

Immunomodulatory Potential of Probiotics and Prebiotics as Adjuvants in Rats Vaccinated with Inactivated Poliovirus: Comprehensive Immunological and Histopathological Evaluation

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Abstract

Objective: Poliomyelitis remains a global public health concern, particularly in developing countries, where vaccine responses are suboptimal due to limited mucosal and cell-mediated immunity. Recent research has shown that probiotics and plant-derived prebiotics are novel mucosal adjuvants that enhance both innate and adaptive immune responses following vaccination. The current study aimed to evaluate the ability of prebiotics (ginger rhizomes) and probiotics (*Lactobacillus acidophilus* 20552s) as mucosal adjuvants to improve immunity following polio vaccination in rats.

Methods: This study examined forty rats equally allocated into four different groups: a control group (POG), and three experimental groups receiving the oral polio vaccine along with either ginger rhizomes prebiotics (PRE), *Lactobacillus acidophilus* 20552s probiotics (PRO), or a mixture of both (MIX). Serum levels of IFN- γ , TPA, and IL-5 were analyzed. Histopathological assessments of the liver, spleen, and kidneys were also performed.

Results: The results showed that the TPA level of the MIX group was significantly higher than in the other groups ($P < 0.05$), while all experimental groups had lower serum IFN- γ levels than the POG group ($P < 0.05$). Interestingly, the MIX group had the highest IL-5 concentration compared with the POG and PRO groups, with statistical significance ($P < 0.05$). Histopathological analysis revealed liver interstitial inflammatory cell infiltration and dilated, congested central veins in the kidneys in the POG group, suggesting potential physiological protection from the treatments in the other groups.

Conclusion: Collectively, the coadministration of PRO and PRE as adjuvants to the polio vaccine enhanced immune responses in rats. This novel approach has the potential to improve vaccine efficacy and preserve organ integrity after vaccination, offering promising strategies for future vaccine development and administration.

Keywords: *Lactobacillus acidophilus* 20552s, ginger rhizomes, prebiotics, probiotics, polio vaccine, rats, immunomodulatory

Introduction

The World Health Organisation (WHO) has declared polio a global public health emergency since 2014. Each year, more than a million under-five-year-old children die from diseases that vaccination could prevent, with developing countries having more than half of all deaths.¹ Significant progress has been made in the global eradication of polio by the Global Polio Eradication Initiative (GPEI). Young children are the main victims of the highly contagious disease polio.² The experience of developing nations has shown that, in the fifty years since oral vaccines were first introduced, immune responses may be weaker and less reliable than those of more developed ones.³ To stop invasive pneumococcal infections, pathogens need to cling to host tissues, or colonise. As a result, it is a practical strategy to enhance mucosal immunity.^{4,5}

When given in the right doses, live bacteria known as probiotics can improve the host's health.⁶ Research conducted in lab settings and on real species has demonstrated the variety of effects probiotics can have on both innate and adaptive immune responses. Probiotics can interact with immune cells

and intestinal tract lining cells to directly impact the immune system or indirectly by changing the gut microbiota's makeup.⁷ The potential of probiotics as a novel adjuvant for vaccines that improve mucosal and systemic immune responses is becoming more widely acknowledged.^{8,9} In order to improve host health, prebiotics are indigestible food ingredients that encourage the proliferation and/or activity of one or a small group of bacterial species that were previously present in the intestine.¹⁰ Ginger (ginger rhizomes), a widely used spice and traditional medicinal treatment, contains various bioactive chemicals that have demonstrated anti-inflammatory and immune-modulating properties.^{11,12} It has also been reported to have gingerols and shogaols. Studies have shown that ginger can boost immunity and protect the stomach mucosa.¹³

Further studies on ginger rhizomes as a novel mucosal adjuvant after polio vaccination could yield promising results. More studies are needed in order to evaluate ginger's effect on the immune system and its role in boosting mucosal immunity. The effects of plant-derived and probiotic investigative mucosal adjuvants for the enhancement of host health after

polio immunisation in rats had not been examined using rhizomes of *Zingiber officinalis* Roscoe (ginger) and *Lactobacillus acidophilus* 20552s probiotic strain. We measured total poliovirus antibodies, IL-5, and IFN- γ responses in the blood. The study also examined histopathological features of the liver, spleen, and kidney in male albino rats. The main goal was to determine some immunomodulatory effects of *Lactobacillus acidophilus* 20552s (probiotic) as well as ginger rhizomes (prebiotic), separately and in combination.

Methods

Chemical Sources

The inactivated trivalent polio vaccine (411-89-314 D antigen units/ml) was provided by the Netherlands Vaccine Institute in Bilthoven, Netherlands. The lyophilized *Lactobacillus acidophilus* 20552s bacteria were obtained from the Egyptian Microbial Culture Collection (EMCC) at the Cairo Microbiological Resources Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Ginger rhizomes were purchased from the local market and identified by the Department of Pharmaceutical Biotechnology, Faculty of Biotechnology, Misr University of Science and Technology, Egypt.

GC/MS Analysis of Ethanolic Ginger (*Zingiber officinale*) Rhizomes Extract

First, 200 g of finely macerated ginger rhizomes were extracted in 1000 cc of 95% ethanol for 48 hours. Then, a rotating evaporator was used to concentrate and dry the extract while simultaneously lowering the pressure. The resulting dried extract of *Zingiber officinale* was suspended in sterile 0.9% NaCl and stored at -20°C until pharmacological investigations were conducted. The ginger rhizomes were analysed using gas chromatography and a mass spectrometer (GC/MS). The GC-MS analysis was conducted using the Clarus 500 Perkin-Elmer (Auto System XL) Gas Chromatograph. It was connected to an Elite-1 Elite mass detector and a Turbomas 5.2 spectrometer (100% Dimethyl polysiloxane capillary column, 300 m x 0.25 mm x 1 μm). The instrument's temperature was initially adjusted to 110°C and remained there for two minutes. Subsequently, the oven temperature was elevated to 280°C and maintained for nine minutes, with a 5°C increase per minute. Helium flow of 1 ml/min and injection port temperature of 250°C were provided. The injection ratio of the samples was 10:1. Chemical components were identified by GC-MS (mass spectrum scan range of 45–450 MHz). The fragmentation modes pattern of mass spectra was compared with that stored in the spectrometer of the National Institute of Standards and Technology Mass Spectral database (NIST-MS) to determine m/z and fragmentation. The relative peak area percentage in the chromatogram was calculated to check the percentage of each component, as previously indicated by Yousfi et al. (2021).

