Chapter

Perspective Chapter: Natural Adjuvants for Mucosal Vaccines—The Promise of Tomatine as an Inherent Adjuvant in Tomatoes

Nazmul Kazi, John W. Kindt Jr, Indika Kahanda, Christopher da Costa, Robert Carnahan, Hugh Mason, Brenda A. Wilson and S. Indu Rupassara

Abstract

Certain natural immunostimulatory compounds inherent to plants, animals, and microorganisms, in both terrestrial and aquatic ecosystems, have been reported to enhance the immunogenicity of vaccines by conferring an adjuvant effect and/or possessing potent immunomodulatory properties acting as immunogens themselves. In this chapter, we summarize the current state of vaccine adjuvant development and application, encompassing a range of immunomodulatory compounds that improve protective immune responses and enhance vaccine efficacy. We place special focus on the effectiveness of tomatine, inherent to tomatoes, as a natural immunostimulant. We discuss the adjuvant- and immunomodulatory-properties of tomatine and its advantages in plant-based vaccine production, cost-effectiveness, development, safety profiles, and applications compared to other adjuvants and vaccine delivery systems. This chapter provides a futuristic overview and insights into the promise of tomatine for the development of safer, easily-scalable, sustainable, and more efficient vaccines.

Keywords: adjuvant, tomatine, tomato, immunomodulatory properties, immunostimulants, immunogens, bioactive compounds, mucosal vaccines, vaccine development

1. Introduction

Vaccines play a critical role in the prevention and control of infectious diseases, significantly impacting global health outcomes. While vaccines primarily consist of antigens that stimulate an immune response, antigens alone do not always elicit a robust and durable immune system. The inclusion of adjuvants can effectively enhance the immunogenicity of vaccines. Adjuvants are substances that stimulate and

potentiate the immune response of vaccines, leading to improved immune protection and longer-lasting immunity. Since the early twentieth-century, the use of adjuvants in vaccines has been well-established, starting with alum, an aluminum salt-based adjuvant derived from naturally occurring minerals. Adjuvants have emerged as valuable tools to optimize the immune response to vaccine antigens and enhance vaccine efficacy. They generate a robust priming response and reduce the number of vaccine doses needed for extended immunity. Adjuvants can also augment specific branches of the immune response, offer broader cross-protection, and improve vaccine responses in vulnerable populations, such as infants and the elderly [1]. These capabilities make adjuvants essential in developing effective vaccines against infectious diseases. Despite their long-standing use and proven benefits, current adjuvants face certain challenges. One significant challenge lies in the extensive processing required to optimize the effectiveness of adjuvants, leading to complex and costly manufacturing processes [2]. Also, adjuvants such as squalene (derived from shark liver oil) raise sustainability and ethical concerns. To overcome these limitations and meet the increasing demand for effective vaccines, there is a growing interest in exploring natural compounds with immunostimulatory properties as potential adjuvants, particularly from natural and renewable sources requiring minimal processing, thereby reducing the environmental impact [2–9].

Tomatine, a glycoalkaloid compound naturally found in tomatoes having both adjuvant- and potent immunomodulatory-properties (acting as an immunogen itself), stands out among adjuvants due to its inherent presence in one of the most widely consumed fruits globally. Unlike many other natural adjuvants, tomatine does not require meticulous processing, making it easily accessible, cost-effective, and scalable for vaccine development in a sustainable way [10–15]. Consequently, tomatine shows remarkable potential for the development of mucosal (such as orally- and nasally-administered) vaccines, especially for tomato-based vaccine formulations. Exploiting the inherent presence of tomatine in tomatoes opens up new avenues for the development of mucosal vaccines, mainly oral vaccines that leverage the edible nature of tomatoes as bioreactors [16], especially when such subunit vaccines are incorporated with viral vectors. Mucosal vaccines have gained increasing attention due to their ability to induce robust immune responses at mucosal surfaces such as the respiratory and gastrointestinal tracts. These vaccines offer several advantages, including convenient and non-invasive delivery, enhanced local immune responses, and the potential to block pathogen entry at the site of infection [15–17].

This chapter aims to explore the properties and mechanisms of action of tomatine as a natural adjuvant intrinsic to tomatoes, emphasizing its potential advantages, especially in tomato-based vaccine production. Harnessing the inherent adjuvant properties of tomatine could pave the way for innovative plant-based mucosal vaccines that offer convenient administration, improved immunogenicity, and enhanced protection against globally problematic mucosally active infections such as respiratory and gastrointestinal diseases. This advancement toward safe, efficient, and cost-effective mucosal vaccine technologies may lead to expanded immunization coverage, improved vaccine accessibility, and significant global health benefits, particularly in resource-limited settings or during outbreaks where rapid vaccination development and delivery becomes crucial.

We will start with a discussion of adjuvants and the landscape of their importance in vaccines. Then we will introduce tomatine as a natural adjuvant inherent in tomatoes and discuss the advantages and limitations of its use in mucosal vaccines.

