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# Investigation of the Potential Immunomodulatory Effects of Some Medicinal Plants on the Immune Response of Layer Birds Vaccinated with a Live *Salmonella gallinarum* Vaccine

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ABSTRACT

Plant-derived immunomodulators modulate adaptive immune responses to vaccines. The present study aims to investigate the acquired immune responses elicited by live attenuated *Salmonella gallinarum* vaccine complemented by some herbal extracts. Sixty chicks (one day old) were divided into six groups; Unvaccinated (NEG), *Vernonia amygdalina* (VA), *Garcinia kola* (GK), *Curcuma longa* (CL), *Zingiber officinale* (ZO), and polyherbal (PH) groups. The NEG group received distilled water, while the other groups were administered live attenuated *Salmonella gallinarum* vaccine intramuscularly (0.2 mL/bird) at 5 and 9 weeks. Aqueous extracts of the plants were administered singly, and in combination (2 mL/bird orally) at 7 and 11 weeks. At 8, and 12 weeks, circulating immunoglobulin G (IgG) antibody titres were evaluated using haemagglutination (HT) test and *enzyme linked immunosorbent assay (*ELISA). Cell-mediated response was evaluated using the delayed-type hypersensitivity response (DTHR) test.
After primary immunization, IgG titres were significantly higher in the vaccinated groups (0.515 - 0.716) compared to the unvaccinated (NEG) group (0.364). After secondary immunization, there was a boost in the IgG titres, with the PH group exhibiting the highest IgG titre (0.768). The haemagglutination test showed the highest agglutination in the VA group (100%), followed by GK (80%), ZO/PH (60%), and CL (40%). Cell-mediated immunity after 24 h showed an insignificant increase in paw size of the vaccinated chicks, with the CL group showing the highest increase in paw size (3.2%). The results showed that the herbal extracts had immunomodulatory effects with some extracts enhancing the acquired immune responses while others suppressed it.

***Keywords*:** *Salmonella gallinarum*, Cellular, Humoral, Herbal extracts, Immunomodulation

## Introduction

Natural remedies have been a valuable source of bioactive compounds for centuries, and have been used ever since in the development of new treatments for communicable diseases, cancer, hypertension, diabetes, etc. Many plant-derived phytochemicals, such as alkaloids, glycosides, and polyphenols, have been shown to possess bioactive properties to mitigate the raging threat of antimicrobial resistance.[1]The emergence of microbial resistance to antibiotics is a global risk to economic development. Nonetheless, plants are being explored as a rich source of lead compounds to combat this challenge. Plant-derived immunomodulators, in particular, have shown great promise in this regard. Immunomodulators are compounds that can modulate adaptive immunity to infections, and those derived from plants have been found to possess unique properties that can help address antimicrobial resistance (AMR).

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Immunomodulators derived from plants can mitigate AMR by enhancing the innate immune responses to infection, inhibiting the growth of resistant microorganisms, reducing inflammation and tissue damage and improving the efficacy of existing antimicrobial agents. The use of plant-derived entities as medicines has many advantages, including natural origin, availability, affordability, safety, minimal side effects and sustainability. Plant-derived immunomodulators have been investigated for their potentials to increase or modulate the immune response to vaccines, making them a promising adjuvant to vaccination. Researchers have proposed that the concurrent administration of imunomodulators with vaccines, or before or after vaccination, will boost immune responses, reduce vaccine dosing and improve vaccine safety.[2] *Curcuma longa*, commonly called turmeric is a flowering plant that belongs to the family Zingiberaceae. The plant is widely distributed in Africa and Asia and grows all year round. Studies have shown that *Curcuma longa* increases the expression of interleukin-2 and interferon-gamma, which are crucial cytokines involved in the Th-1 (T-helper 1) immune response pathway. The Th-1 pathway is essential for cell-mediated immunity and the elevation of IL-2 and IFN-γ expression suggests that *Curcuma longa* may have immunomodulatory effects, and enhance the body's natural defense against infections and diseases.[3] The T-helper cells equally help B cells to make good blocking antibodies. Curcumin, a polyphenol extracted from *Curcuma longa* activates dendritic cells, natural killer cells, and macrophages. These effects are utilized in presenting peptides to T cells, kill infected cells and activate Bcells, which are essential for adaptive immunity. Curcumin has also been reported to block the inflammatory functions of signaling proteins, like TNF-α, IL-1β and suppress the metabolic pathways involved in inflammation,

