponin adjuvant activity was first correlated with the aldehyde group attached to the core aglycone. Formation of bond (Schiff base) between the aldehyde group of saponin and free amino groups of APCs was the first stage of immune-activating mechanism.[39] The proportion and conformation of the aldehyde group in the saponin molecule plays a crucial role in maintaining the integrity and strength of Th1 response. Axial aldehyde shifts the immune system toward the stimulation of humoral immune responses, whereas equatorial aldehyde produces cell-mediated immune responses.[70]

### Lipophilic acyl groups

Acyl residues of saponin can enhance the activation of CTL against exogenous antigens. Deacylation of saponins, such as QS-18 and QS-21, shows reduced antibody production and Th1 response compared to the acylated saponin, suggesting that the acyl residues are important for the activation of CTL-mediated immune response.[39]

### Sugar chains

The sugar chain linked at the C-28 position is essential for the initiation of immune-activating mechanism, as well as for the toxic hemolytic effect.[71] The amphipathic nature of saponins is the result of sapogenin (hydrophobic) and sugar chain (hydrophilic) presence in their chemical structure. The balance between these sapogenin (hydrophobic) and sugar chain (hydrophilic) properties is important for maintaining the adjuvanticity of saponins.[39]

### Biological activities

Saponins are a diverse group of glycosides with a wide range of biological properties. They are thought to be the main constituents of many plant-based drugs and traditional medicines, responsible for various pharmacological properties.[72]

### Hemolytic activity

Saponins have the ability to lyse the erythrocyte membrane. This property has led to the development of hemolytic assays to detect the presence of saponins in drugs or plant extracts. Hemolytic properties are generally attributed to the interaction between the saponins and the sterols in the erythrocyte membrane.[73] The undesirable hemolytic activity of the saponin molecules is mainly due to the presence of saccharide side chain and the acyl residues in the aglycone.[39] Cell membrane cholesterol enhances saponin attachment and induces pore formation. The level of cell permeabilization is highly influenced by the concentration and structure of saponin molecules.[74–77] As a result, formation of membrane pores allows ionic transportation[74] and protein mobility[78] between the inter- and intra-cellular spaces.

### Anti-inflammatory activity

Many saponins isolated from plant sources have an inhibitory effect on inflammation. Aescin, a triterpenoid saponin mixture isolated from *Aesculus hippocastanum* L. (Hippocastanaceae), is known to have anti-inflammatory, anti-edematous, and venotonic properties.[79] The loniceroside C, a triterpenoid saponin isolated from the *Lonicera japonica* Thunb. aerial parts (Caprifoliaceae), the medicinal plant known for centuries as an anti-inflammatory agent, demonstrated its anti-inflammatory activity *in vivo* when tested with croton oil in the mouse ear edema model.[80] A novel steroidal saponin isolated from the *Agave attenuate* Salm-Dyck (Agavaceae) leaves was evaluated for its anti-inflammatory activity using the capillary permeability assay.[81] The steroidal saponin inhibited the increase in vascular permeability caused by acetic acid, a standard model for the inflammatory reaction at first stage. Kim and coworkers[82] investigated the ginseng (Panax ginseng C.A. Mey., Araliaceae) saponins for their anti-complementary activity. Ginsenoside Ro and oleanolic acid showed that these saponins have the highest anti-complementary activity. They suggested that the anti-inflammatory activity of these saponins is mediated through the classical pathway of inflammation to anti-complementary action. A good correlation between the radical scavenging activity and a weak cytotoxicity against the murine monocytic macrophage cell line was produced *in vitro* by the hydroalcoholic extract of *Silene vulgaris*.[83] The saponin-enriched fraction of *S. vulgaris* showed lower *in vitro* hemolytic activity and cell cytotoxicity in the VERO cells and this could be studied further as a newer source of saponin adjuvant.[84]

### Antibacterial/antimicrobial activity

Saponins have also been shown to possess antimicrobial activity. Three butanol-extractable 5β-spirostan-3β-ol saponins have been shown to have antimicrobial activity against both prokaryotic and eukaryotic organisms at low cell densities. However, these saponins did not inhibit dense populations of microbial growth.[85] Saponins with tetraglycoside have stronger activity compared to the saponins with triglycoside. A new saponin jujubogenin, isolated from *Colubrina retusa* L. (Rhamnaceae), had antimycobacterial activity at minimum inhibitory concentration (MIC) of 10 μg/ml when tested against *Mycobacterium intracellulare*.[86] It has been documented that triterpenoid saponins from other sources, such as *Maesalanceolata, Maesachisia*, and *Maesaindica*, display direct virucidal activity against Newcastle disease virus, vaccinia virus, and herpes simplex virus.[87] Several saponins, for example aescine from *Aesculus hippocastanum*, primula saponin from *Primula veris*, saikosaponin A from *Bupleurum falcatum*, theasaponin from *Thea sinensis*, and gymnemic acid from *Gymnema sylvestre*, showed antagonist activity against influenza A2 virus.[88,89]Arganine C, the saponin isolated from the fruit of *Tieghemella heckelii* Pierre ex A. Chev. (Sapotaceae), showed antiviral activity against HIV virus.[90] Arganine C saponin strongly inhibited HIV entry into the cells during the cell fusion test, and did not show substantial cytotoxicity to HeLa-CD4$^+$ cells. Mixture of maesasaponin isolated from *Maesa lanceolata* Forssk. (Myrsinaceae) has been reported to have anti-herpes simplex type 1 virus (HSV-1) and type 1 poliovirus[91] properties. Triterpenoid saponin isolated from the family Fabaceae also showed antiviral activity against herpes viruses. Activity of anti-herpes simplex virus has been found to be related to the chemical structure of fabaceous saponins. The sugar moiety of this saponin has a glucosyl unit in the central part, exerting a better antiviral activity.[92] Simoes et al.[93] tested two triterpenoid saponins, oleanane and ursane from Brazilian and Chinese plants, for their antiviral activity. The oleanane-type saponins inhibited the DNA synthesis of herpes simplex virus type 1, whereas the ursane-type saponin inhibited the capsid protein synthesis of HSV type 1.