2. Adjuvants

Adjuvants are ingredients used in vaccines to enhance the magnitude and durability of the immune response elicited [18], helping vaccines work more effectively by creating a stronger and more protective immune response in individuals receiving the vaccine. They can be broadly defined as substances that improve the immunogenicity or antigen-specific immune response when co-administered with antigens [2, 3, 19, 20]. Adjuvants can be naturally occurring or synthetic compounds that assist the body in producing a robust, and sometimes directed, immune response to the specific disease targeted by the vaccine [18]. Some vaccines contain live attenuated or inactivated pathogen preparations (i.e., weakened or killed microbes or viruses), which naturally contain adjuvants due to their heterogeneous compositions that include particulate forms of proteins, lipids, and oligonucleotides, and thereby contribute to the production of strong, long-lasting immune responses [2]. However, peptide-based subunit vaccine antigens are incapable of triggering a robust and durable immune response on their own. Adjuvants play a major role in stimulating and enhancing the efficacy of such vaccines, especially in immunocompromised individuals such as infants, the elderly, and those who are suffering from chronic diseases and disorders [1, 17].

2.1 Importance and properties of adjuvants in vaccines

Over the last two decades, it has become evident to researchers that solely focusing on increasing antibody responses may not always be sufficient to ensure the effectiveness of vaccines [1]. Adjuvants have emerged as valuable tools in this regard, capable of addressing various challenges, optimizing, and sometimes customizing the immune response to vaccine antigens. One notable advantage of adjuvants lies in their capacity to generate a robust priming response, particularly in individuals who have not been previously exposed to the antigen. By reducing the number of vaccine doses needed to confer lasting protection, adjuvants expedite the development of immunity and enhance the overall efficiency of vaccination programs. Moreover, adjuvants have displayed promising abilities in extending the duration of immune responses offering sustained defense against infectious agents, which proves particularly crucial for diseases requiring long-term immunity.

The latest developments in molecular biological- and nano-technologies have introduced micro- and nano-particles, emulsions, and various immunostimulators for further improving antigen efficacy by playing the roles of carriers, depots, and/or potentiators. **Figure 1** illustrates a broad overview of such mechanisms of action of adjuvants in enhancing immune responses when administered either parenterally (e.g., injectable delivery) or mucosally (e.g., orally) [21–25]. The properties of adjuvants crucial to their immunostimulatory effects can be classified into two main categories: immune potentiators and delivery systems [3]. Immune potentiators directly activate key immune cells through specific receptors, such as Toll-like receptors (TLRs), thereby providing the necessary inflammatory context for optimal antigen-specific immune activation. On the other hand, delivery systems primarily function to localize vaccine components and target them to antigen-presenting cells (APCs), facilitating their interaction with cells of the immune system [3].

Adjuvants demonstrate the potential to augment specific branches of the immune response, such as cell-mediated immunity (CMI) [1]. This attribute proves critical for

