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Immunomodulatory effects of epiphytic *Loranthus micranthus* leaf extracts collected from two host plants: *Psidium guajava* and *Parkia biglobosa*

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Abstract

Background Immunological abnormalities are implicated in the pathogenesis of many chronic diseases. Due to the drug-related adverse effects of currently available orthodox immunomodulators, natural immunomodulators are being looked upon as potential agents to replace them in therapeutic regimens. This research aimed to investigate the immunomodulatory potential of *L. micranthus* extracts epiphytic on *Psidium guajava* (LMPGE) and *Parkia biglobosa* (LMPBE).

Methods Phytochemical screening and acute toxicity testing were carried out to identify the phytoconstituents and safety profiles of the extracts. The extracts' innate and adaptive immunomodulatory potentials were determined in experimental animals using in vivo leucocyte mobilization, delayed-type hypersensitivity (DTH) response, hemagglutination antibody titre, and cyclophosphamide-induced myelosuppression models. Levamisole was used as the standard drug throughout the study.

Results Compared to LMPBE, LMPGE contained significantly ($p < 0.05$) more tannins, cyanogenic glycosides, saponins, reducing sugars, glycosides, flavonoids, and alkaloids. Furthermore, the groups treated with the extracts had a significant ($p < 0.05$) increase in the total number of leucocytes, neutrophils, basophils, and antibody titers relative to the untreated control. In the same way, the treatment raised TLC in cyclophosphamide-intoxicated rats, with 250 mg/kg b. w. of LMPGE and LMPBE recording 9712.50 ± 178.00 and $8000.00 \pm 105.00 \times 10^9 /L$, respectively, compared to $3425.00 \pm 25.00 \times 10^9 /L$ in the untreated group. Overall, LMPGE was more effective.

Conclusions The findings from this study suggest that *L. micranthus* epiphytic in *Psidium guajava* and *Parkia biglobosa* has possible immune stimulating potential.

Keywords Antibodies, Cell-mediated immunity, Humoral immunity, Immunomodulatory, *Loranthus micranthus*, Phytochemicals

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Background

The past decade has experienced a rise in the development of several immunomodulators, which are primarily used as immunoadjuvants to enhance vaccine efficacy; immunostimulants to induce or stimulate immune mediators and cellular components of immune responses for the treatment of infectious diseases and immunodeficiency disorders such as AIDS; immunosuppressants to suppress immunological responses after therapy for autoimmune diseases, hypersensitivity reactions, and organ transplants [1]. Various synthetic and recombinant drugs, including levamisole, pentoxifylline, thalidomide, and isoprenaline, are immunomodulators prescribed in clinical settings [2]. Administration of levamisole enhances the maturation of thymocytes, improves DTH in immunosuppressed patients, and restores T-suppressor and T-helper cell activity [3].

However, the adverse side effects of levamisole, which include but are not limited to right heart failure, pulmonary hypertension, vasculitis, inflammatory leukoencephalopathy, agranulocytosis, retiform purpura, arthralgia, vasculopathy, and leukoencephalopathy [4–6], limit their intended use. Consequently, immunomodulatory agents with better safety and therapeutic effectiveness are still needed [1]. The increased shift in research attention from orthodox medicine to alternative herbal systems is due to their high safety profile, accessibility, therapeutic efficacy, and multi-targeted approach [7, 8]. According to a WHO report, more than 70% of the population in developing countries rely on medicinal plants due to their better safety profile and improved efficacy [9]. In addition, the high cost of orthodox immune modulators limits their effective usage by most patients, leading to a high morbidity and mortality rate from immune-mediated diseases. The only hope for such patients is the discovery of natural agents within their environment, which will have little or no cost. Interestingly, research on medicinal plants will provide valuable entities for developing immunomodulatory agents to supplement or replace current chemotherapy [10, 11]. Previous studies demonstrated that phytochemicals derived from plants, such as tannins, alkaloids, glycosides, polysaccharides, triterpenoids, and flavonoids, have immunomodulatory properties [12, 13]. Several plants contain these phytochemicals, including African mistletoe (*Loranthus micranthus*) [14, 15].