HPLC Analysis of Polyphenol Compounds Extracted from *Zingiber officinale* Rhizomes

An Agilent 1260 series instrument was used to carry out HPLC analysis with a Zorbax Eclipse plus C8 column (4.6 mm x 250 mm i.d., 5 μm) for separation. The mobile phase,

flowing at 0.9 ml/min, consisted of water (A) and acetonitrile with 0.05% trifluoroacetic acid (B). A linear gradient elution program was implemented as follows: 82% A from 0–1 min, decreasing to 75% A by 11 min, further reducing to 60% A by 18 min, then returning to and maintaining 82% A from 18–24 min, ensuring comprehensive separation and analysis of the sample components.

Experimental Animals

Forty male albino rats (eight weeks old, 200–250 g body weight) were housed in individual cages supplied by VACSERA, Egypt. Standard laboratory settings included a 12-hour light/dark cycle, a temperature of $25^{\circ}\text{C} \pm 2$, and a relative humidity of 65–70%. The rats were fed a conventional diet of bread, barley, milk, and unfettered access to water throughout the experiment. Before receiving any treatments, the rats were allowed to acclimate to the laboratory conditions for one week to ensure their adaptability and reduce stress during the experiment. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University of South Valley, Faculty of Sciences, Egypt. Ethical approval No: (003/05/23).

Probiotic Culture Conditions, Media Composition and Enumeration of Probiotic Microorganisms

It was cultivated by sub-culturing on MRS agar (meat peptone 10 g/l, dextrose 20 g/l, yeast extract 5g/l, BEEF extract 10 g/l, disodium phosphate 2 g/l, sodium acetate 5 g/l, ammonium citrate 2 g/l, magnesium sulfate 0.1 g/l, manganese sulfate 0.05 g/l, tween 80 1g/l and agar 15 g/l). Then, it was incubated at 37°C for 48 hours in an anaerobic jar and maintained on Man-Rogosa-Sharpe (MRS) agar. The viability of probiotic cultures was determined and expressed as colony-forming units (CFU/ml) on MRS agar. Serial dilutions were prepared in a sterile physiological solution before plating onto MRS agar. The plates were incubated at 37°C for 24 h.

Immunisation Protocol and Treatments

For this study, mouse and rat groups (40 rats) were divided into four groups (10 rats assigned to each group). First group (POG): Rats were administered a single intraperitoneal injection of the polio vaccine (120 mg/kg bw dissolved in saline solution). Second group (PRE): it was administered ginger rhizomes (28.5 mg/kg) orally twice daily for 30 days. Third group (PRO): *Lactobacillus acidophilus* 20552 (28.5 mg/kg). Fourth group (Mix): both ginger rhizomes and *L. acidophilus* at the same dosage and frequency. All three groups (PRE, PRO, and Mix) received the same vaccination as the POG group, with the additional treatments being the distinguishing factor in the study.

Blood and Tissues Sampling

Before blood collection, rodents underwent an overnight fasting period. Subsequently, they were administered a combination of ketamine and xylazine for anesthesia. Blood samples were obtained via the retro-orbital plexus technique and immediately transferred into sterile tubes without anticoagulant. To facilitate serum separation, the blood specimens were left to clot at room temperature for 30 minutes. The samples were subsequently transferred into Eppendorf tubes and subjected to centrifugation at 3000 rpm for 20 minutes at a temperature

of 4°C. After serum separation, samples were stored at -20°C until biochemical assays could be conducted. The rats were sacrificed, and their organs were removed right away. The kidneys, liver, and spleen tissues were then removed and cleaned in cold PBS (phosphate buffer saline). Neutral buffered formalin (10%) was used to preserve the spleen, kidney, and liver specimens until the histopathological examinations.

Assessing IL-5 and IFN- γ

The levels of serum interleukin-5 (IL-5) and interferon-gamma (IFN- γ) were assessed using commercial ELISA kits (Sigma-Aldrich USA) following the manufacturer's protocol. The levels of IFN- γ and IL-5 in the rat serum were determined using a sandwich ELISA kit following the methods of (Matowicka-Karna et al., 2009; Schmitz et al., 2007), respectively. The absorbance was measured at 650 nm using an ELISA plate reader (Biotek®, USA). The ELISA reader-controlling software (Masterplexreaderfit) converts the digital data of the raw absorbance value into a standard curve, from which the cytokine concentrations of unidentified samples may be estimated directly.

Immune Antibodies Assays

Total Anti-Poliovirus antibody serum levels were assessed at 7, 14, 21, 28 and 35 days post-vaccination. Following the method,¹⁴ total poliovirus antibodies were detected. A mouse antibody specific to type 1 PV (from Abcam, catalogue number ab47802) was diluted 1:1,000 and added to detect the protein. Then, a secondary antibody that binds to mouse antibodies and is linked to horseradish peroxidase (from Cell Signalling Technology, catalogue number 7076) was also diluted 1:1,000 and added. The reaction was stopped using hydrosulfuric acid, and the resulting amount was quantified using TMB solution (catalogue no. 555214, BD Biosciences) on a FilterMax F5 microplate reader (F5, Molecular Devices).

Histopathological Investigation

The spleen, renal and hepatic tissue samples were extracted from the abdominal cavity of five rats in each group. The samples were preserved in 10% neutral buffered formalin for 72 hours. Then, specimens were fixed in ascending grades of ethyl alcohol (70–100%), cleared in xylene, and embedded in paraffin following the method (Eurell and Frappier, 2013). The specimens were cleaned, fixed, and embedded in paraffin blocks. Five-micron-thick paraffin sections were prepared using a microtome (Leica RM 2155, England), and the obtained sections were stained with hematoxylin and an eosin stain (H&E). Representative photomicrographs were captured with a digital camera (Leica EC3, Leica, Germany) connected to a microscope (Leica DM500). Histological analysis was carried out using a Leica DM500 brightfield light microscope (Leica Microsystems, Wetzlar, Germany). Microscopic observations were performed at various magnifications using plan achromatic objectives as follows: 4 $\times$ (NA 0.10) for general tissue architecture, 10 $\times$ (NA 0.25) for intermediate views, and 40 $\times$ (NA 0.65) for detailed cellular observations. Images were captured using an integrated digital camera system attached to the microscope and analysed using Leica LAS EZ software (Version X.X). Calibration of the microscope and imaging system was performed before image acquisition. A scale bar (typically 50 μ m or 100 μ m, depending on magnification) was

added to all captured images using the software's calibration tools to ensure accuracy.

Statistical Analysis

The data were expressed as means $\pm$ SE. Data analyses were performed using one-way analysis of variance (ANOVA) using SPSS 21 (IBM, USA). The level of statistical significance was set at $P < 0.05$.