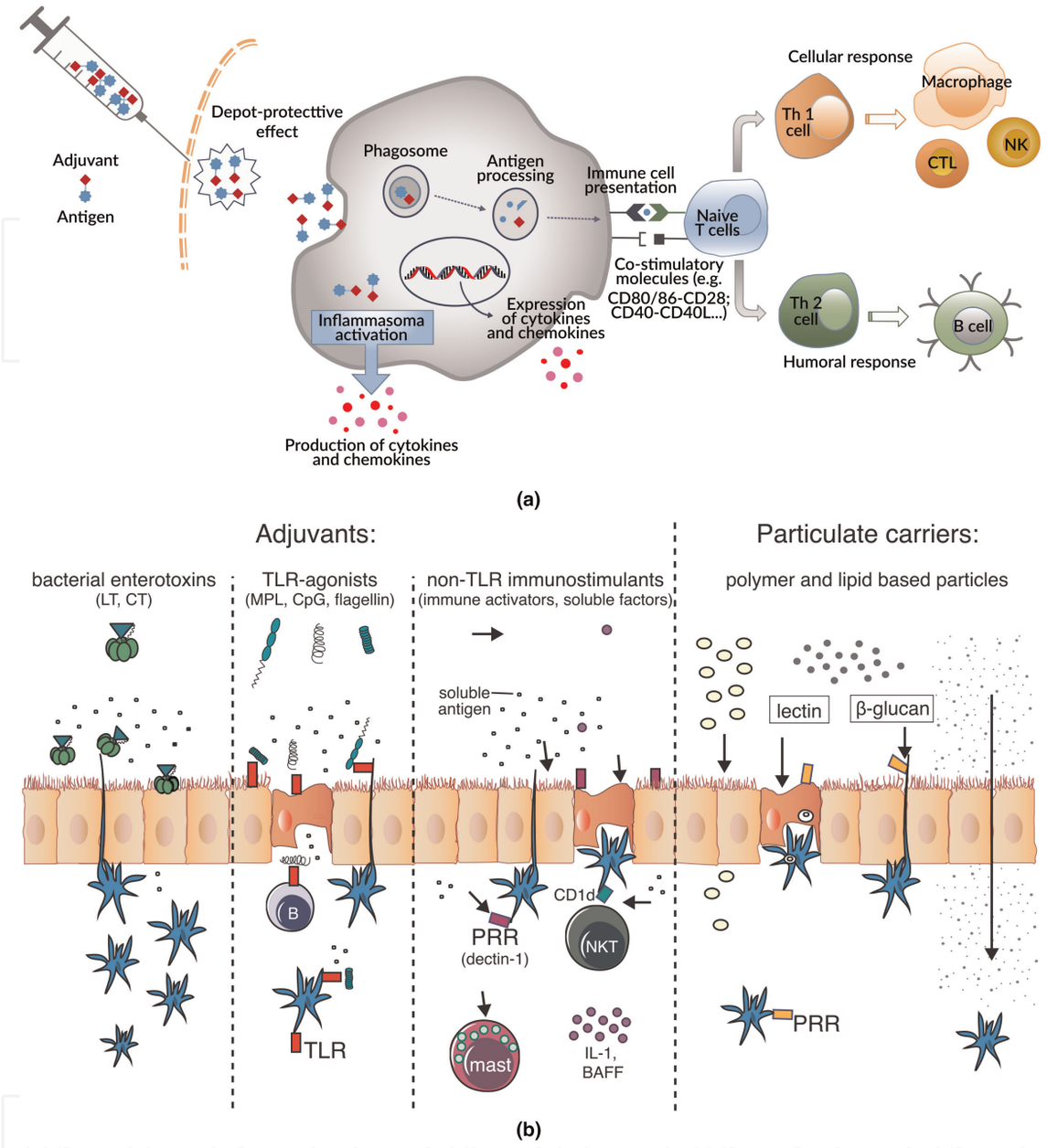

Figure 1.
Mechanism of action of vaccine-adjuvants: (a) parenterally delivered (adapted from [21]), and (b) mucosally delivered (taken from [22]). Adjuvants act as carriers, depots, and/or stimulators in enhancing the immunogenicity of the antigens (LT: Heat-labile enterotoxin from Escherichia coli, CT: Cholera toxin, TLR: Toll-like receptor).

many infectious diseases in which CMI plays a vital role, as adjuvants can elicit robust immune responses and provide heightened protection. Additionally, adjuvants enable the formulation of vaccines that offer broader cross-protection, effectively enhancing the immune response against multiple and variable antigens. This advantage is especially valuable in combating rapidly evolving pathogens where bolstering the overall efficacy of vaccines is critical. Furthermore, adjuvants hold the promise of improving vaccine responses in populations that exhibit diminished responsiveness to immunization, such as infants, the elderly, and individuals with compromised immune systems. These immunocompromised groups often require a more potent immune system stimulation to develop a strong protective response. Adjuvants bridge this gap

by augmenting the immunogenicity of vaccines and ensuring adequate protection in vulnerable populations [1, 8, 17, 21, 22].

2.2 Current landscape of adjuvants

Modern vaccine development has benefited from the tremendous knowledge that has been accumulated over the past couple of decades regarding how our immune system works, and the factors that direct the type and extent of stimulation of particular immune responses [26]. This knowledge along with biotechnological advancements has enabled the creation of vaccines based on highly purified recombinant antigens formulated together with immunostimulant additives, rather than using the entire microorganism which potentially leads to Enhanced Respiratory Disease (ERD) [2]. Modern adjuvant development focuses on selectively adding well-defined molecules, formulations, or both to enhance and shape vaccine-induced responses while maintaining safety [7–9]. In these cases, adjuvants are used to stimulate the immune system and ensure a sufficiently strong immune response. Adjuvanted vaccines may cause stronger local and systemic reactions compared to non-adjuvanted vaccines, however, they have been used safely for decades. Several adjuvants are employed in human vaccines licensed for use in the United States and/or Europe, with aluminum salts being one of the most commonly employed adjuvants for over 70 years [2, 18]. Adjuvants currently licensed for clinical use are listed in **Table 1**.

An increasing number of adjuvants formulated or combined with investigational vaccines have been developed in recent years that are currently in preclinical or

Adjuvant	Developer	Composition	Mechanism of Action	Use in Currently Licensed Vaccines
Alum	Multiple developers	Aluminum Hydroxide or Aluminum Phosphate	Macrophage and dendritic cell NLRP3 inflammasome activation independent of TLR[#] signaling	Multiple vaccines[*]
AS01 $_B$	GSK[**]	MPL[***], QS21, and liposomes	NK cell secretion of interferon-gamma locally, with activation of macrophages, dendritic cells, and CD8 T-cells in draining lymph nodes. QS21 activates caspase-1 dependent Interleukins 1 β and 18, resulting in Th1 biased cellular responses, as antibody responses through poorly understood mechanisms	Shingrix® (Shingles)
AS03	GSK[**]	Squalene, α-tocopherol and surfactant stabilizer: Tween (polysorbate) 80 as surfactant	Activation of human monocytes and macrophages and generation of a local chemokine response in draining lymph nodes. Both CD4 T-cell and antibody responses are enhanced	Q-Pan H5N1 (Influenza)
AS04	GSK[**]	Alum and MPL[***]	TLR activation by MPL that is prolonged by alum, resulting in enhanced antigen presentation by dendritic cells	Cervarix® (HPV[##])

Adjuvant	Developer	Composition	Mechanism of Action	Use in Currently Licensed Vaccines
MF59®	Sequirus	Squalene; Tween (polysorbate) 80, and Span 85 as surfactants	Activation of macrophages and dendritic cells at the injection site. Both CD4 T-cell and antibody responses are enhanced	FLUAD® (influenza)
CpG-1018®	Dynavax Technologies	22-mer synthetic single-stranded DNA that mimics bacterial and viral genetic material	TLR-9 activation and induction of innate immune responses through type 1 interferon	Heplisav-B (Hepatitis B)
Alhydroxi-quim II	Virovax, LLC	TLR7/TLR8 ligand (adsorbed in alum)	Activation of TLR-7 and TLR-8 receptors in regional lymph nodes	COVAXIN (COVID-19)
Matrix-M	Novavax	QS21, QS7 saponins from Quillaja saponaria Molina bark; Cholesterol; Phospholipids	Recruitment and activation of T-cells and, B-cells by inducing the influx of antigen-presenting cells	Covovax, Novaxovid (COVID-19)

[*]Havrix (hepatitis A), Engerix-B (Hepatitis B), Gardasil 9 (HPV[##]), Bexsero and Trumenba (Meningitis B), Prevnar 13 (Pneumococcal), COVID-19 vaccines (Sinopharm and Sinovac), etc.[**]GSK – GlaxoSmithKline.[***]MPL - Monophosphoryl lipid.[#]TLR - Toll-like receptor.[##]HPV - Human Papillomavirus.

