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# Plant-Derived Saponins from Soybean (Glycine max), Fenugreek (Trigonella foenum-graecum), Soapnut (Sapindus mukorossi), and Shikakai (Acacia concinna): A Comprehensive Review of Their Immunoadjuvant Potential and Applications in IgY Production

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## ABSTRACT

Plant-derived immunoadjuvants have attracted considerable attention as natural alternatives to synthetic adjuvants due to their safety, biodegradability, and immunostimulatory properties. Among these, Soybean (Glycine max), Fenugreek (Trigonella foenum-graecum), Soapnut (Sapindus mukorossi), and Shikakai (Acacia concinna) are rich sources of saponins and other bioactive compounds that possess significant pharmacological activities. These plants exhibit antimicrobial, antioxidant, anti-inflammatory, and immune-enhancing properties that contribute to improved host defense mechanisms. Saponins derived from these plants have been reported to stimulate humoral and cellular immune responses, making them promising candidates as vaccine adjuvants and immunostimulants. Recent studies have demonstrated their potential applications in animal health, aquaculture, and functional food development. This review summarizes the phytochemical composition, pharmacological activities, immunomodulatory properties, and potential applications of these four medicinal plants, highlighting their importance as sustainable natural immunoadjuvants..

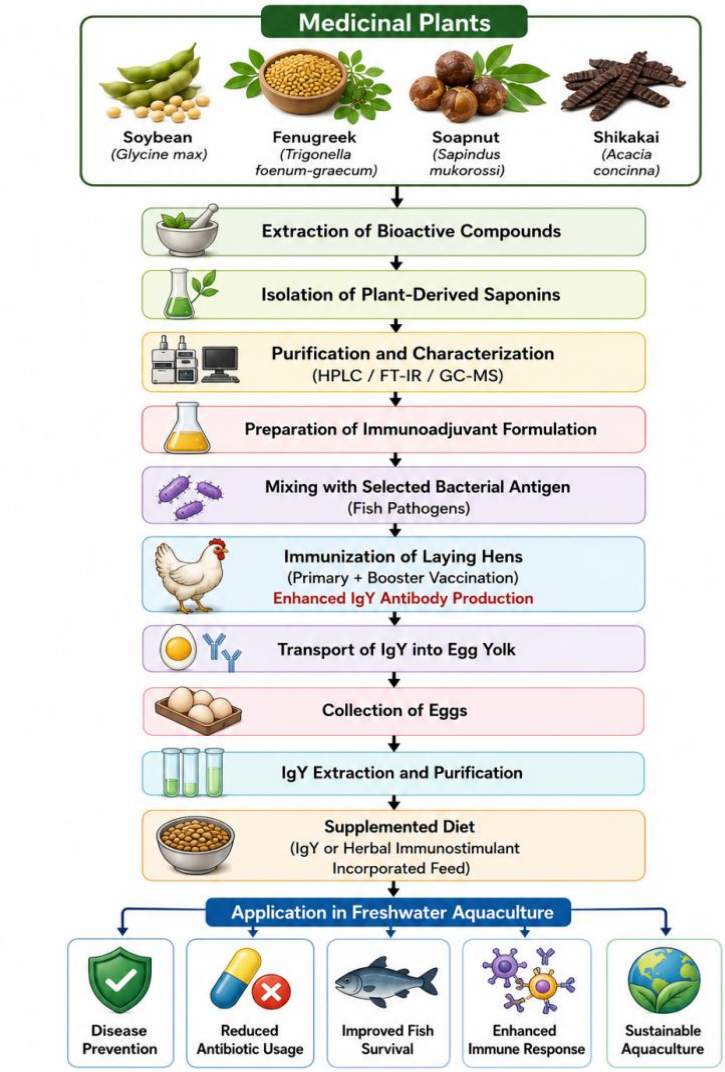
**Keywords:** Soybean, Fenugreek, Soapnut, Shikakai, Saponins, Immunoadjuvant, IgY, Aquaculture..