Results

GC/MS and HPLC of Ethanolic Ginger (*Zingiber officinale*) Rhizomes

The GC chromatogram of ginger oil demonstrated a complex mixture of volatile compounds (Figure 1A). The phytochemical profile of the ginger extract was analysed using HPLC at a detection wavelength of 280 nm, as shown in Figure 1B. Multiple peaks were detected, based on comparison with known standards. The major identified compounds included gallic acid, caffeic acid, chlorogenic acid, ferulic acid, p-coumaric acid, and rosmarinic acid among the phenolic acids, and naringenin, quercetin, kaempferol, daidzein, and hesperetin from the flavonoid group.

Sharp and well-resolved peaks were evident in the figure for several compounds. Notably, rosmarinic acid (retention time 11.807 min), quercetin (17.292 min), and hesperetin (21.175 min) had high peak intensities. Full chromatographic data are provided in Supplementary Tables 1–3.

Total Poliovirus Antibodies (TPA)

Total Poliovirus Antibodies (TPA) over five time points demonstrated a significant immunostimulatory effect of the MIX formulation (ginger + probiotics). By day 7, TPA levels increased in the MIX group. This increase continued through days 14 and 21. The MIX group showed the highest antibody titers ($P < 0.05$). However, the PRO group also displayed intermediate levels. By days 28 and 35, the MIX and POG groups showed similar TPA levels. Regarding the PRE group, the lowest response was evident throughout the experiment (Figure 2).

Inflammatory Cytokines (IFN- γ and IL-5)

Supplemented groups showed significantly reduced levels of pro-inflammatory IFN- γ compared to the POG group ($P < 0.05$), indicating a dampening of vaccine-induced inflammation (Figure 3).

Hepatic Histopathological Changes

Histopathological examination of liver tissues showed differences among treatment groups (Figure 4A–4D). In the POG group, liver sections had a dilated and congested central vein, and inflammatory cell infiltration in the portal area (Figure 4A). The MIX group (Figure 4D) demonstrated preserved hepatic architecture.

Splenic Histopathological Changes

Figure 5A–5D show the histological effects of various treatments on rat splenic tissues. In the vaccinated group (POG, Figure 5A), slight alterations were observed. This included mild changes in the periarteriolar lymphoid sheath and

Sample Chromatograms

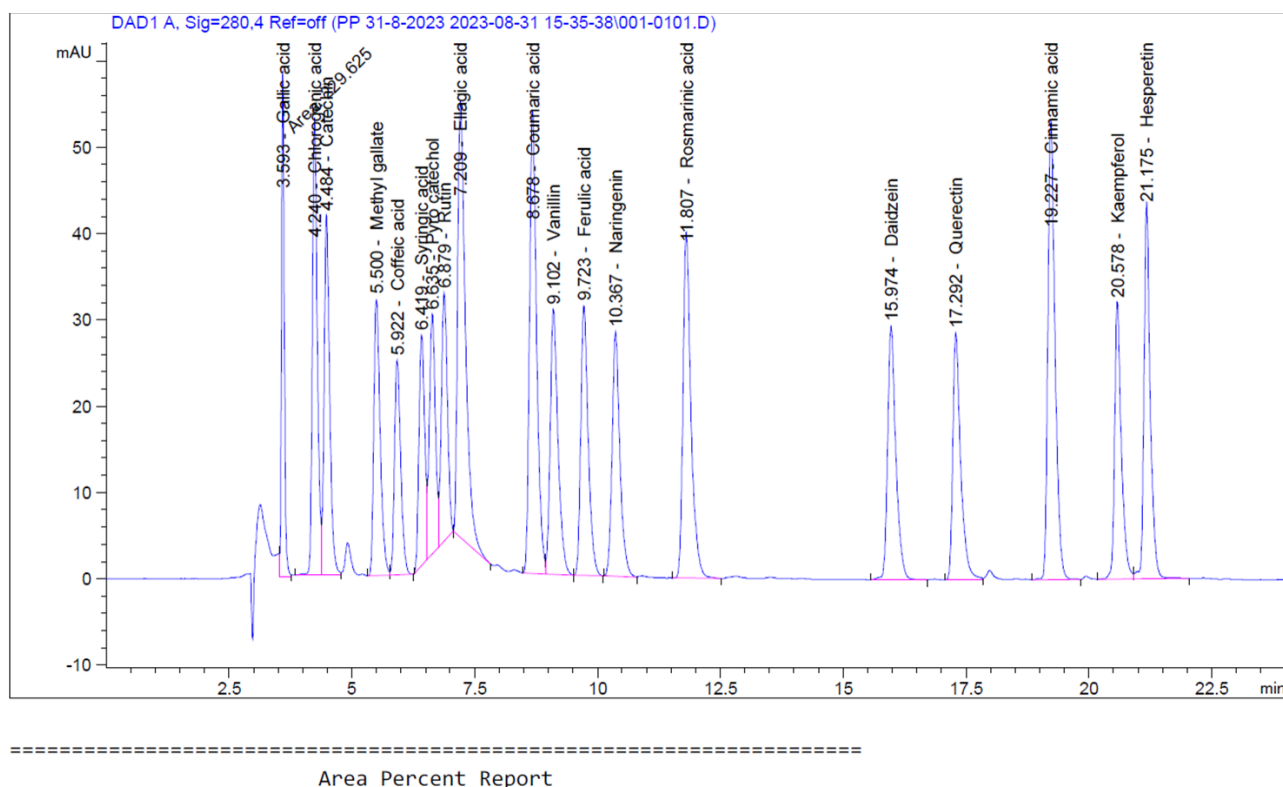
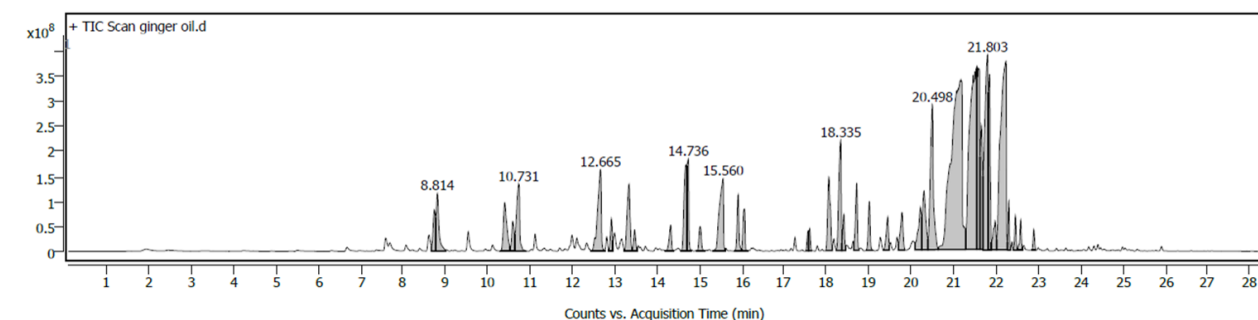


Fig. 1A The GC-MS spectra analysis of the ginger rhizome extract. Fig. 1B Quantification of polyphenol compounds from *Zingiber officinale* rhizomes extract using HPLC.

minor lymphoid depletion within the splenic follicles. Similar changes were seen in the PRE (Figure 5B) and PRO (Figure 5C) groups. In these groups, lymphoid depletion levels are comparable to those of the POG group. In contrast, in the MIX group (Figure 5D), there was normal splenic architecture and intact lymphoid follicles with no evidence of lymphocyte loss.