including the NF-KB signaling pathway.[4] *Vernoniaamygdalina* is a small plant that is native to tropical Africa and belongs to the order, Asterales, family Asteraceae, genus Vernonia and species amydalina.[5]It is popularly used in folkloric medicine for its therapeutic potential owing to its bioactive constituents.[6,7] Momoh *et al*. (2012)[8] studied the effects of a combination of *V.amygdalina* extract and Immunaceat different doses on CD4+ cell count of AIDS patients on ART-regimen. The results showed a dose-dependent increase in CD4+ cell count.[8] In a study by Im *et al*. (2016)[9] *V. amygdalina* was shown to enhance immune responses of rats. Studies have also shown that the concurrent administration of *Vernonia amygdalina* with hepatitis B vaccine reduced the dosing frequency of hepatitis B vaccine from 3 doses to 2 doses, evoked a higher antibody titre than the conventional vaccine, and increased haematological indices.[10,11]

*Zingiber officinale* is a flowering plant that originated from Southeast Asia. The rhizome of the plant known as the ginger root, is employed for its culinary and medicinal properties.[12] Compounds like gingerol, shogaols, zingiberene, and zingerone have been isolated from *Zingiber officinale*.[13,14] Some studies have shown that *Zingiber officinale* or its compounds have immunomodulatory effect. For, example, an aqueous extract of *Zingiber officinale* have been shown to reduce the stimulation of the inflammatory cytokines interleukin-1β (IL-1β), TNF-α, and interleukin-6(IL-6), and potentially eased symptoms associated with various inflammatory disorders.[15] Additionally, gingerol, a bioactive compound of ginger was shown to increase the expression of T-helper 1 (Th-1), and T-helper (Th-17), interferon gamma (IFN-γ),and activate the P38 Mitogen-Activated Protein Kinase (MAPK) signaling pathway, which helps in regulating immune responses and inflammation.[16] Zingerone, another bioactive compound found in ginger has been shown to inhibit the expression of innate receptors such as Toll-like receptor 4 (TLR4), an important molecule in innate immunity responsible for recognizing pathogen-associated molecular patterns and activation of immune responses, suppression of MyD88-dependent signaling, which is a central pathway in innate immune responses and inhibition of MAPK-activation and expression of inflammatory gene in lipopolysaccharide (LPS)-induced liver failure of C57BL/6 mice.[17] Studies have also shown that G*arcinia kola* and garcinoic acid exhibited immune-modulatory properties by decreasingsignificantly the production of pro-inflammatory cytokines induced by the coronavirus spike glycoprotein, and decreased the expression of phosphor-1kBα and phosphor-p65 in cell-culture assays leading to a reduction in NF-KB-dependent luciferase activation after stimulation with the S1 spike protein.[18-21] A study by Olajide *et al*. (2021)[22] showed that pre-treatment of cells with the extract containing garcinoic acid controlled the damage to adjoining lung epithelial cells, suggesting a protective effect against cellular damage caused by the S1 spike protein. Fowl typhoid, a disease of birds, is caused by *Salmonella enterica* subspecies *enteric* serovar Gallinarum biovar Gallinarum. It is an acute or chronic septicaemic disease with high mortality rates, usually exceeding 80%. Fowl typhoid is prevalent in developing countries, where biosecurity measures are usually inadequate.[23,24] The commercially available fowl typhoid vaccine is inactivated, it stimulates a strong humoral response but cannot elicit T cell response essential for killing infected cells. This limitation justifies the need for more effective vaccines and control strategies to combat fowl typhoid. It is important to note that B cells and T cells contribute greatly to protecting the host against intracellular bacteria like Salmonella, and future vaccine developments should focus on robust T cell responses to ensure optimal protection against fowl typhoid.[25] The vaccine candidate used in this experiment was a live, attenuated *Salmonella gallinarum* vaccine complemented with plant immunomodulators. Live bacterial vaccines have several advantages, including; (i) resemblance to natural infection where they mimic the natural infection process, which can stimulate a more comprehensive immune response, (ii) they are orally administered, which is a more convenient and less invasive route than injectable vaccines.[26,27] This study investigated how immunosuppressant or stimulatory plants can affect live-attenuated vaccines. This may be especially significant for children exposed to plant immunomodulators *in utero*, as they may be at risk of infections due to T-cell suppression.