## INTRODUCTION

The increasing demand for environmentally friendly and sustainable disease management strategies has stimulated extensive research on plant-derived immunoadjuvants. According to Francis et al. (2002), natural saponins obtained from medicinal plants possess strong immunostimulatory activities and have been widely investigated as alternatives to synthetic vaccine adjuvants. Researchers have reported that plant-based immunoadjuvants can enhance both innate and adaptive immune responses while minimizing adverse side effects commonly associated with conventional adjuvants. According to Cheeke (2000), saponins are naturally occurring glycosides distributed in numerous plant species and are characterized by their surface-active properties and biological activities. Several studies have observed that saponins stimulate macrophage activation, cytokine production, antibody synthesis, and lymphocyte proliferation, thereby enhancing host immune competence. Consequently, plants rich in saponins have gained considerable importance in pharmaceutical, veterinary, and aquaculture applications.

Among the various medicinal plants, Soybean (Glycine max), Fenugreek (Trigonella foenum-graecum), Soapnut (Sapindus mukorossi), and Shikakai (Acacia concinna) represent valuable natural sources of bioactive saponins and secondary metabolites. According to Price et al. (1987), these plants contain diverse phytochemicals including triterpenoid saponins, flavonoids, phenolic compounds, alkaloids, and tannins, which contribute to their pharmacological activities. Researchers have reported that these compounds possess antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties.. .:

Recent investigations have demonstrated that plant-derived saponins can function as effective vaccine adjuvants by enhancing antigen presentation and stimulating antibody production. According to **Sun et al. (2009)**, saponin-based adjuvants significantly improve humoral immune responses and increase antigen-specific antibody titers. Such properties are particularly important for the production of chicken egg yolk antibodies (IgY), where enhanced immune stimulation can result in improved antibody yield and efficacy. In aquaculture, disease outbreaks caused by bacterial pathogens continue to pose serious challenges to sustainable production. Researchers have reported that natural immunoadjuvants can enhance disease resistance, improve immune status, and reduce dependence on antibiotics. Therefore, the exploration of medicinal plants rich in saponins offers promising opportunities for developing eco-friendly immunostimulatory agents for animal health and aquaculture applications.




**Mechanism**

**2. Soybean (*Glycine max*)**

**Botanical Description**

**Soybean (*Glycine max* L. Merr.)** belongs to the family Fabaceae and is one of the most extensively cultivated leguminous crops worldwide. According to **Hartman et al. (2011)**, soybean originated in East Asia and is presently cultivated in numerous countries due to its high nutritional and economic value. The plant is an annual herb characterized by trifoliate leaves, erect stems, and protein-rich seeds.

**Phytochemical Composition**

Researchers have reported that soybean seeds contain abundant proteins, lipids, carbohydrates, vitamins, minerals, and bioactive phytochemicals. According to **Kumar et al. (2010)**, soybean is particularly rich in isoflavones, phenolic compounds, phytosterols, and triterpenoid saponins. These compounds contribute significantly to its biological and pharmacological activities.

**Saponins and Immunomodulatory Properties**

According to **Guang et al. (2014)**, soybean contains group A and group B soyasaponins, which exhibit potent immunostimulatory activities. Studies have demonstrated that soybean saponins enhance macrophage activation, increase cytokine secretion, and stimulate antibody production. Researchers observed that dietary supplementation with soybean saponins significantly improved immune responses in experimental animals.

**Antimicrobial Activity**

Several studies have reported that soybean extracts possess antibacterial and antifungal activities against a wide range of pathogenic microorganisms. According to **Wang et al. (2008)**, soybean-derived bioactive compounds inhibit microbial growth by disrupting cell membranes and interfering with essential metabolic pathways. These antimicrobial properties make soybean a promising candidate for disease control applications.

**Antioxidant Activity**

According to **Messina (2016)**, soybean is an excellent source of antioxidant compounds including isoflavones and phenolic acids. Researchers observed that these compounds effectively scavenge free radicals, reduce oxidative stress, and protect biological tissues from oxidative damage. Such antioxidant activities contribute to improved health and disease resistance.

**Potential as an Immunoadjuvant**

Recent investigations have demonstrated that soybean saponins can function as effective natural adjuvants. According to **Sun et al. (2009)**, soybean-derived saponins enhance antigen-specific antibody responses and improve vaccine efficacy. Consequently, soybean has attracted considerable attention as a sustainable source of immunoadjuvants for veterinary and aquaculture applications.