Table 1.
Adjuvants are currently licensed for clinical use. (adapted from [18, 27]).

clinical stage development and are not yet licensed. Some of these are listed in **Table 2**, which is not an exhaustive list.

Many vaccine adjuvants are capable of enhancing and shaping antigen-specific immune responses. More recent adjuvant formulations are designed to selectively add well-defined components to improve the immunogenicity of vaccine antigens while

Adjuvant	Developer	Composition	Mechanism of Action	Investigational Vaccine
Squalene in Water Emulsion (SWE)	VFI*/ SEPPIC	Squalene (shark-derived); Proprietary surfactants (SEPPIC); Water for injection; Citrate buffer	Secretion of proinflammatory cytokines and chemokines; induction of damage-associated molecular patterns (DAMP)-signaling; recruitment of innate immune cells, which capture the antigen and migrate to draining lymph nodes; induction of plasmablasts and memory B cells; T cell activation by antigen-loaded dendritic cells, with a generation of a mixed Th0/Th1/Th2 phenotype	COVID-19; Influenza; RSV; Ebola; Polio; Rabies; group A Streptococcus
SQ	VFI	Emulsion; Cholesterol; Saponin		HIV infection; Group A Streptococcus; Malaria
LQ	VFI	Liposomes; Cholesterol; Saponin		
SQX (AS02 like with TLR4 agonist)	VFI	Emulsion; Cholesterol; Saponin; Immunomodulator		
LXQ (AS01 like with TLR4 agonist)	VFI	Cholesterol; Saponin; Immunomodulator		

Adjuvant	Developer	Composition	Mechanism of Action	Investigational Vaccine
Montanide	SEPPIC/VFI	Mineral oil; non-mineral oil; Surfactants	Activation of innate immune responses and recruitment of antigen-presenting cells	COVID-19
Advax™	Vaxine Pty Ltd., Australia	Delta Inulin (natural plant-derived polysaccharide)	A Th0 adjuvant (neither Th1 nor Th2-biased). Acts through enhancement of antigen-presenting cell function, leading to enhanced B- and T-cell activation	Influenza; Japanese encephalitis; HIV infection
PIKA**	Yisheng Biopharma (Singapore) Pte. Ltd	Stabilized chemical analog of double-stranded RNA	TLR-3 agonist	Rabies (IPRV#); SARS-C0V-2 spike protein subunit vaccine
CpG ODN## (including CpG 55.2, CpG7909, CpG10101, CpG10104)	Dynavax; Shionogi; InvivoGen	Single-stranded synthetic DNA molecules containing cytosine and guanine deoxynucleotides with a phosphodiester or phosphorothioate link	TLR-9 activation and induction of innate immune responses through type 1 interferon	HIV infection; Malaria; Hookworm disease; Influenza; Pneumococcal disease, Hepatitis C; Hepatitis B

VFI - Vaccine Formulation Institute.PIKA - Polyinosinic Polycytidylic Acid.#IPRV - Inactivated and purified rabies vaccine.##ODN - Oligodendronucleotides.*

Table 2.
Unlicensed adjuvants used in investigational vaccines. (adapted from [18, 27]).

maintaining safety. Adjuvants used in licensed human vaccines include aluminum salts, oil-in-water emulsions, virosomes, saponins, liposomes, and monophosphoryl lipid A preparations with aluminum salt. The selection of adjuvants is based on various factors such as the nature of the vaccine antigen, desired immune response, target population age, and route of administration [1, 2, 7, 8].

2.3 Sources of adjuvants

The majority of the adjuvants are derived from various compounds found in natural sources—such as plants, fungi, animals, marine organisms, insects, and microorganisms—and harness unique immunostimulatory properties [7–9, 14, 15, 28, 29]. Alum has been employed as an adjuvant in human vaccines since 1932 and served as the sole adjuvant in licensed vaccines for nearly seven decades [5]. Alum encompasses various aluminum compounds, namely aluminum phosphate, aluminum hydroxide, and alum-precipitated vaccines [30]. Aluminum compounds are derived from potassium aluminum sulfate (a natural mineral salt) and have been favored as adjuvants. Originally, their mechanism of action was thought to be due to their capacity to create a depot at the injection site. This depot facilitates the gradual release of antigens and supports effective antigen presentation to immune cells, contributing to an enhanced immune response [23]. However, this depot model has more recently been challenged based on some *in vivo* studies [31, 32].