## Materials and Methods

*Reagents*

Distilled water (National Commission for Energy Research and Development (NCERD), University of Nigeria, Nsukka), Pullorum antigen stained antigen polyvalent, Salmonella polyvalent Groups B and D SPA, negative SPA serum (Charles River, Wilmington, MA, USA). Group B and D Antibody ELISA test kit from Biochek, Netherlands.

*Culture media*

Nutrient agar and broth (Lab M, Lancashire, UK), Salmonella-Shigella Agar (Oxoid, England). The culture media were prepared following the manufacturer's instructions.

*Collection and identification of plant materials*

*Zingiber officinale* (Ginger), *Vernonia amygdalina* (Bitter leaf), *Garcinia Kola* (Bitter kola), and *Curcuma longa* (Turmeric) were collected from Nsukka, Enugu State, Nigeria in the month of October, 2020. The whole plant was identified and authenticated by Mr. Felix Nwafor, a plant taxonomist of the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka. The voucher specimens were deposited at the University of Nigeria Herbarium and the voucher numbers are UNN/11721, UNN/11722, UNN/11723, UNN/11724 for *Zingiber officinale* Rosc., *Curcuma longa* L., *Vernonia amydalina* Del. and *Garcinia kola* Heckel, respectively.

*Salmonella gallinarum*

Live attenuated *Salmonella gallinarum* serovar enteriditis biovar gallinarum was a donation from Dr Moses Gyang, National Veterinary Research Institute, Vom, Jos, Nigeria. *S. gallinarum* virulent strain 9.

*Chicks*

Sixty (one day old) Lohmann layer chicks of average weight 42 g were obtained from Agrited farms, Ogun State. The birds were handled according to the guidelines of University of Nigeria Research Ethics Committee (FPSRA/UNN/24/0085).

*Bacteria collection, identification, and culture*

The bacterium was collected from a field case of Fowl typhoid occurring in Vom, Jos. The organism was mutagenized by passaging twenty times in a foreign host. A loopful of the stored organism was aseptically inoculated into nutrient broth and incubated at 37ºC overnight, after which a loopful was streaked on MacConkey agar to confirm viability and presence of Salmonella. Biochemical tests using MacConkey agar and Salmonella-Shigella agar were done to confirm the presence of *Salmonella gallinarum*. An overnight culture of the live attenuated *Salmonella gallinarum* was inoculated in 10 mL of sterile nutrient broth (Lab M, Lancashire, UK). This was diluted 1/100 with nutrient broth and then incubated at 37℃ for 2 h (log phase). Serial dilutions to $10^{-10}$ were prepared on nutrient agar and incubated at 37℃ for 24 h. The original and diluted cultures were counted. Surface viable count was done to enumerate the number of colonies per mL. The original cell population (cells/mL) was calculated using the formula below:

$$\text{Original cell population (cells/mL)} = \frac{Meancountpermlx\frac{1}{dilution}factor}{1\ ml}$$

*Vaccination*

Sixty chicks (one day old) were divided into six groups of 10 birds each. The groups include; I: Unvaccinated (NEG), II: *Vernonia amygdalina* (VA), III: *Garcinia kola* (GK), IV: *Curcuma longa* (CL), V: *Zingiber officinale* (ZO), and VI: polyherbal (PH) groups. At 5 weeks old, the chicks in groups II – VI were vaccinated with 0.2 mL/bird (2.5 x $10^7$cfu/mL) of the live attenuated *Salmonella gallinarum* vaccine intramuscularly. The unvaccinated group was

given water only during the vaccinations. The vaccination was repeated at 9 weeks of age for the secondary vaccination.