**3. Fenugreek (*Trigonella foenum-graecum*)**

Botanical Description

*Trigonella foenum-graecum* L., commonly known as fenugreek, belongs to the family Fabaceae and is one of the oldest medicinal plants cultivated throughout Asia, Europe, and North Africa. According to Petropoulos (2002), fenugreek has been traditionally used as a medicinal herb, spice, and functional food because of its diverse pharmacological properties. Researchers have described that the plant is an annual herb characterized by trifoliate leaves, white flowers, and golden-yellow seeds rich in biologically active compounds. Acharya et al. (2006) reported that fenugreek is widely cultivated in India, where it serves both nutritional and therapeutic purposes. The seeds are particularly valued because they contain high concentrations of proteins, dietary fiber, steroidal saponins, alkaloids, flavonoids, vitamins, and essential minerals. According to Basch et al. (2003), the medicinal value of fenugreek has attracted considerable scientific attention owing to its immunomodulatory, antimicrobial, antioxidant, and anti-inflammatory activities.

Phytochemical Composition

Fenugreek contains numerous secondary metabolites that contribute to its therapeutic efficacy. According to Yadav and Baquer (2014), the seeds are rich in steroidal saponins, diosgenin, yamogenin, gitogenin, trigonelline, flavonoids, coumarins, polyphenols, and galactomannan. Researchers have reported that diosgenin represents the major steroidal sapogenin responsible for many biological activities of fenugreek. Basch et al. (2003) observed that fenugreek also contains amino acids, including 4-hydroxyisoleucine, which contributes to its metabolic and immunological functions. According to Wani and Kumar (2018), the synergistic interaction among these phytochemicals provides significant antioxidant, antimicrobial, hypoglycemic, and immune-enhancing properties.

Immunomodulatory Properties

Fenugreek has been extensively investigated for its immunomodulatory potential. According to Kaviarasan et al. (2007), fenugreek seed extracts stimulate macrophage activation, increase cytokine production, and enhance both humoral and cellular immune responses. Researchers have demonstrated that steroidal saponins present in fenugreek function as natural immunoadjuvants capable of improving antigen presentation and antibody production. Yadav and Baquer (2014) reported that dietary supplementation with fenugreek significantly enhances lymphocyte proliferation and improves host defense mechanisms. According to Al-Habori and Raman (2002), fenugreek-derived bioactive compounds regulate inflammatory mediators and reduce oxidative stress, thereby supporting immune homeostasis.

Antimicrobial and Antioxidant Activities

Several investigations have demonstrated the antimicrobial activity of fenugreek against bacterial and fungal pathogens. According to Randhir et al. (2004), phenolic compounds and flavonoids inhibit microbial growth by disrupting cellular metabolism and membrane integrity. Researchers have observed that fenugreek extracts exhibit inhibitory activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Pseudomonas aeruginosa*. Basch et al. (2003) reported that the antioxidant activity of fenugreek is primarily attributed to flavonoids and polyphenolic compounds capable of scavenging reactive oxygen species. These antioxidant properties reduce oxidative damage and improve cellular protection under stress conditions.

Applications in Aquaculture

Recent studies have reported that fenugreek supplementation improves growth performance, digestive enzyme activity, immune response, and disease resistance in cultured fish. According to Hoseinifar et al. (2020), dietary inclusion of fenugreek enhances lysozyme activity, phagocytosis, and antioxidant enzyme activity in aquaculture species. Researchers have demonstrated that fenugreek-derived saponins improve intestinal health and beneficial gut microbiota while reducing pathogen colonization. Therefore, fenugreek has emerged as a promising natural immunoadjuvant for sustainable aquaculture.

**4. Soapnut (*Sapindus mukorossi*)**

Botanical Description

*Sapindus mukorossi* Gaertn., commonly known as soapnut or soapberry, belongs to the family Sapindaceae. According to Singh and Singh (2010), soapnut is widely distributed throughout India, Nepal, China, and Southeast Asia. Researchers have reported that the fruit pericarp contains exceptionally high concentrations of triterpenoid saponins, making it one of the richest natural sources of plant saponins. Traditionally, soapnut has been used in Ayurvedic medicine for the treatment of skin diseases, inflammation, respiratory disorders, and microbial infections.

Phytochemical Composition

According to Huang et al. (2008), soapnut fruits contain triterpenoid saponins, flavonoids, sugars, fatty acids, sterols, and polysaccharides. Researchers have identified numerous sapindosides that possess remarkable biological activities. Singh and Singh (2010) reported that these bioactive compounds contribute to the plant's antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects.