Renal Histopathological Changes

Figure 6A–6D showed renal histopathological alterations across treatment groups. In the POG group (Figure 6A), kidney sections had interstitial inflammation, constricted tubular lumens, and degenerative changes such as karyolysis and karyorrhexis in tubular epithelial cells. In contrast, renal tissues from the PRE, PRO, and MIX groups (Figure 6B–6D respectively) showed normal histological architecture, including well-preserved glomeruli, Bowman's capsules, and renal tubules.

Discussion

Immunomodulatory agents such as probiotics and prebiotics have been studied to enhance vaccine efficacy and reduce potential adverse effects. This study evaluated immune responses and histological changes in selected organs of rats following polio immunisation. It focused on the immunomodulatory effects of ginger and probiotics, individually and in combination. The findings provide new insights into approaches for improving the histopathological architecture of the spleen, liver, and kidneys.

It is worth noting that modulation of immune responses to improve vaccine efficacy has been important in recent years. For instance, Lei et al., 2017¹⁵ reported that a prebiotic-enriched diet enhanced influenza vaccine-specific antibody responses in male mice. This could be attributed to the probiotics and prebiotics' ability to boost antibody production. The effect of

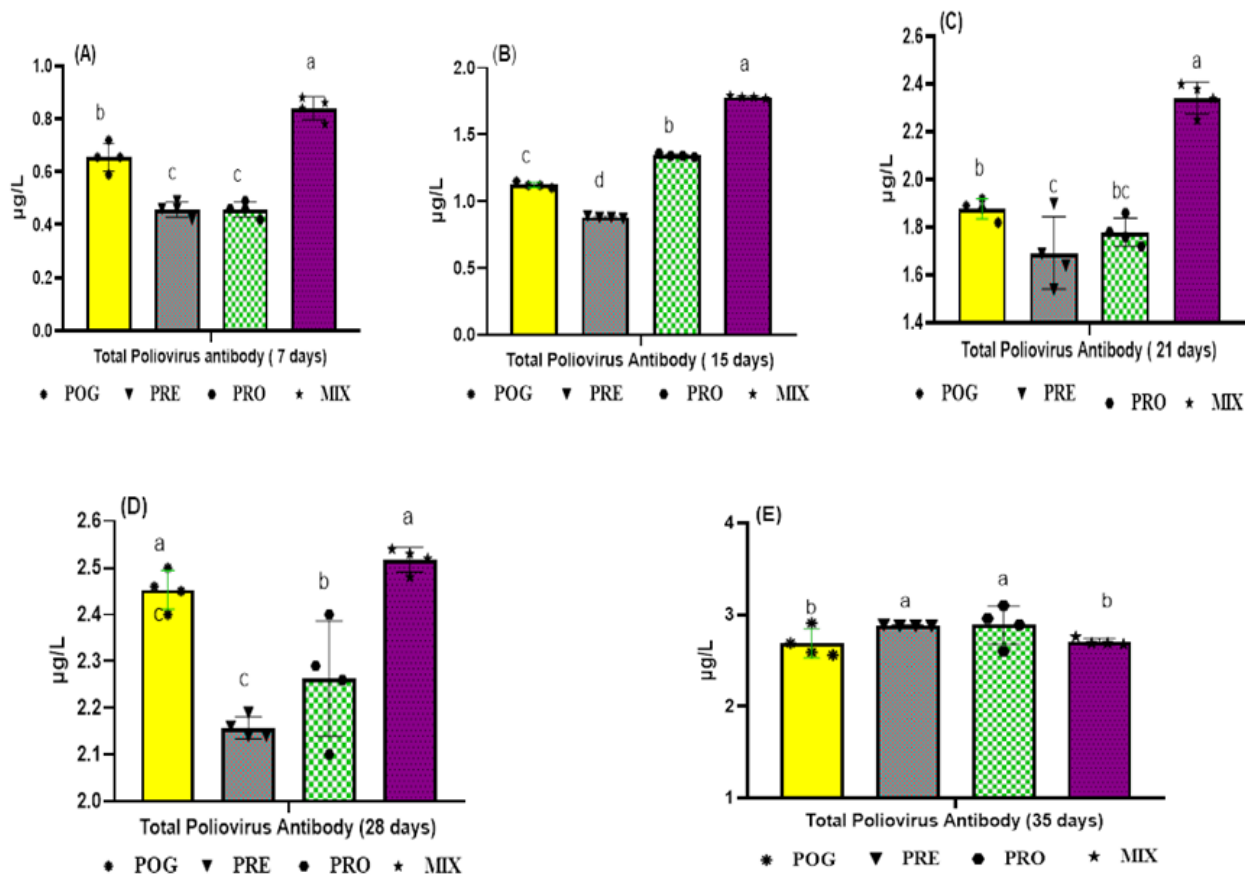


Fig. 2 Serum TPA levels were measured at day 7 (A), 14 (B), 21 (C), 28 (D), and 35 (E) post-treatment. Rats were intraperitoneally injected with a single dose of polio vaccine (120 mg/kg; POG group). The PRE, PRO, and MIX groups received the same vaccination protocol along with oral administration of ginger extract (28.5 mg/kg), *Lactobacillus acidophilus* 20552s (28.5 mg/kg), or their combination, respectively, twice daily for 30 days. Data are expressed as mean $\pm$ SE; * ($P < 0.05$).