AS03 (**Table 1**), an adjuvant used in some vaccines, contains α-tocopherol (Vitamin E) and squalene. The fat-soluble compound α-tocopherol is a naturally occurring compound found in vegetable oils, nuts, and seeds that acts as an antioxidant. Squalene is another natural compound commonly sourced from shark liver oil and hydrogenation from plant-derived sources [6]. AS04 combines an aluminum salt with monophosphoryl lipid A (MPL) [20]. MPL is a detoxified form of lipopolysaccharide (LPS) extracted from the outer membrane of certain Gram-negative bacteria, such as Salmonella enterica serovar Minnesota in this case. Utilization of MPL derived from this natural source contributes to the adjuvant's immunostimulatory properties. The enhanced immunogenicity of the adjuvant MF59 is derived from a combination of the natural product squalene and the synthetic surfactants, Tween-80 and Span 85, used to stabilize the emulsion [1].

Saponins are naturally occurring compounds found in various plants and possess potent immune-enhancing effects. When combined with liposomes, which are synthetic or naturally occurring vesicles composed of a lipid bilayer, these natural adjuvants further enhance antigen presentation and stimulate the immune system [9]. For instance, AS01 combines two immunostimulants: MPL and QS-21, a saponin extracted from the bark of the Quillaja saponaria tree [20]. Lectin, a class of proteins with carbohydrate-binding properties, is another plant-derived adjuvant [33].

Fungi provide a diverse array of natural adjuvants. Polysaccharides found in fungal cell walls, called β-glucans, have been extensively studied for their immunostimulatory effects [8]. These β-glucans can activate various immune cells, such as macrophages and dendritic cells, leading to enhanced immune responses. Zymosan is another fungal cell wall component with adjuvant properties that can stimulate immune cells via pattern recognition receptors [8].

Marine-derived polysaccharides, such as fucoidan and carrageenan, have also demonstrated adjuvant properties [34]. Insect-derived proteins, such as hemocyanin, have been investigated as adjuvants in vaccine development. Hemocyanin, derived from the hemolymph of certain insects, possesses immunostimulatory properties and can enhance immune responses [28]. Additionally, insect venom components, such as melittin from bee venom, have been studied for their adjuvant potential [28, 35].

CpG-1018, the only licensed adjuvant that is fully synthetic, is composed of synthetic cytosine-phosphoguanine (CpG) motifs, which emulate the genetic material found in bacteria and viruses [1]. While CpG motifs naturally occur in bacterial and viral DNA, the CpG motifs utilized in vaccines are artificially created and not sourced from natural origins.

2.4 Processing and formulation of adjuvants

Most natural compounds presenting adjuvant properties cannot be used directly in their natural form and undergo a meticulous process to optimize their adjuvant properties, enhance stability, and ensure safety in vaccine formulations [2, 7–9]. The direct application of these adjuvant compounds in vaccines may present challenges, such as variability in potency, stability issues, and potential safety concerns [36]. To overcome these limitations, extensive research and development efforts are focused on refining, formulating, and purifying these natural compounds to harness their adjuvant potential effectively, before they can be used in vaccines [4, 9, 26].

During the refinement process, natural compounds undergo isolation to separate the active immunostimulatory components [37]. For example, saponins derived from

plant sources are subjected to refinement techniques to obtain specific saponin fractions with enhanced adjuvant activity [38]. These refined fractions can be further modified or combined with other components to optimize their immunostimulatory effects [2].

The formulation plays a crucial role in maximizing the adjuvant properties and stability of natural compounds [2]. Adjuvants are often combined with specific carriers, emulsions, liposomes, or other delivery systems to enhance their stability, solubility, and controlled release of antigens [3]. For example, one common formulation approach is the use of oil-in-water emulsions, such as MF59, which contains squalene, Tween-80, and Span 85. As mentioned earlier, the combination of squalene with surfactants enhances the stability and dispersibility of the adjuvant, allowing for efficient antigen presentation and stimulation of the immune system. The use of liposomes, which are synthetic or naturally occurring vesicles composed of a lipid bilayer, is another formulation strategy used in adjuvant processing [2]. These liposome formulations are designed to encapsulate and improve the delivery of antigens by promoting the interaction between the antigen-adjuvant complexes and antigen-presenting immune cells to efficiently uptake the antigen and enhance the overall immune response [36].

Importantly, safety considerations are paramount in the development of adjuvants. While natural compounds are derived from natural sources, they may still possess undesirable toxicities or unwanted side effects, either due to inherent properties or contaminants [9, 26, 39]. For instance, lipopolysaccharide (LPS), a natural adjuvant derived from bacterial cell walls, can elicit strong inflammatory responses that may be undesirable in vaccine formulations. In this case, the purification process is needed to remove or reduce unwanted impurities and potentially toxic components while maintaining the desired adjuvant properties to ensure that the final product meets stringent quality and safety standards [39]. The steps may include filtration, chromatography, or chemical treatments to eliminate contaminants and unwanted substances.

3. Tomatine as a natural immunostimulant inherent to tomatoes

The active glycosteroid alkaloid tomatine (also called lycopersicin) (**Figure 2a**) is an immune stimulator found in tomatoes and tomatillos, especially at high levels in the leaves and unripe fruit of Lycopersicon pimpinellifolium, a wild tomato species [10–15]. Tomatine belongs to a class of compounds called glycoalkaloids. Glycoalkaloids are naturally occurring in plants of the Solanaceae family, including potatoes, tomatoes, peppers, tomatillos, and eggplants. Notably, tomatine is exclusive to tomatoes and tomatillos [10]. In nature, tomatine functions as a toxicity-based defense mechanism, protecting against viral and bacterial pathogens, preventing infestation by arthropods, and acting as a deterrent to discourage the consumption of unripe tomatoes [40].