Immunomodulatory Properties

Soapnut-derived saponins have attracted considerable attention as vaccine adjuvants. According to Sun et al. (2009), triterpenoid saponins stimulate antigen-presenting cells, activate macrophages, and promote cytokine secretion. Researchers have demonstrated that these saponins enhance antibody production by improving antigen uptake and stimulating both Th1 and Th2 immune responses. Sparg et al. (2004) observed that soapnut saponins exhibit strong adjuvant properties comparable to commercially available vaccine adjuvants while producing fewer adverse reactions.

Antimicrobial and Antioxidant Activities

According to Huang et al. (2008), soapnut extracts exhibit broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. Researchers have reported inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. Singh and Singh (2010) observed that the antioxidant compounds present in soapnut effectively neutralize free radicals and inhibit lipid peroxidation. These biological activities contribute significantly to improved immune protection.

Potential as a Natural Vaccine Adjuvant

Researchers have increasingly recognized soapnut saponins as potential alternatives to synthetic adjuvants. According to Sun et al. (2009), purified plant saponins enhance vaccine efficacy by stimulating prolonged antibody responses and cellular immunity. These characteristics make soapnut an attractive candidate for improving IgY production in laying hens and for disease prevention in aquaculture.

**5. Shikakai (*Acacia concinna*)**

Botanical Description

*Acacia concinna* (Willd.) DC., commonly known as Shikakai, belongs to the family Fabaceae (Mimosoideae). According to Kirtikar and Basu (2005), the plant is widely distributed throughout tropical India and has long been utilized in traditional Ayurvedic medicine. Researchers have reported that various parts of the plant, particularly the pods, leaves, and bark, contain valuable phytochemicals with medicinal properties.

Phytochemical Composition

According to Anonymous (2001), *Acacia concinna* contains abundant triterpenoid saponins, flavonoids, tannins, alkaloids,

phenolic compounds, glycosides, and organic acids. Researchers have observed that these secondary metabolites exhibit synergistic biological activities responsible for the therapeutic efficacy of the plant. Flavonoids and phenolic compounds provide antioxidant activity, whereas saponins contribute to immune stimulation.

Pharmacological Activities

Numerous pharmacological investigations have demonstrated that *Acacia concinna* possesses antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, and immunomodulatory activities. According to Kalaivani et al. (2011), methanolic extracts of the plant inhibit several pathogenic bacteria and fungi. Researchers have reported that the antioxidant activity effectively reduces oxidative stress by scavenging free radicals and enhancing endogenous antioxidant defense systems.

Immunomodulatory Potential

According to Sparg et al. (2004), plant saponins isolated from *Acacia* species stimulate macrophages, lymphocytes, and antibody-producing plasma cells. Researchers have demonstrated that saponins enhance antigen presentation and improve vaccine-induced immune responses. These findings suggest that *Acacia concinna* may serve as a valuable natural immunoadjuvant for enhancing chicken IgY antibody production.

**7. Comparative Analysis of Plant-Derived Saponins as Immunoadjuvants**

Plant-derived saponins have emerged as promising natural immunoadjuvants because of their ability to stimulate both innate and adaptive immune responses. According to Francis et al. (2002), saponins activate antigen-presenting cells, promote cytokine secretion, and enhance antibody production without causing significant toxicity. Researchers have reported that soybean, fenugreek, soapnut, and shikakai each contain structurally distinct saponins that differ in their biological potency and pharmacological properties.

Soybean contains soyasaponins that exhibit moderate immunostimulatory activity while providing excellent nutritional benefits. Fenugreek contains steroidal saponins, particularly diosgenin, which have been reported to possess strong anti-inflammatory and immune-enhancing effects. Soapnut is recognized as one of the richest natural sources of triterpenoid saponins and demonstrates exceptionally high adjuvant activity comparable to commercial vaccine adjuvants. Shikakai contains triterpenoid saponins with significant antioxidant, antimicrobial, and immunomodulatory properties.

According to Sun et al. (2009), the effectiveness of saponin-based immunoadjuvants depends on their chemical structure, concentration, purity, and compatibility with vaccine antigens. Researchers have demonstrated that combining plant-derived saponins with bacterial antigens significantly improves humoral immune responses and increases antigen-specific antibody titers. Francis et al. (2002) observed that these natural compounds also stimulate macrophage activation, lymphocyte proliferation, and complement-mediated immune responses.