Loranthus micranthus, an obligate semi-parasitic plant of the family *Loranthaceae*, is native to Nigeria, America, Europe, and other parts of Africa [14]. It grows on various host trees and shrubs, including *Azadirachta indica*, *Kola acuminata*, *Pentaclethra macrophylla*, and *Persia americana* [16]. Nevertheless, it has been spotted on other trees, including *Psidium guajava* (known as “guaba” in Yoruba, “goba” in Hausa, and “gova” in Igbo) and *Parkia*

biglobosa (called “Ugba” in Igbo, “dawadawa” in Hausa, and “Iru” in Yoruba). *L. micranthus* has a strong reputation in traditional medicine for its use in treating various ailments, notably high blood pressure, diabetes, cardiovascular illnesses, and conditions affecting the human immune system, as well as headaches, epilepsy, infertility, agglutination, menopausal syndrome, and rheumatism [17]. The pharmacological properties reported in the plant are not limited to antidiabetic, antihypertensive, hypolipidemic, and antimicrobial activities [16, 18]. Leveraging on the high ethnomedicinal and pharmaceutical actions of mistletoe and the paucity of information on the comparative immunomodulatory activity of *L. micranthus* epiphytic on *P. guajava* and *P. biglobosa*, which are known to have numerous established health benefits. This study investigated the immunomodulatory effects of *L. micranthus* leaves collected from *P. guajava* and *P. biglobosa* on rats’ innate and adaptive immune responses and cyclophosphamide-induced myelosuppression.

Methods

Plant collection and identification

The plant material (*L. micranthus* leaves) was collected from Orba in Nsukka Local Government Area, Nigeria, from different wild host trees, *P. guajava* and *P. biglobosa*. Plant taxonomist Alfred Ozioko identified and collected the leaves. The voucher specimen of the leaves was registered in the herbarium of the International Centre for Ethnomedicine and Drug Development (INTERCEDD) Nsukka, Enugu State, Nigeria (voucher number INTERCEDD/1603).

Experimental animals

The experimental animals (160 Wistar albino rats and 36 Swiss mice) used for this research were procured from the same source. They were kept in stainless steel cages (five animals per cage) under the same environmental conditions to eliminate any confounding factors that might influence the experiment’s outcome. The animals were fed pelletized feed (Vital Feed Nig. Ltd.) and tap water ad libitum. Standard laboratory conditions and protocols recommended by the National Institute of Health Guidelines for the Use and Care of Laboratory Animals (revised 1985, Pub No. 85–23) (1985) were strictly followed throughout the experiment. After the investigation, the death of the animals was humanely induced with an intraperitoneal injection of 200 mg/kg of sodium pentobarbital following the American Veterinary Medical Association (AVMA) guidelines [19].

Antigen preparation

The antigen was prepared from fresh sheep red blood cells (SRBCs). The SRBC was washed three times in

pyrogen-free, sterile normal saline by centrifugation for 10 min at 3000 x g rpm. Then, the concentration of the washed SRBCs was adjusted to 109 cells/mL with normal saline by counting the cells using a Neubauer chamber in a light microscope [20]. After that, concentrated SRBC was used for the challenge and immunization.

Drugs

The drugs employed as a standard immunostimulant and immunosuppressant were levamisole (25 mg/kg), procured from ICI Pakistan, and cyclophosphamide (25 mg/kg), sourced from Cadila Healthcare Limited, Germany, respectively. In addition, sheep red blood cells (SRBCs) were used as antigens for immunization and challenge.

Preparation and extraction of the plant material

L. micranthus leaves were washed and air-dried for 6 days after being obtained from *P. guajava* and *P. biglobosa*. Then, they were pulverized in a laboratory grinder, and 200 g of each leaf was extracted for 72 h in a conical flask with 2.5 L of methanol (Sigma Cat. No. 107018) and distilled water in a ratio of 80:20. The methanol was diluted to increase the polarity, which is suitable for the extraction of phytochemicals present in the leaves [21, 22]. The macerate was filtered through Whatman 1 filter paper (paw size 11 m) and concentrated at 45 °C under reduced pressure (≤ 10 mmHg) using a rotary evaporator (Model Modulyo 4k, Edward England) to obtain extracts of *L. micranthus* epiphytic on *P. guajava* and *P. biglobosa* designated as LMPGE and LMPBE, respectively.