3.1 Adjuvant properties

Tomatine shares similarities with saponins, which are recognized for their membrane-disrupting qualities [13] and ability to trigger strong immune responses [28]. It has undergone evaluation in both preclinical and clinical trials, and as shown in **Figure 2b** and **c**, can act as a potent adjuvant to elicit CD8+ T cell responses for

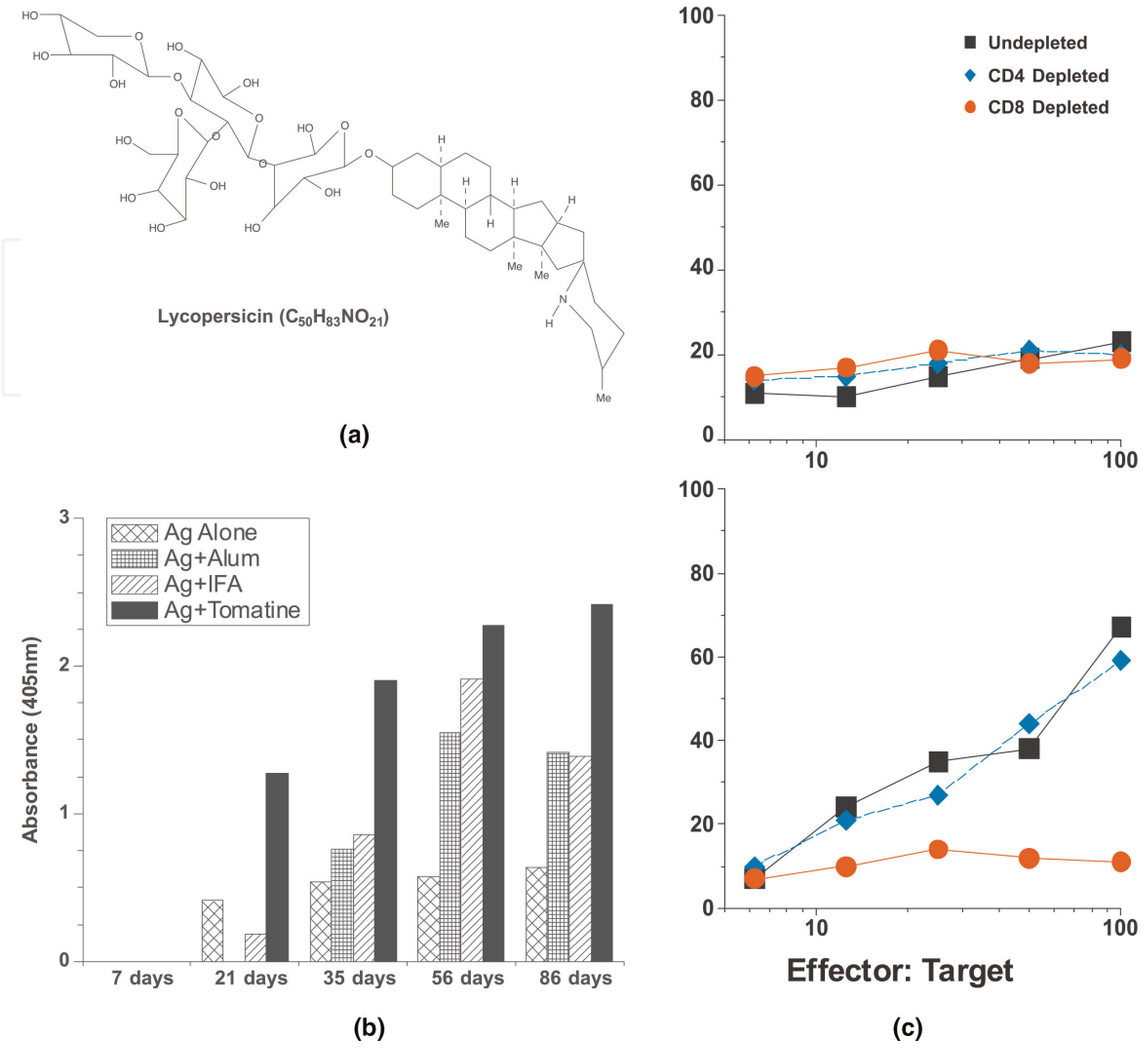

Figure 2.
Properties of tomato-derived α-tomatine. (a) the structure of lycopersicin (α-tomatine) intrinsic to tomatoes, (b) comparison of total combined IgG and IgM anti-ovalbumin (anti-ova) antibody titers in mice immunized with ova alone and ova formulated with different adjuvants shows superior and persistent cellular immune response triggered by tomatine compared with alum and incomplete Freund's adjuvant (IFA), and (c) antigen-restricted CD8 + cytolytic T-cell responses generated by anti-ova-tomatine immunization in mice (figures taken from [11]).

specific antigens [11, 28, 41]. Immunization with ovalbumin (Ova) formulated with tomatine elicited significantly higher and sustained antibody titers (see **Figure 2b**), surpassing those induced by conventional reference standard adjuvants, Alum and incomplete Freund's (IFA) [11]. Tomatine as an adjuvant for a pre-erythrocyte circumsporozoite protein vaccine candidate provided protective immunity against malaria by eliciting antigen-specific CD8+ T cell responses [41]. Furthermore, tomatine has demonstrated effectiveness in promoting strong, specific immune responses involving both humoral and cellular components, thereby contributing to the defense against malaria [13] and Francisella tularensis [11].