*Administration of the herbal extracts*
Freshly washed roots of *Garcina kola*, *Curcuma longa* and *Zingiber officinale* were dried using an oven at 40ºC, while fresh washed leaves of *Vernonia amygdalina* were air-dried. The dried roots and leaves were pulverized and packaged in airtight containers until ready for use. The powdered plant samples (1g each) were macerated in 100 mL of distilled water and filtered. The filtrate was concentrated to dryness to obtain the dried aqueous extracts. At 7 and 11 weeks old, the vaccinated birds were administered 2 mL each of 0.025 g/mL of the different aqueous extracts orally. The chicks in the polyherbal (PH) group were administered 2 mL each of a mixture of all the plant extracts.

*Blood sample collection for antibody titre evaluation*
At 8 and 12 weeks, blood sample was collected from the jugular vein of each bird using a sterile syringe and emptied into marked ethylene-diamine-tetra-acetic acid (EDTA) tubes. Sera were prepared from the blood samples to determine the circulating antibody (IgG) titres by plate agglutination test and *enzyme linked immunosorbent assay (*ELISA).

*Haemagglutination test*
Serum samples from all the birds were tested with one drop of crystal violet Pullorum antigen stained polyvalent antigen. A drop of the serum samples of the immunized birds from the different groups, one group at a time, was placed next to the drop of antigen. The drops of antigen and serum were mixed on a tile using a glass rod which was wiped clean between samples. The tiles were rotated with gentle rocking for 2 min and signs of agglutination observed. The results were compared with the Salmonella positive polyvalent Groups B &D SPA and the negative SPA serum.

*Enzyme linked immunosorbent assay (ELISA)*
Blood was collected from the jugular vein and the serum was separated after the blood clotted. The sera were stored at -26ºC until ready for assay. A commercial ELISA kit (Biochek, Netherlands) was used according to the manufacturer's instructions to obtain optical density (OD) values. The OD values from the different groups were compared to the standard positive and negative sera.
*Delayed type hypersensitivity response (DTHR)*
DTHR was induced in the layer birds using 85-kb virulent strain of *Salmonella gallinarum* 9 as the antigen. The birds in the different groups were sensitized by subcutaneous injection of 0.2 mL of 1 x$10^7$ cells/mL of an 85-kb virulent strain of *Salmonella gallinarum* (University of Nottingham, UK) (day 0) in the plantar region of the right foot paw and challenged on day 5 by subcutaneous injection of the same amount of antigen into the left foot paw. The plant extracts (2 mL of 0.025 g/mL) were administered orally 3 days prior to sensitization and continued until the challenge. The oedema produced by antigenic challenge in the left paw was taken as the difference in the paw thickness before and 24 h after the challenge. The paw thickness was measured with a vernier caliper.

*Statistical analysis*
All data were expressed as the mean ± SD. Sigma plot 11.0 was used to determine the significant differences applying a one-way analysis of variance (ANOVA) test.

## Results and Discussion

*Vaccine preparation*
Live attenuated *Salmonella gallinarum* strain was used. Serial dilutions were performed to estimate the original cell population (2 x $10^8$ cfu/mL). Each dose contained 0.2 mL of 2.5 x $10^7$ cfu/mL of the weakened strain. The vaccine was administered immediately after preparation without freeze-drying. The biochemical tests confirmed the presence of pure cultures of *Salmonella gallinarum*. The cells were cultivated at the exponential growth stage and showed distinct, pure colonies. The vaccine schedule consisted of two intramuscular injections at 5 weeks and 9 weeks of age. Live attenuated vaccines typically evoke strong humoral and cellular immunity, but this weakened vaccine candidate required adjuvants due to a possible defective somatic antigen during attenuation. Adjuvants can enhance the immune response by activating T cells, increasing dendritic cell migration, antigen processing, and co-stimulatory molecule expression.[28] Overall, this vaccine candidate stimulated an immune response against *Salmonella gallinarum* and other Group D Salmonella strains by colonizing internal lymphoid tissues and activating appropriate immune cells.