Qualitative phytochemical analysis

Harborne [23] and Trease and Evans [24] experimental procedures were used to screen the extracts for phytochemicals as follows:

Steroids: 2 mL of chloroform (Sigma Cat. No. 107024) and 1 mL of sulfuric acid (BDH Cat. No. BDH3072) were added to 0.1 g of each extract. The formation of a reddish-brown ring at the interface indicated the presence of steroids.

Tannins: A quantity of 0.1 g of each extract was added to deionized water (10 mL) and filtered before adding 3 drops of ferric chloride (BDH Cat. No. BDH7318). The formation of a greenish-brown precipitate (ppt) indicated the presence of tannins.

Soluble Carbohydrate: Each extract (0.1 g) was separately boiled with 2 mL of distilled water and filtered. Subsequently, a few drops of Molisch's reagent were added to the filtrate. Conc. sulfuric acid was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicated the presence of carbohydrates.

Cyanogenic glycosides: A few drops of ferric chloride (5%) and 2 mL glacial acetic acid (Sigma Cat. No. 100066) were added to 0.1 g of each extract. The formation of the brown ring at the interface after adding 1 mL of conc. Sulfuric acid confirmed the presence of cyanogenic glycosides.

Saponins: Each extract (0.1 g) was added to deionized water (200 mL) and shaken vigorously for 15 min. The formation of the foam layer indicated the presence of saponins.

Reducing Sugar: Five milliliters (5 mL) of a mixture of equal parts of Fehling's solution A (Sigma Cat. No. 89474) and B (Sigma Cat. No. 12603) were added to 0.5 g of each extract and then heated in a water bath for 5 min. Brick-red precipitate showed the presence of reducing sugar.

Glycosides: Dilute sulfuric acid (5 mL) was added to 0.1 g of each extract in a test tube, which was later boiled for 15 min in a water bath. The test tube was cooled and neutralized with a 20% potassium hydroxide solution (Sigma Cat. No. BDH9262). Then, 10 mL of a mixture of equal parts of Fehling's solutions A and B was added and boiled for 5 min. A more dense brick-red precipitate indicated the presence of glycoside.

Flavonoids: The presence of flavonoids in each extract was determined by boiling 0.1 g of the extract in 10 mL of ethyl acetate (Sigma Cat. No. BDH1123) for 3 min. Then, 4 mL of the cooled filtrate was shaken with 1 mL of dilute ammonia solution (Sigma Cat. No. BDH7615). An intense yellow coloration confirmed the presence of flavonoids.

Alkaloids: Alkaloids were qualified by boiling 0.5 g of each extract with 6 mL of 1% aqueous HCl (Sigma Cat. No. 113136). A reddish-brown ppt formed after adding 2 drops of Wagner's reagent to the filtrate indicated the presence of alkaloids.

Terpenoids: Chloroform (2 mL) was added to 0.1 g of each extract in a test tube. The formation of a reddish-brown color at the interface after adding conc. Sulfuric acid (1.5 mL) indicated the presence of terpenoids.

Resin: Acetic anhydride solution and 1 mL of conc. Sulfuric acid were added to 1 g of each extract. There was no formation of orange-to-yellow coloration to indicate the presence of resin.

Fats and oils: A small quantity of the leaf extract was pressed between two filter papers. There was no oil stain on the filter paper to confirm the presence of fats and oils.

Quantitative phytochemical analysis

The identified phytochemicals were further quantified with a UV-vis spectrophotometer (Jenway 6305, Bibby Scientific Ltd., UK) using the methods of Harborne [23] and Trease and Evans [24]. The quantities of each phytochemical were extrapolated from the standard curve of their respective standard compounds, and the results were expressed in mg/100 g.

Test for steroids: Each extract (1 g) was macerated with 20 mL of ethanol (Sigma Cat. No. 108543) and filtered. Subsequently, 2 mL of chromagen solution was added to the filtrate (2 mL), and the solution was left to stand for 30 min before measuring the absorbance at 550 nm. The concentration of steroids was extrapolated from the testosterone standard curve.

Test for tannins: Each extract (1 g) was macerated with 50 mL of methanol and filtered. Then, 0.3 mL of 0.1N ferric chloride in 0.1N HCl and 0.3 mL of 0.0008M potassium ferricyanide were added to 5 mL of the filtrate, and the absorbance was read at 720 nm. The tannin content was extrapolated from a tannic acid standard curve.