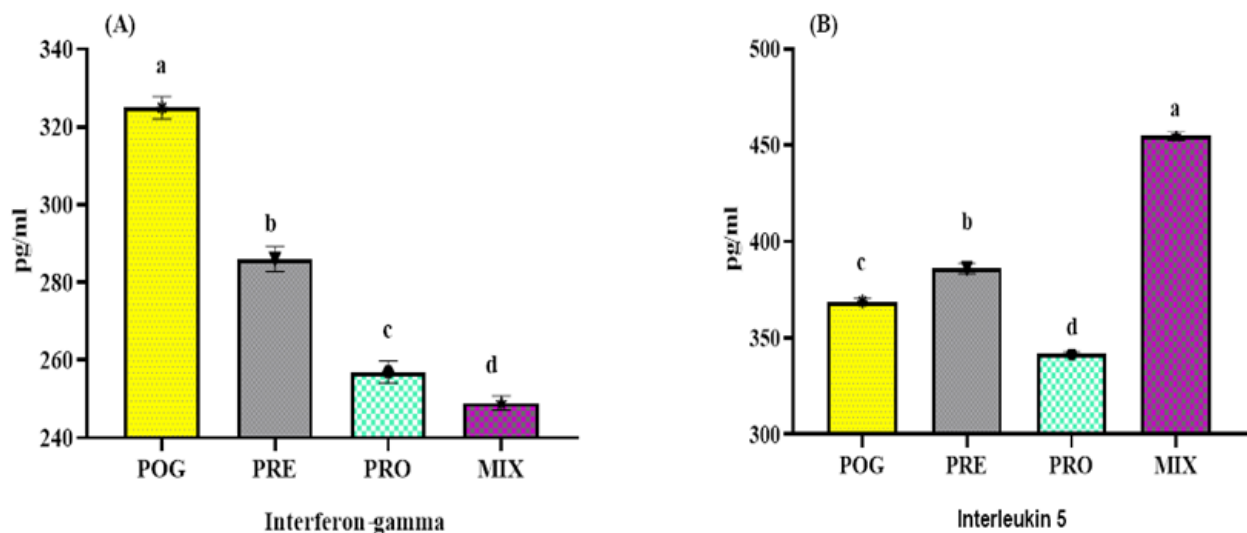


Fig. 3 Serum levels of interferon-gamma (A) and interleukin-5 (B) in rats vaccinated intraperitoneally with a single dose of polio vaccine (120 mg/kg; POG group). The PRO, PRE, and MIX groups received the same vaccination protocol along with oral administration of *Lactobacillus acidophilus* 20552s (28.5 mg/kg), ginger extract (28.5 mg/kg), or their combination, respectively, twice daily for 30 days. Data are presented as mean $\pm$ SE. Values with different superscripts (a–d) differ significantly ($P < 0.05$).

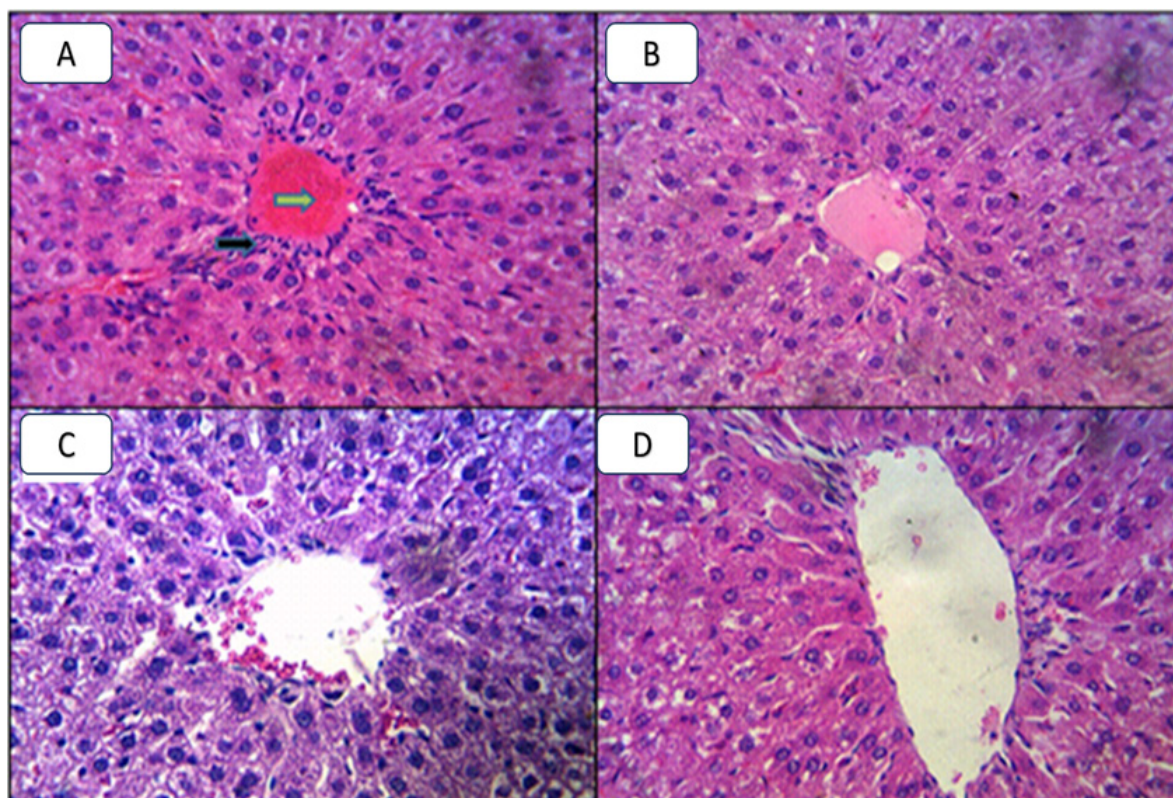


Fig. 4 Representative histological sections of liver tissue from rats injected intraperitoneally with a single dose of polio vaccine (120 mg/kg; POG group, Figure 4A). The PRE (Figure 4B), PRO (Figure 4C), and MIX (Figure 4D) groups received the same vaccination plus oral administration of ginger rhizomes (28.5 mg/kg), *Lactobacillus acidophilus* 20552s (28.5 mg/kg), or their combination, respectively, twice daily for 30 days. Sections stained with hematoxylin and eosin (H&E), magnification 400 $\times$.