*Haemagglutination after immunization*
The haemagglutination test is a technique used to detect the presence of antibodies against a particular antigen, in this case, the *Salmonella gallinarum* vaccine.[31,36] The haemagglutination test is an important tool in ensuring the quality and efficacy of vaccines, particularly in resource-limited settings where access to advanced diagnostic techniques may be limited. By detecting the presence of antibodies, this test helps determine the immune response to other vaccines and inform vaccine development and distribution strategies. There is a significant disparity between vaccine production and consumption in Africa. This disparity emphasizes the need for increased investment in local vaccine production, distribution, and delivery infrastructure to address the significant demand and ensure equitable access to vaccines for the African populations.[29] Access to easy and cost-effective vaccine technology is therefore crucial in addressing emerging pandemic. Scalability is also essential to meet the demand for vaccines during a pandemic. Additionally, personnel training and ultra-cold chain infrastructure are critical components in the vaccine development and distribution process. Well-trained personnel are necessary for vaccine manufacturing, quality control, and administration, while ultra-cold chain infrastructure ensures the vaccines remain effective during transportation and storage. Collaborative efforts among governments, industry, and global health organizations to share resources, expertise, and risks are expedient. The haemagglutination test results indicate that unvaccinated birds (NEG group) showed no agglutination, as expected, while the vaccinated birds (VA, GK, ZO, PH, and CL groups) showed varying degrees of agglutination, indicating the stimulation of antibodies against the *Salmonella gallinarum* antigen. The *Vernonia amydalina* (VA) group showed the highest agglutination (100%), suggesting a strong immune response. The other vaccinated groups (GK, ZO, PH, and CL) showed lower agglutination levels, ranging from 80% to 40%. All vaccines induced an immune response, but to varying degrees (Figure 1). The finding that *Vernonia amygdalina* showed 100% agglutination and has immune-boosting properties is consistent with previous reports.[30] Cytokine regulation is a crucial aspect of immune system function, and ability of VA to strengthen the immune system through this mechanism is a valuable attribute. These results suggest that VA could be a promising adjuvant or component of vaccine strategies, particularly in combination with other vaccines or immune-modulating agents. Further research could explore the optimal combination and dosing regimens to achieve the strongest immune response.
*Antibody titres after immunization*
The Enzyme-Linked Immunosorbent Assay (ELISA) results indicate that all groups showed antibody (IgG) titres higher than the negative control(0.364), and the positive control (0.422) after primary immunization. The average optical density (OD) values after primary immunization and administration of herbal extracts were; ZO (0.716 ± 0.17) > PH (0.642 ± 0.22) > VA (0.633 ± 0.16) > GK (0.585 ± 0.30) > CL (0.5148 ± 0.32) (Figure 2). These results suggest that all vaccinated groups developed an immune response, as indicated by antibody titres higher than the standard negative control. The herbal extracts used in this study enhanced the immune response, with ZO showing the highest OD value, followed by PH, VA, GK, and CL.The