The comparative evaluation of these four medicinal plants indicates that each possesses unique phytochemical characteristics suitable for immunoadjuvant development. Soybean offers nutritional and moderate immune benefits, fenugreek provides potent steroidal saponins and antioxidant compounds, soapnut exhibits superior vaccine adjuvant properties owing to its abundant triterpenoid saponins, while shikakai contributes strong antimicrobial and immunomodulatory activities. Collectively, these medicinal plants represent sustainable, biodegradable, and cost-effective alternatives to synthetic immunoadjuvants for enhancing chicken egg yolk antibody (IgY) production and improving disease management in freshwater aquaculture.

**8. Applications of Plant-Derived Saponins in IgY Production**

The production of chicken egg yolk immunoglobulin (IgY) has emerged as an effective, economical, and animal-friendly approach for passive immunization against infectious diseases. According to Schade et al. (2005), IgY antibodies possess high antigen specificity and can be produced in large quantities without causing harm to experimental animals. Researchers have reported that the success of IgY production largely depends on the selection of appropriate immunoadjuvants capable of enhancing antigen presentation and stimulating strong humoral immune responses. Plant-derived saponins have attracted considerable attention because of their potent immunostimulatory properties and excellent safety profile.

According to Sun et al. (2009), saponins function as effective vaccine adjuvants by promoting antigen uptake, activating antigen-presenting cells, and stimulating cytokine secretion. Researchers have demonstrated that these mechanisms significantly increase B-cell activation, plasma cell differentiation, and antibody synthesis in immunized hens. Francis et al. (2002) reported that saponins enhance both Th1 and Th2 immune responses, resulting in higher antibody titers and prolonged immune memory. These immunological properties make plant-derived saponins suitable candidates for improving IgY production against bacterial, viral, and parasitic pathogens.

Soybean (*Glycine max*) saponins have been reported to improve humoral immunity by stimulating macrophage activation and increasing cytokine production. According to Guang et al. (2014), soyasaponins enhance antigen-specific antibody production while exhibiting low toxicity. Researchers have observed that soybean-derived saponins improve vaccine efficacy by increasing antibody persistence and immunological memory. Similarly, fenugreek (*Trigonella foenum-graecum*) contains

steroidal saponins such as diosgenin that have been shown to stimulate lymphocyte proliferation and enhance antibody responses. According to Yadav and Baquer (2014), fenugreek supplementation improves immune competence through modulation of cytokine expression and activation of immune cells.

Soapnut (*Sapindus mukorossi*) has been identified as one of the richest natural sources of triterpenoid saponins with remarkable adjuvant activity. According to Sparg et al. (2004), soapnut saponins stimulate dendritic cells, macrophages, and lymphocytes, thereby enhancing antigen presentation and antibody production. Researchers have demonstrated that purified soapnut saponins produce antibody responses comparable to commercially available vaccine adjuvants while causing minimal adverse reactions. Likewise, *Acacia concinna* (Shikakai) contains biologically active saponins and flavonoids that exhibit immunomodulatory effects. According to Kalaivani et al. (2011), extracts of *A. concinna* stimulate immune cell activation and improve humoral immune responses, making them potential adjuvants for IgY production.

Recent studies have suggested that combining plant-derived saponins with bacterial antigens significantly enhances the production of pathogen-specific IgY antibodies. According to Schade et al. (2005), higher IgY concentrations improve passive immunization against infectious diseases and reduce dependence on antibiotics. Researchers have reported that IgY antibodies produced using natural immunoadjuvants exhibit greater neutralizing capacity, improved pathogen agglutination, and enhanced therapeutic efficacy. Therefore, plant-derived saponins represent promising natural immunoadjuvants for developing sustainable IgY-based immunotherapies.

**9. Applications of Plant-Derived Immunoadjuvants in Aquaculture**

Aquaculture has become one of the fastest-growing food-producing sectors worldwide; however, infectious diseases continue to limit production efficiency and economic sustainability. According to FAO (2024), bacterial diseases caused by *Aeromonas*, *Vibrio*, *Edwardsiella*, and *Streptococcus* species account for significant production losses in freshwater and marine aquaculture. Researchers have reported that excessive use of antibiotics has resulted in antimicrobial resistance, environmental contamination, and food safety concerns. Consequently, natural immunoadjuvants have gained considerable importance as environmentally friendly alternatives for disease prevention.