### Immunomodulatory activity

Bushneva and coworkers[94] showed that pectic polysaccharide named silenan, isolated from the aerial parts of *S. vulgaris*, possessed immunomodulatory activity. Ghonime and coworkers[95] confirmed the immunomodulatory activity of the *Silene* species. In a study conducted by Rivera and coworkers,[96] porcine parvovirus (PPV) vaccines containing Rb1 fraction of ginseng was evaluated for inducing Th1 or Th2 type of immunity in mice. The study revealed the production of large amounts of cytokines, including IFN-γ, IL-2, IL-4, IL-10, and TNF-α, and stimulated titers of antigen-specific IgG1, IgG(2a), and IgG(2b).

### Antitumor activity

A number of pharmacological properties have been attributed to saponins, such as immunomodulative potential by the cytokine interplay[39] and cytotoxic effects on cells from the malignant tumors.[97] Saponins have surface-active properties due to the amphiphilic nature of their chemical structure. The mechanisms suggested for saponin's anticarcinogenic properties include direct cytotoxicity, immune-modulatory activity, bile acid binding, and cancer-induced cell proliferation normalization.[98] Saponins have been shown to not only increase antibody responses but also induce helper and cytotoxic T-cell responses.[99] QS-21 saponin adjuvants show strong Th1 reactions by stimulating cytokine production (IL-2 and IFN-γ) as well as specific CTL via MHCI against the exogenous antigens and cancer cells.[39] The conformation of aldehyde in the QS-21 saponin determines the integrity of Th1 immune responses. Cellular immune responses are highly stimulated by the equatorial conformation of triterpene aldehyde.[70]

## Saponins as adjuvant candidates for COVID-19 vaccine

COVID-19, a disease caused by the novel SARS-CoV-2 coronavirus, based on genetic and clinical evidence, appears to follow a mechanism similar to SARS and MERS. The outbreak of this disease has led to a pandemic endangering global public health and posed high challenges to contain it.[100–103] The production of vaccines is the most promising method for prevention and elimination of this highly contagious respiratory disease. Coronaviruses (CoVs) are large enveloped viruses that carry single-stranded positive-sense RNA genome. The viral membrane is studded with spikes of glycoproteins which give coronaviruses a crown-like appearance. Four types of coronaviruses are identified and they include alpha, beta, gamma, and delta. Severe acute respiratory syndrome (SARS) virus (SARS-CoV), Middle East respiratory syndrome (MERS)

virus (MERS-CoV), and SARS-CoV-2 belong to the betacoronavirus class. The genomic sequence of SARS-CoV-2 demonstrated a similar but distinct composition compared to the SARS-CoV and MERS-CoV genomes. SARS-CoV-2 binds to target cells located in the lower respiratory system to cause viral pneumonia, similar to SARS-CoV and MERS-CoV, but can also affect the GI tract, CNS, heart, kidneys, and liver and lead to multiple organ failure.[104,105]

Currently, scientists are racing toward the development of safe and effective vaccines to prevent COVID-19. According to the WHO (as on September 17th, 2020), 36 candidate vaccines are in different phases of clinical evaluation whereas, 146 candidate vaccines are in the pre-clinical evaluation stage. Several platforms, including non-replicating viral vector vaccine, inactivated vaccine, RNA or DNA vaccine, protein subunit vaccine and virus-like particle vaccine, with (like Matrix M, Advax, MF59, CpG 1018, GlaxoSmithKline adjuvants) or without adjuvants are being investigated. Nine candidate vaccines are in the phase 3 of the clinical trial. These include the ChAdOx1-S (University of Oxford/AstraZeneca), Adenovirus Type 5 Vector (CanSino Biological Inc./Beijing Institute of Biotechnology), Adeno-based (rAd26-S + rAd5-S) (Gamaleya Research Institute), Ad26COVS1 (Janssen Pharmaceutical Companies), three inactivated vaccines (Sinovac, Wuhan Institute of Biological Products/Sinopharm, Beijing Institute of Biological Products/Sinopharm), LNP-encapsulated mRNA (Moderna/NIAID), and 3 LNP-mRNAs (BioNTech/Fosun Pharma/Pfizer). Spike protein and its fragments, including S1, S2, RBD, and N were the prime targets for MERS and SARS vaccine development. Similarly, SARS-CoV-2 regions are expected to be considered for COVID-19 vaccines as important targets.[106,107] Spike protein-based vaccines would induce antibodies to block not only viral receptor binding, but also virus genome uncoating. The S protein has a major role in the induction of protective immunity during the infection with SARS-CoV -2 by generating neutralizing antibodies and T-cell responses. Thus, full-length or functional domains of S glycoprotein are believed to be the most promising candidates for SARS-CoV-2 vaccine composition.[1] The identification of immunodominant region among the subunits and domains of S protein is critical for developing an effective vaccine against the coronavirus. Further investigations are needed to determine the immunodominant regions of SARS-CoV-2 to facilitate the vaccine development.[100]