average optical density after secondary immunization, and administration of herbal extracts were higher than the values obtained after primary immunization, indicating a boosted immune response following the secondary immunization. The order of OD values after secondary immunization were; PH (0.768) > CL (0.743) > VA (0.670) > ZO (0.665) > GK (0.571) (Figure 2). The negative control (0.429) and positive control (0.602) values were lower than all the vaccinated groups except GK. These findings indicate that PH which is a combination of all the herbal extracts, showed the highest OD value of 0.768 after secondary immunization, indicating a strong immune response. This suggests that the combination of herbal extracts in PH had a synergistic effect, leading to an enhanced immune response compared to the individual herbal extracts. This is a promising finding, as it indicates that a combination of herbal extracts may have a more significant impact on immune system stimulation than using individual extracts. Onah *et al* (2019)[10] revealed that the level of antibody response in mice immunized with hepatitis B vaccine (HV) and *V.amygdalina* (VA) had a significant ($P$<0.05) increase compared to hepatitis B vaccine group, *V. amygdalina* group or distilled water.[10] ZO also showed potentiating effect, consistent with the plate agglutination test. In a research conducted by Onuigbo *et al*. (2017), ZO-primed Newcastle disease virus vaccine had a potentiating effect with an increased overall mean antibody titre.[32] ZO can alter the immune system by stimulating granulocytes, macrophages, complement, and certain T-lymphocytes and enhance immune reactions by increasing effector substances.[35] The consistently lower OD values for *Garcinia kola* (GK) after both primary and secondary immunizations suggest that it may have a suppressive effect on the humoral immune response, as previously reported. This means that GK may have an immunomodulatory effect, but in this case, it appears to be reducing the immune response rather than enhancing it. This finding is important, as it highlights the complex and potentially variable effects of herbal extracts on the immune system. While some extracts like PH (the combination of all herbal extracts) may enhance the immune response, others like GK may have a suppressive effect. GK's suppressive effect could be explored in situations where immune suppression is desired, such as in autoimmune diseases or organ transplantation. Additionally, we could also investigate whether combining GK with another herbal extracts or immunomodulator could mitigate its suppressive effects and enhance overall immune responses. Onah *et al*. (2018)[2] showed that *Garcinia kola* had a suppressive effecton the immunoglobulin G when the mice were given Hepatitis B virus subunit vaccine. The treatment groups were comparable at the given dose and were not statistically different. In other words, the results suggest that the different herbal extracts used in this study did not have a significantly different effect on the immune response after primary immunization, at the dose administered. This indicates that the treatment groups were equivalent in terms of their effects on the immune response at this stage. A P-value of 0.213 indicates that the observed differences between the groups were not statistically significant, suggesting that the differences are due to random sampling rather than a real effect. After the secondary immunization, the statistical analysis revealed that the differences in mean values among the treatment groups were greater than would be expected by chance. This suggests that the secondary vaccination enhanced the immune response, and the herbal extracts had a more pronounced effect on the immune system after the secondary vaccination. Significant differences existamong the treatment groups, implying that some herbal extracts may be more effective than others in enhancing the immune response. This observation highlights the importance of secondary vaccination in enhancing the immune response.

*Delayed Type Hypersensitivity Response (DTHR)*

The results obtained from the delayed type hypersensitivity (DTH) response as seen in Table 1 indicated that the herbal extracts inhibited the DTH response evoked by the antigen in all the treatment groups. The cell-mediated immunity, measured by the DTH response, showed an insignificant increase in paw size in the vaccinated groups, in the following order; CL (3.2%) > VA (2.0%) > PH (1.63%) > GK (1.14%) > ZO (1.05%). The negative control (NEG) group had the highest percentage increase in paw size of 5.30%, indicating a stronger DTH response compared to the vaccinated groups. These findings suggest that the herbal extracts may have immunosuppressive effects on cell-mediated immunity, as measured by the DTH response. The inhibition of the DTH response in the treatment groups may indicate a reduced ability to mount a cell-mediated immune response, which is an important aspect of immune function. The results also suggest that the negative control group, which did not receive any herbal extracts, had a more robust DTH response because the antigen sensitized the T-lymphocytes to release pro-inflammatory cytokines without inhibition from any extract. DTHR is a cell-mediated response by interferon-gamma producing CD4+ or CD8+ cells.[33,34] The response takes about 24-72 h to activate the T cells, which then leads to the recruitment of monocytes and lymphocytes.[37] This delayed response is the hallmark of cell-mediated immunity and allows for the activation, proliferation, and differentiation of T cells, including CD4+ and CD8+ T cells which then coordinate the immune response by producing cytokines and recruiting other immune cells to the site of antigen exposure. The low cell-mediated response exhibited by the extracts could be related to the dose of the extracts administered to the chicks. DTHR is a dose-related reaction, high doses inhibit the sensitized T-lymphocytes by the antigen from releasing pro-inflammatory cytokines leading to oedema.[38,39]