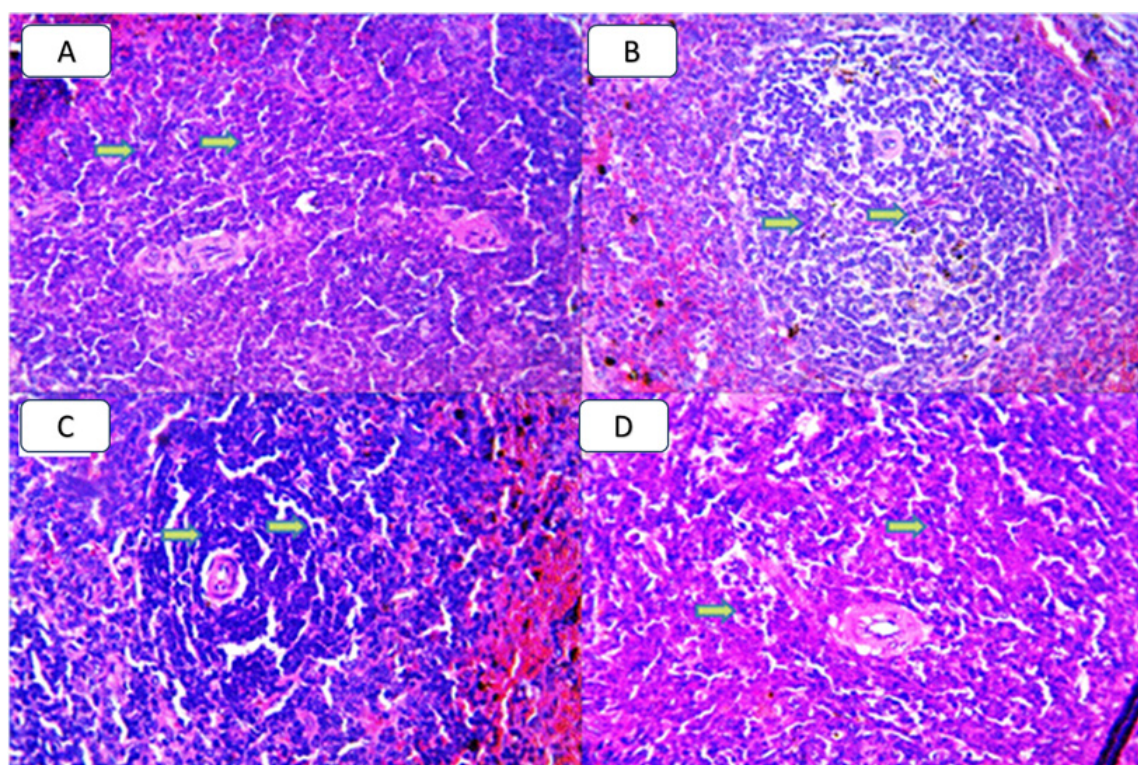


Fig. 5 Representative histological sections of spleen tissue from rats injected intraperitoneally with a single dose of polio vaccine (120 mg/kg; POG group, Figure 5A). The PRE (Figure 5B), PRO (Figure 5C), and MIX (Figure 5D) groups received the same vaccination protocol plus oral administration of ginger rhizomes (28.5 mg/kg), *Lactobacillus acidophilus* 20552s (28.5 mg/kg), or their combination, respectively, twice daily for 30 days. Sections stained with hematoxylin and eosin (H&E), magnification 400 $\times$.

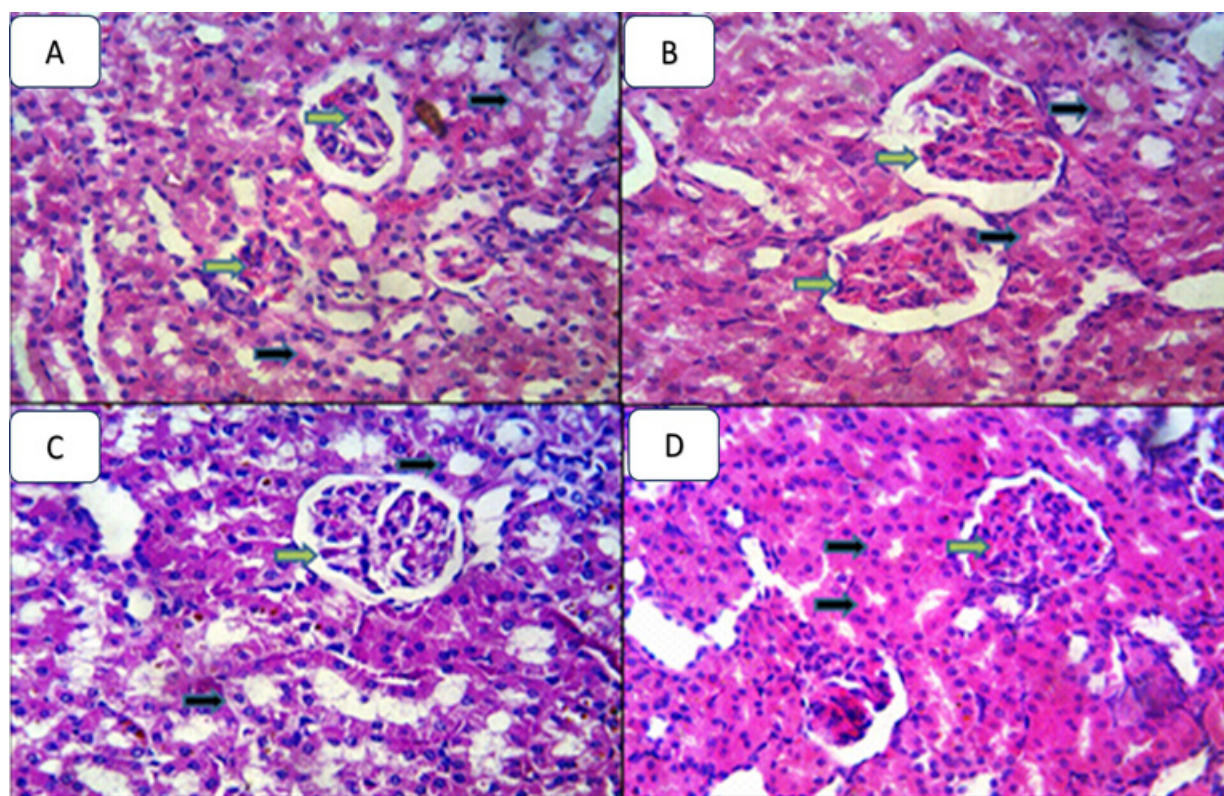


Fig. 6 Representative histological sections of kidney tissue from rats injected intraperitoneally with a single dose of polio vaccine (120 mg/kg; POG group, Figure 6A). The PRE (Figure 6B), PRO (Figure 6C), and MIX (Figure 6D) groups received the same vaccination along with oral administration of ginger rhizomes (28.5 mg/kg), *Lactobacillus acidophilus* 20552s (28.5 mg/kg), or their combination, respectively, twice daily for 30 days. Sections stained with hematoxylin and eosin (H&E), magnification 400 $\times$.

this combination was sex-specific and was confined to only male mice. Similarly, Wright et al. (2016) demonstrated that *Lactobacillus paracasei* ssp. CRL431 significantly increased IgA titers over five weeks following oral poliovirus immunisation. Compared to the placebo group, the probiotic-treated rats had a higher rise in neutralising antibodies. However, changes in IgM and IgG antibody levels specific to poliovirus serotypes 2 and 3 were minimal.¹⁶