Studies have demonstrated the safety and tolerability of tomatine in mice, as it does not provoke certain reactions at the administration site [28]. For instance, when tomato fruit and potato tuber were evaluated as means to produce a mucosal vaccine against the Norwalk virus, orally delivered tomato was much more effective than potato, perhaps in part due to the presence of tomatine in the tomato fruit [16].

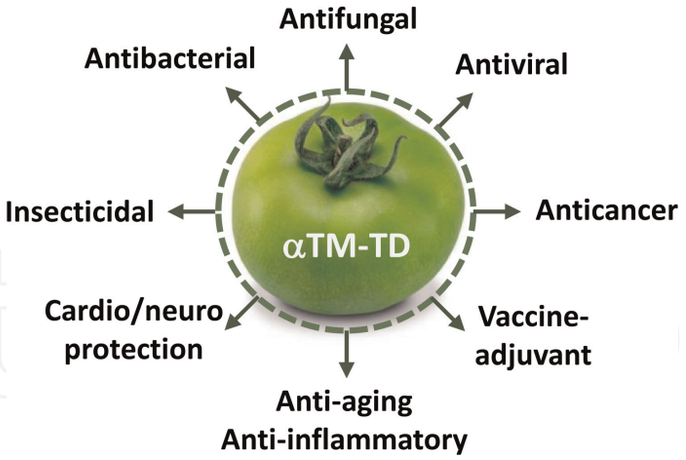

Figure 3.
Functional properties of tomatine (taken from [15]).

3.2 Immunomodulatory properties

As depicted in **Figure 3**, tomatine has a number of functional properties with demonstrated beneficial effects in different areas of research, including both immunomodulatory- and adjuvant-properties, acting as an immunogen itself, in addition to potentiating the efficacy of other immunogens [10–12, 14, 15, 29]. Tomatine's antiviral properties have also been investigated, with studies demonstrating its ability to restrict the spread of certain viruses, including dengue, chikungunya, and porcine epidemic diarrhea viruses [15]. These findings suggest a potential role for tomatine in antiviral strategies and warrant further exploration of its mechanism of action against viral pathogens.

In the context of cancer, tomatine has shown promising results in inhibiting cell invasion and melanoma-dependent angiogenesis by reducing the release of vascular endothelial growth factor (VEGF) and interfering with tumor-stimulating effects on capillary tube formation, thus exerting suppressive effects on melanoma progression [29]. Moreover, tomatine has exhibited potent anti-inflammatory and anticancer effects both *in vitro* and *in vivo*, particularly against androgen-independent prostate cancer [15, 42], hepatocellular carcinoma [43], non-small cell lung cancer [44], breast [45], and ovarian cancer [46].

Beyond its anticancer potential, tomatine displays notable antifungal activity. Additionally, it functions as an effective cholesterol binder, which also facilitates the uptake of protein antigens [15]. These properties demonstrate its dual role, both as an immunomodulator and as a vaccine adjuvant enhancing the delivery of protein antigens or therapeutic oligonucleotides.

3.3 Advantages and limitations

Tomatine offers a significant advantage in the development of tomato-based vaccines due to its inherent presence in one of the most widely consumed fruits worldwide. Tomato plants have a remarkable ability to adapt to a wide range of climatic conditions, spanning from 12 to 32°C, which enables their cultivation on a global scale [47–49]. Tomatoes are not only consumed raw but also processed into various forms such as juice, paste, soups, ketchup, sauces, and more. The Food and Agriculture

Organization[1] of the United Nations reports that in 2021, global tomato production exceeded 189 million metric tons. The intrinsic presence of tomatine in tomatoes provides a significant advantage compared to various other immunostimulatory compounds such as saponins that may require additional processing steps during manufacturing/formulation [8]. Using tomatine as an adjuvant in tomato-based mucosal vaccine production, especially in oral vaccine production, offers the further advantage of bypassing all the extensive refinement, formulation, and purification processes typically required for other naturally derived adjuvant compounds. Unlike many adjuvants that need meticulous processing to extract/isolate and optimize their properties, tomatine is inherently present in tomatoes and does not require additional refinement or purification steps, thereby significantly reducing the complexity, time-intensiveness, and cost associated with adjuvant processing.

Tomatine, in tomato-based vaccines, essentially eliminates the need for formulation or formulation-related challenges. Other adjuvants often require specific carriers, emulsions, liposomes, or other delivery systems to enhance their stability, solubility, and controlled release of antigens. In contrast, tomatine provides a ready-to-use adjuvant within the plant tissues, especially when the fruits are used to express the recombinant vaccine antigens. This inherent formulation eliminates the necessity of developing complex delivery systems and reduces the risk of formulation-associated issues, ensuring a more straightforward and streamlined manufacturing process.

Most other adjuvants have proprietary formulations, including a lack of transparency surrounding the manufacturing process—besides the additional cost of these processes—that makes it difficult for lower-income areas to access these more complicated vaccine products. In contrast, tomato-based vaccines have a relatively simple formulation process, such as homogenization (blending) and lyophilization (freeze-drying), facilitating the maintenance of the efficacy of antigenic proteins at room temperature storage for up to 2 years [50].