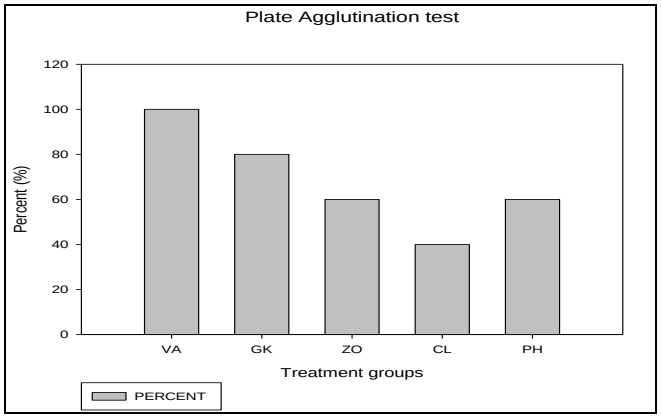


**Figure 1:** Plate agglutination of the vaccinated groups given herbal extracts post vaccination.
Key: *Vernonia amydalina* (VA); *Garcina Kola* (GK); *Zingiber officinale* (ZO); *Curcuma longi* (CL); Polyherbal (PH)

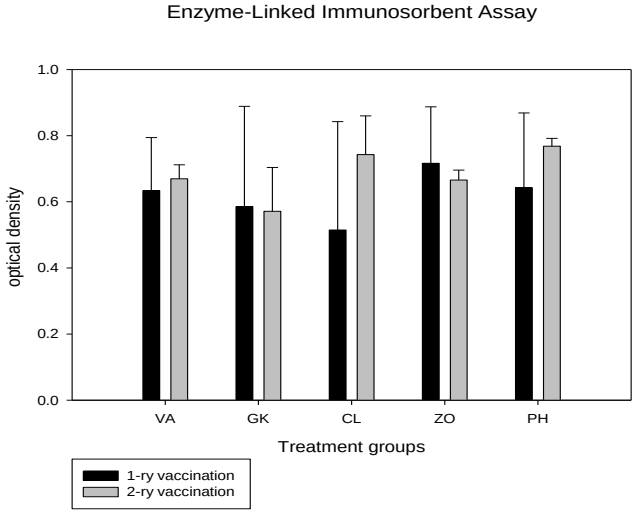


**Figure 2:** Comparison of the IgG titres of the various treatment groups

**Table 1:** Percentage inhibition of the delayed-type hypersensitivity response

| Treatment Group | Number in a group | DTHR Oedema($cm^3$) | Mean Inhibition (%) |
|---|---|---|---|
| VA | 10 | 0.035± 0.009 | 98.00 |
| GK | 10 | 0.020 ± 0.002 | 98.86 |
| ZO | 10 | 0.018± 0.008 | 98.95 |
| CL | 10 | 0.060 ± 0.170 | 96.80 |
| PH | 10 | 0.030±0.000 | 98.37 |
| NEG | 10 | 0.095±0.080 | 94.70 |

*Vernonia amydalina* (VA); *Garcina Kola* (GK); *Zingiber officinale* (ZO); *Curcuma longi* (CL); Polyherbal (PH); Negative control (NEG).

## Conclusion

The herbal extracts used in this study had immunomodulatory effects, with some extracts enhancing the immune response while others suppressed it. The combination of herbal extracts (PH) showed the highest immune-enhancing effect, implying a potential synergistic effect. *Garcinia kola* (GK) extract showed a suppressive effect on the immune response, indicating potential anti-inflammatory properties. DTHR was highly inhibited in all the treatment groups, suggesting that the herbal extracts may have suppressed the T-lymphocytes or that the test duration was short.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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