Furthermore, Interleukin-5 (IL-5) plays a vital role in eosinophil production and maturation of B cells into antibody-secreting plasma cells.¹⁷ Elevated IL-5 levels are associated with eosinophilia and various conditions, including helminth infections, malignancies, allergic diseases, and asthma.¹⁸ In the current study, administration of probiotics and prebiotics significantly reduced IL-5 serum levels in vaccinated rats. This observation aligns with findings which showed that *Lactobacillus acidophilus* LAVR1-A1 decreased IL-5 levels in infants vaccinated against tetanus.^{19,20} In addition, research showed that ginger has demonstrated anti-inflammatory properties by downregulating proinflammatory cytokines and upregulating anti-inflammatory cytokines such as IL-10 and IL-5.²¹

Despite that, the increase in serum IFN- γ post-vaccination is a marker of proinflammatory activity and immune activation, excessive levels of IFN- γ may be detrimental. For instance, premature infants showed impaired IFN- γ responses to polio vaccination.²² The choice of IL-5 and IFN- γ as the two cytokines of interest in this study correlates with their

roles as proxies for the Th2 and Th1 immune responses. IL-5 supports the maturation of eosinophils and the differentiation of B cells into antibody-secreting plasma cells. Thus, IL-5 is a significant cytokine for both humoral and anti-inflammatory responses.²³ On the other hand, IFN- γ is the central Th1-response cytokine that contributes to macrophage activation and antigen presentation, and thus, it serves as a surrogate marker for cellular immunity and inflammation.²⁴

The selection of the current cytokines in the study is supported by evidence which illustrate that *Lactobacillus acidophilus* could increase IL-5 and decrease IFN- γ in allergy and infection models.¹⁹ In addition, Grzanna et al., 2005 found that some phytochemicals from ginger increase IL-5 and IL-10 and suppress IFN- γ . This constitutes their two-sided anti-inflammatory and immune stimulating activity.²⁵

Both ginger (*Zingiber officinale*) and *L. acidophilus* demonstrated immunoprotective and anti-inflammatory effects in our animal models. For example, in cisplatin-treated rats, ginger reduced oxidative stress and inflammation. Combined administration of ginger and *L. acidophilus* improved antibody production and decreased inflammatory markers, likely through promoting tolerogenic responses and restoring intestinal immune balance. These synergistic effects may help explain the superior immunological outcomes observed in the combined treatment (MIX) group. Another study tested different concentrations of ginger extract in the lab and found that 0.4% not only supported the growth of the probiotic but also helped it thrive. Rats that received the cobiotic showed

improvements in colon tissue structure, reduced oxidative damage, and better gut barrier function. Clear drop in inflammation markers and lower expression of genes linked to cancer progression, such as COX-2, iNOS, and c-Myc.²⁶

The synergistic effects of probiotics and prebiotics, known as symbiotics, are mediated through multiple mechanisms. These include selective stimulation of beneficial gut microbiota, production of short-chain fatty acids (SCFAs), enhancement of gut barrier integrity, and modulation of the gut-brain-immune axis.²⁷ Prebiotics not only nourish probiotics but also improve their functionality within the gastrointestinal tract.²⁸

Recent evidence suggests that some phenolic compounds, such as epigallocatechin gallate (EGCG), promote the growth of *Lactobacillus* species. Although studies specifically involving ginger components are limited, *Lactobacillus* species have superior metabolic activity on phenolic acids compared to traditional prebiotics such as inulin. This could be attributed to their phenol decarboxylase and inducible phenol reductase enzymes.²⁹

The bitter ginger extract contains seven phenolic compounds as identified by high-performance liquid chromatography (HPLC) analysis. These compounds include myricetin, caffeic acid, kaempferol, gallic acid, sinapic acid, quercetin, and chlorogenic acid, which was found in the highest concentration in aqueous extracts. D-limonene, eucalyptol, p-cymene, rosefuran, linalool, isoborneol, terpinen-4-ol, α-terpineol, myrtenal, citronellol, cuminaldehyde, neral, geraniol, and α-citral are some of the diverse bioactive compounds further identified by gas chromatography-mass spectrometry (GC-MS). The presence of these compounds is responsible for ginger's diverse therapeutic effects.

As probiotics receive more attention as new vaccine adjuvants, there is increased research on the gut microbiome's role in modulating mucosal and systemic immunity during and after vaccination. Studies conducted on animals, including some on broiler chickens, indicated that ginger could enhance cellular immunity, mitigate inflammation, and boost the antibody response post vaccination.³⁰

Limitations and Recommendations

This study lacks advanced immunological assessments such as flow cytometry, more comprehensive cytokine profiling for reasons beyond IL-5 and IFN-γ, or gut microbiota analysis. These aspects would have illustrated the mechanisms involved in the immune change that was noted. Based on the gut-immune axis and the responses to the vaccines, further research should integrate immune cell phenotype analysis and microbiome sequencing. Moreover, the histopathological evaluation in this study was descriptive and did not include any quantitative scoring, which is prone to

subjectivity. Increasing the objectivity, reproducibility, and scientific rigor of histological evaluations of tissues could be achieved with the use of a blinded semi-quantitative histological scoring system, which would be more reliable if conducted by several independent pathologists. Increasing the mechanistic understanding of the findings and enhancing the translational value would be achieved by addressing the noted gaps in further research.

Conclusion

This study showed that *Lactobacillus acidophilus* 20552s and ginger-derived prebiotic compounds had clear protective effects on liver, kidneys, and spleen from the tissue damage of polio infection in rats. The intervention reduced the inflammation and the histopathological damage that is usually seen with poliovirus infection. The protective and reparative effects on the tissues were accompanied by modulation of important immune markers like reduction of the proinflammatory IFN-γ and increase of the IL-5, suggesting protective immunoregulation. Prebiotic and probiotic compounds produced an improvement when used together, suggesting an additive or synergistic effect. These results are a strong endorsement for the use of synbiotics in vaccines to enhance immune responses and control inflammation. However, the failure to pinpoint the precise molecular or cellular mechanisms involved is a shortcoming in this study. Those pathways in question would be more illuminating if sophisticated immunological testing and microbiota profiling were applied to the clinical or human vaccine trials.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Disclaimer

No AI-generated content was used in drafting the manuscript. All text, analyses, and interpretations were produced by the authors based on their expertise and scientific judgment. To ensure transparency and compliance with editorial standards, the manuscript has been screened using AI content detection tools (GPTZero) and shows no indication of AI-generated authorship. ■

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Supplementary Tables

Table 1. The main identified components of *Zingiber officinale* rhizomes extract by GC-MS