According to Ringø et al. (2020), plant-derived immunoadjuvants enhance innate immunity by stimulating macrophages, neutrophils, complement proteins, lysozyme activity, respiratory burst activity, and phagocytosis. Researchers have observed that dietary supplementation with saponin-rich medicinal plants significantly improves disease resistance and survival in cultured fish and shrimp. Hoseinifar et al. (2018) reported that herbal immunostimulants also improve feed utilization, digestive enzyme activity, intestinal microbiota composition, and antioxidant defense mechanisms.

Soybean, fenugreek, soapnut, and shikakai have demonstrated promising applications in aquaculture nutrition. According to Dawood et al. (2019), dietary soybean saponins improve immune function, antioxidant status, and intestinal integrity in aquatic animals. Researchers have reported that fenugreek supplementation enhances growth performance, feed conversion efficiency, and resistance against bacterial pathogens. Similarly, soapnut saponins exhibit strong antimicrobial activity by inhibiting the growth of pathogenic bacteria and preventing disease outbreaks. *Acacia concinna* extracts have also been reported to improve immune responses and antioxidant enzyme activities while reducing oxidative stress during bacterial infections.

Plant-derived immunoadjuvants have also shown considerable potential as vaccine adjuvants in aquaculture. According to Sun et al. (2009), saponin-based adjuvants enhance antigen-specific immune responses and increase vaccine efficacy by stimulating both humoral and cellular immunity. Researchers have demonstrated that vaccination combined with natural immunoadjuvants results in improved survival following pathogen challenge experiments. Such findings indicate that plant-derived saponins can serve as sustainable immunostimulants for improving fish health and reducing reliance on chemotherapeutic agents.

Recent investigations have emphasized the integration of herbal immunoadjuvants into functional aquafeeds. According to Ringø et al. (2020), functional feeds containing plant-derived immunostimulants improve gut health, beneficial microbial populations, nutrient utilization, and immune competence. Researchers have concluded that these natural products contribute significantly to sustainable aquaculture by enhancing productivity while minimizing environmental impacts and antibiotic usage.

**10. Future Perspectives and Conclusion**

Natural plant-derived immunoadjuvants are increasingly recognized as valuable alternatives to synthetic vaccine adjuvants because of their safety, biodegradability, and broad-spectrum biological activities. According to Francis et al. (2002), medicinal plants rich in saponins provide promising opportunities for enhancing vaccine efficacy, antibody production, and disease resistance in both veterinary and aquaculture applications. Researchers have reported that continued exploration of medicinal plants will facilitate the discovery of novel immunostimulatory compounds suitable for commercial vaccine development.

Recent advances in molecular biology, nanotechnology, proteomics, metabolomics, and immunology have greatly expanded

the understanding of plant-derived immunoadjuvants. According to Sun et al. (2009), purification of specific saponin fractions and structural modification of these compounds may further improve their immunological efficacy while minimizing toxicity. Researchers have demonstrated that nanoformulations containing plant saponins enhance antigen delivery, prolong antigen release, and improve immune responses. Such innovations provide exciting opportunities for developing next-generation vaccines and antibody production systems.

The integration of plant-derived immunoadjuvants with IgY technology represents a promising strategy for controlling infectious diseases in aquaculture. According to Schade et al. (2005), pathogen-specific IgY antibodies offer effective passive immunization without contributing to antimicrobial resistance. Researchers have observed that combining IgY antibodies with natural immunoadjuvants significantly enhances protective efficacy against bacterial pathogens while maintaining environmental sustainability.

Among the medicinal plants reviewed, soybean provides nutritionally valuable soyasaponins with moderate immunostimulatory activity, fenugreek contributes steroidal saponins and antioxidant compounds, soapnut serves as an exceptionally rich source of highly potent triterpenoid saponins, and shikakai provides multifunctional phytochemicals with antimicrobial and immune-enhancing properties. According to Sparg et al. (2004), these plants collectively represent excellent sources of natural immunoadjuvants for veterinary vaccines and antibody production systems.

In conclusion, plant-derived saponins obtained from *Glycine max*, *Trigonella foenum-graecum*, *Sapindus mukorossi*, and *Acacia concinna* possess significant pharmacological, antimicrobial, antioxidant, and immunomodulatory activities. Researchers have consistently demonstrated their potential to enhance IgY antibody production, improve vaccine efficacy, and strengthen disease resistance in aquaculture species. Future research should focus on purification of active saponin fractions, molecular mechanisms of immune modulation, large-scale production technologies, and commercial application of these natural immunoadjuvants for sustainable aquaculture and veterinary medicine..

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