In general, the tomatine content in green (unripe) tomato fruits can be up to 10,000 times higher than in red tomatoes [39], so appropriate fruit selection may need to be factored into the vaccine manufacturing process. However, we speculate that with the advancements in modern technologies, we can genetically manipulate the plants to adjust the expression levels of tomatine in green as well as mature fruits to be at optimal/desired concentrations for the vaccine formulation, offering precise control over adjuvant content to be used in unmodified/natural form for optimized vaccine development. This will make vaccine manufacturing possible with the most natural possible adjuvant formulated into the most natural possible delivery vehicle (tomato-fruit) for the vaccine antigen.

Manufacturing oral vaccines using tomatoes provides several benefits. This oral route of administration is convenient, cost-effective, and non-invasive, eliminating the need for injections, and thereby reducing potential needle-associated concerns [51]. This user-friendliness can contribute to increased vaccine acceptance and compliance, especially in populations where needle phobia or vaccine hesitancy is prevalent. The use of tomatoes for oral vaccine production aligns with the concept of utilizing edible plants as bioreactors. This approach capitalizes on the edible nature of tomatoes, allowing for direct consumption of the vaccine without the need for additional processing steps simplifying the production process of oral vaccines.

[1] https://www.fao.org/

Tomatine has been proven safe for oral use in a preliminary study, which investigated its chronic oral toxicities as a potential food preservative due to its antifungal activity. Diets containing varying concentrations of tomatine from 0.0025 to 0.04% weight-based (w/w) were administered to weanling female and male albino rats for 200 days, with the highest concentration well above any potential amount added to food. Throughout the study, the rats exhibited normal growth rate, health, and general appearance, and no abnormalities were observed during the autopsy. Organ weights were within the normal range, and histopathologic examinations of tissues revealed no changes indicative of toxicity related to the experimental diets [52].

Even though potential tomato-allergy can be a limitation for tomato-based vaccines, the remarkably low prevalence of tomato allergies further enhances the viability of using tomatine as an adjuvant in tomato-based vaccines. Research indicates that only a tiny percentage of the population is allergic to tomatoes. While no study was conducted to determine the true percentage of allergy to tomatoes for the global population, it is estimated to be less than 1% [53]. Another study found that only 1.5% of the population in northern Europe is allergic to tomatoes, although tomato allergy appears to be more prevalent in southern Europe, particularly in Italy and Spain, where up to 16% of adults in Italy are allergic to tomatoes [49]. Individuals allergic to tomatoes may also exhibit allergies to other fruits within the nightshade family, such as tomatillos [54]. However, allergenic gene-silenced tomato fruits can exhibit a stable reduction of allergenic potency, leading to decreased skin reactivity and inheritance of reduced allergenicity in subsequent generations, showing the potential for the development of tomatoes with reduced allergenicity [55, 56]. This potentially minimizable allergenicity would result in avoiding the likelihood of adverse reactions in vaccine recipients, widening the percentage of the population that can benefit from tomato-based vaccine formulations.

The concept of tomato-based, orally-administered vaccines holds promise for easier storage, distribution, and administration, particularly in resource-limited settings or during outbreaks where rapid vaccine development and dissemination becomes crucial. As tomatoes are cultivated and consumed in large quantities worldwide, the availability of tomatine intrinsic to tomatoes also remains equally abundant and accessible as an immunostimulant. Therefore, it is not limited by scarcity or geographical constraints. This makes tomatine a natural, cost-effective, easily-scalable, and sustainable option, especially for tomato-based vaccine production, making it a highly promising avenue for expanding immunization coverage worldwide.

4. Conclusion

Tomatine is an inherent adjuvant in tomatoes, a crop-plant grown worldwide, and holds great promise for the development of mucosal vaccines against numerous globally problematic infections such as respiratory and gastrointestinal diseases. Its natural presence in tomatoes simplifies the vaccine production process of tomato-based vaccines, eliminating the need for extensive processing. Tomatine's inherent immunomodulatory- and adjuvant-properties, i.e., acting both as an immunogen itself and an agent potentiating other immunogens, combined with the advantages of highly-simplified tomato-based oral vaccine formulation and administration, offer opportunities for convenient storage, long shelf-life, distribution, administration/delivery, and improved immunogenicity. Its abundance and accessibility make tomatine a natural, cost-effective, and easily-scalable option for vaccine production, with the

potential to expand immunization coverage globally, especially with tomato-based vaccine formulations.

By embracing tomatine as a natural adjuvant usable in unmodified form, we can address key challenges in vaccine formulation, storage, distribution, and accessibility. Capitalizing on the inherent properties of tomatine and the edible nature of tomatoes opens new avenues for oral vaccine technology, particularly in resource-limited settings or during outbreaks where rapid vaccination becomes crucial. By advancing tomato-based oral vaccine technology with tomatine as an inherent adjuvant, we can protect populations worldwide from infectious diseases in a non-invasive manner, and improve global health outcomes.

Author details

Nazmul Kazi[1†], John W. Kindt Jr[2†], Indika Kahanda[1], Christopher da Costa[3,4], Robert Carnahan[5], Hugh Mason[6], Brenda A. Wilson[7] and S. Indu Rupassara[2*]

1 University of North Florida, Jacksonville, FL, USA

2 FruitVaccine, Inc., Champaign, IL, USA

3 Coalition for Epidemic Preparedness Innovations, Washington, DC, USA

4 Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, MA, USA

5 Vanderbilt University Medical Center, Nashville, TN, USA

6 Arizona State University, Tempe, AZ, USA

7 The University of Illinois Urbana-Champaign, Champaign, IL, USA

*Address all correspondence to: indurupassara@gmail.com

† These authors contributed equally.

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