Component name	Formula	RT	Component name	Formula	RT
D-Limonene	C ₁₀ H ₁₆	8.74	δ-Elementene	C ₁₅ H ₂₄	17.569
Eucalyptol	C ₁₀ H ₁₈₀	8.814	Citronellol acetate	C ₁₂ H ₂₂ O ₂	17.603
p-Cymenene	C ₁₀ H ₁₄	10.405	α-Murolene	C ₁₅ H ₂₄	18.055
Rosefuran	C ₁₀ H ₁₄ O	10.594	Copaene	C ₁₅ H ₂₄	18.335
Linalool	C ₁₀ H ₁₈₀	10.731	Geranyl acetate	C ₁₂ H ₂₀ O ₂	18.41
Isoborneol	C ₁₀ H ₁₈₀	12.665	β-Elementene	C ₁₅ H ₂₄	18.713
Terpinen-4-ol	C ₁₀ H ₁₈₀	12.922	7-epi-Sesquithujene	C ₁₅ H ₂₄	19.011
α-Terpineol	C ₁₀ H ₁₈ O	13.34	β-Caryophyllene	C ₁₅ H ₂₄	19.446
Myrtenal	C ₁₀ H ₁₄ O	13.472	Aromandendrene	C ₁₅ H ₂₄	19.783
Citronellol	C ₁₀ H ₂₀ O	14.324	γ-Gurjunene	C ₁₅ H ₂₄	20.218
Cuminaldehyde	C ₁₀ H ₁₂ O	14.679	(Z)-Caryophyllene	C ₁₅ H ₂₄	20.304
Neral	C ₁₀ H ₁₆ O	14.736	Alloaromadendrene	C ₁₅ H ₂₄	20.498
Geraniol	C ₁₀ H ₁₈ O	15.017	Zingiberene	C ₁₅ H ₂₄	21.174
α-Citral	C ₁₀ H ₁₆₀	15.56	α-Himachalene	C ₁₅ H ₂₄	21.546
Bornyl acetate	C ₁₂ H ₂₀ O ₂	15.915	γ-Murolene	C ₁₅ H ₂₄	21.563
2-Undecanone	CH ₃ (CH ₂) ₈ COCH ₃	16.054	γ-Cadinene	C ₁₅ H ₂₄	21.654
cis-γ-Bisabolene	C ₁₅ H ₂₄	22.307	Longifolene-(V4)	C ₁₅ H ₂₄	21.803
α-Gurjunene	C ₁₅ H ₂₄	22.461	Eremophilene	C ₁₅ H ₂₄	21.855
β-Guaiene	C ₁₅ H ₂₄	22.587	γ-Amorphene	C ₁₅ H ₂₄	21.98
E-Nerolidol	C ₁₅ H ₂₆ O	22.896	β-Cedrene	C ₁₅ H ₂₄	22.238

Table 2. Chromatogram peaks of *Zingiber officinale* rhizomes extract analysis using GC-MS

Peak	Start	RT	End	Height	Area	Area %
1	8.671	8.740	8.774	82207231	325286412	5.26
2	8.774	8.814	9.009	115008724	529861452	8.57
3	10.296	10.405	10.536	96299332	555794663	8.99
4	10.536	10.594	10.639	59328495	229001469	3.71
5	10.639	10.731	10.903	134155642	725350047	11.74
6	12.453	12.665	12.756	162957082	990935759	16.04
7	12.859	12.922	12.957	64707856	201577782	3.16
8	13.231	13.340	13.415	133747506	653056922	10.57
9	13.415	13.472	13.529	42376864	140405218	2.27
10	14.204	14.324	14.387	51831064	198131272	3.21
11	14.570	14.679	14.696	171972069	725157197	11.22
12	14.696	14.736	14.799	183787864	599180603	9.70
13	14.937	15.017	15.114	48906093	194686233	3.15
14	15.326	15.560	15.606	145131934	956601718	15.48
15	15.829	15.915	15.989	112603326	340841083	5.52
16	15.989	16.054	16.127	83191119	297704333	4.82
17	17.508	17.569	17.580	39879134	98056043	1.59
18	17.580	17.603	17.660	43897257	109724862	1.77
19	17.979	18.055	18.135	146667134	633115958	10.25
20	18.232	18.335	18.364	219902166	871431855	14.10

(Continued)

Table 2. Chromatogram peaks of *Zingiber officinale* rhizomes extract analysis using GC-MS—Continued

Peak	Start	RT	End	Height	Area	Area %
21	18.364	18.410	18.450	71539271	201993235	3.27
22	18.918	18.975	19.065	134765668	415590083	6.72
23	18.934	19.011	19.090	97021593	295332454	4.78
24	19.343	19.446	19.486	65874067	241844092	3.91
25	19.697	19.783	19.859	74911397	336410865	5.44
26	20.085	20.218	20.252	84321485	389885758	6.31
27	20.252	20.304	20.395	116970183	630959439	10.24
28	20.395	20.498	20.636	289901852	1516878444	24.58
29	21.174	20.218	20.252	338658254	871968853	14.13
30	21.285	21.546	21.581	364946630	4112558311	66.55
31	21.551	21.563	21.626	36330781	150908148	2.44
32	21.626	21.694	21.694	248146902	866640390	14.02
33	21.694	21.803	21.820	390181611	2210421214	35.67
34	21.820	21.855	21.895	350340633	1026527270	16.61
35	21.895	21.980	22.015	58002793	256757893	4.17
36	22.015	22.238	22.272	378344305	545334825	8.77
37	22.272	22.307	22.347	97613254	231698255	3.71
38	22.410	22.507	22.587	68284365	161853286	2.61
39	22.507	22.587	22.627	60234951	159581562	2.58
40	22.852	22.896	22.946	40636514	94913753	1.54

Table 3. **Quantification of polyphenols compounds from officinale rhizomes extract using HPLC**

Item	Area	Conc. (µg/ml)	Conc. (µg/g)
Gallic acid	54.18	4.72	235.96
Chlorogenic acid	35.60	4.82	241.09
Catechin	0.00	0.00	0.00
Methyl gallate	12.60	0.66	32.95
Coffeic acid	67.40	5.52	276.22
Syringic acid	28.85	2.30	114.96
Pyro catechol	0.00	0.00	0.00
Rutin	2.05	0.42	20.79
Ellagic acid	5.76	0.62	30.77
Coumaric acid	55.48	2.06	103.15
Vanillin	74.90	2.86	143.13
Ferulic acid	35.81	2.19	109.31
Naringenin	11.67	1.12	56.13
Rosmarinic acid	5.00	0.55	27.65
Daidzein	81.40	4.75	237.28
Quercetin	29.89	3.78	188.88
Cinnamic acid	114.52	2.12	106.02
Kaempferol	60.29	3.95	197.68
Hesperetin	6.88	0.35	17.70

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