Several animal-testing research and human trials have shown promising trends, focusing on achieving high rates of neutralizing antibodies. Until now, high titers of S-protein neutralizing antibodies in pre-clinical models have been achieved for two vaccines developed by the traditional methods. Efforts are being made to use novel adjuvants that can potentiate humoral, cellular, and memory immune responses to prevent COVID-19 infection and subsequent disease from being established. Both triterpenoid and steroidal saponins show antiviral activity against different viral groups. Aqueous extracts from the Chilean soapbark tree (*Quillaja saponaria* Molina) produce many physiologically active triterpenoid saponins[108] and demonstrate high adjuvant activity for use in animal and human vaccines.[61,109] As earlier discussed, AS01, a novel adjuvant, contains liposomes and two immunostimulants, 3-O-desacyl-4 incl-monophosphoryl lipid A and distilled saponin QS-21. Both immunostimulant compounds in this adjuvant appear to be critical for the stimulation or activation of antigen-specific cellular and humoral immune responses.[110]

Saponin purified from *Quillaja saponaria* alone or introduced as part of the immunostimulating complexes (ISCOMs), proved to be a powerful adjuvant in human cytomegalovirus vaccines,[111] influenza vaccines,[112] or polysaccharide vaccines.[113] Platycodin D (PD) has been evaluated as an adjuvant in vaccine formulations for the recombinant hepatitis B surface antigen[114] and Newcastle disease virus-based recombinant avian influenza vaccine[115] in mice. Con A-, LPS-, and antigen-induced splenocyte proliferation and serum antigen-specific IgG, IgG1, IgG2a, and IgG2b antibodies titers were significantly enhanced by formulations containing PD. The mRNA expression of Th1 and Th2 cytokines in splenocytes was also up-regulated by PD, which remarkably increased the killing activities of natural killer cells from splenocytes in the immunized mice. Thus, PD showed adjuvant activity in both formulations. Platycodin D2 (PD2) improved both cellular and humoral responses to hepatitis B surface antigen (HBsAg) in mice. PD2 also significantly increased the Con A-, LPS-, and HBsAg-induced splenocyte proliferation, as well as enhanced HBsAg-specific IgG, IgG1, IgG2a, and IgG2b antibody levels in HBsAg-immunized mice. Moreover, PD2 promoted the production of Th1 (IL-2 and INF-gamma) and Th2 (IL-4 and IL-10) cytokines from splenocytes in HBsAg-immunized mice.[115]

Recently, the effectiveness of *Quillaja brasiliensis* saponins has also been confirmed in experimental vaccines against bovine herpesvirus type 1 and 5 (BoHV), human poliovirus, and rabies in mice.[116–120] These saponins are able to form micellar nanometric ISCOM-type structures which are even more effective as vaccine adjuvants, generating both humoral and cellular immune responses.[117]

## Conclusions

At present, vaccine development for SARS-CoV-2 causing COVID-19 is the highest priority of the global medical research community. For a vaccine to be safe, effective and of increased immunogenicity, adequate adjuvants are required. QS-21, the most active saponin fraction from Quil A, possesses high potent adjuvant activity with minimal toxicity. Saponin-based adjuvants selectively stimulate Th1 and cytotoxic T cell responses because they direct antigens into endogenous processing pathways and enhance IFN-γ release by dendritic cells. As a result, a robust antibody and cell-mediated immune response is activated. Therefore, more research is needed to develop saponin adjuvanted recombinant spike or RBD protein subunit vaccine. Development of a saponin adjuvanted subunit vaccine for SARS-COV-2 would also help us in tackling future pandemics associated with other novel coronaviruses.

## Acknowledgments

The mini-review paper was supported by the research project, IVRI/PALAM/17-19/013 and for this we gratefully acknowledge the Director, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India.

## Author contributions

RS conceptualized and designed the review. AP and RS wrote the manuscript. KD and RS critically edited the manuscript. GM, BS and KPS collected literature and reviewed the manuscript. All the authors read and approved the final manuscript.

## Disclosure of potential conflicts of interest

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

## Funding

No funding was required to write this review.

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