Test for soluble carbohydrates: Each extract (1 g) was macerated with 50 mL of distilled water and filtered. Afterwards, a saturated aqueous solution of picric acid (Sigma Cat. No. 197378) was added to 1 mL of the filtrate, and the absorbance of the resulting solution was measured at 580 nm. The soluble starch standard curve was used to extrapolate the concentration of soluble carbohydrates in each extract.

Test for cyanogenic glycosides: Alkaline picrate (4 mL) was added to 1 g of each extract, and the mixture was shaken thoroughly before incubating in a water bath at 30°C. Subsequently, the absorbance was read at 490 nm. Cyanogenic glycoside content was extrapolated from the KCN standard curve.

Test for saponins: A quantity of 0.5 g of each extract was macerated in 10 mL of petroleum ether (Sigma Cat. No. 101769). Then, another 10 mL of petroleum ether was added to the same beaker, and the filtrate evaporated to dryness. The residue formed was dissolved in ethanol (6 mL). Chromagen solution was added to 2 mL of the solution before incubating it for 30 min. at room temperature. The absorbance was measured at a wavelength of 550 nm. The contents of saponins in the extracts were calculated from the standard graph of diosgenin.

Test for reducing sugars: Each extract (0.5 g) was macerated with 20 mL of distilled water and filtered. 1 mL of the filtrate was added to 1 mL of alkaline copper reagent. The mixture was boiled for 5 min and cooled. Then, 2 mL of distilled water and 1 mL of phosphomolybdic acid reagent (Sigma Cat. No. 221856) were added, and the absorbance was measured at a wavelength of 420 nm. A

standard glucose curve was used to extrapolate the quantity of reducing sugar in each extract.

Test for glycosides: A quantity (0.5 g) of each extract was macerated with 50 mL of distilled water and filtered. Afterwards, 4 mL of alkaline picrate solution was added to the filtrate (1 mL). The mixture obtained was boiled for 5 min and allowed to cool. The absorbance was read at a wavelength of 490 nm, and the quantity of glycosides in the extracts was obtained from the linalool standard curve.

Test for flavonoids: Each extract (1 g) was macerated with 20 mL of ethyl acetate for 5 min and filtered. To the filtrate (5 mL), 5 mL of dilute ammonia was added and shaken for 5 min. The upper layer was collected, and the absorbance was read at 490 nm. The amount of flavonoids in each extract was extrapolated from the quercetin standard curve.

Test for alkaloids: Each extract (1 g) was macerated with 20 mL of ethanol and 20% sulfuric acid (1:1 v/v). The filtrate (1 mL) was added to 5 mL of 60% sulfuric acid. After 5 min, 5 mL of 0.5% formaldehyde in 60% sulfuric acid was added to the mixture and allowed to stand for 3 h. The absorbance was read at 565 nm. The standard curve of caffeine was used to calculate the alkaloid content of each extract.

Test for terpenoids: Each extract (1 g) was macerated with 50 mL of ethanol and filtered. Then 2.5 mL of a 5% aqueous phosphomolybdic acid solution was added to the filtrate (2.5 mL), followed by the gradual addition of 2.5 mL of conc. Sulfuric acid. The mixture obtained was left to stand for 30 min. and then made up to 12.5 mL with ethanol. The absorbance was taken at 700 nm. The terpenoid standard curve was used to extrapolate the terpenoid quantity in each extract.

Acute toxicity and lethality

Extracts' acute toxicity and lethality were investigated using Lorke's [25] method in two phases using 36 Swiss albino mice. The LD₅₀ was calculated using the following equation:

$$LD_{50} = \sqrt{D_0} \times \sqrt{D_{100}}$$

Do and D100 are the maximum doses of the extracts that caused no mortality and the minimum dose that caused mortality, respectively.

Selection of treatment doses

The doses of the extracts used for the study were chosen within the range of concentrations that gave optimal activity in the preliminary analysis. The acute toxicity test results showed that these doses were safe because they were lower than the maximally tolerated doses. However, the doses of the conventional drugs were translated from

the human dose for each drug using the human equivalent dose conversion formula. Accurately weighed quantities of each extract and standard drug were suspended in Tween 80 to prepare suitable dosages.

Assay of in vivo leukocyte mobilization

The experimental procedure of Ribeiro et al. [26] was used to investigate the effect of the extracts on in vivo leukocyte migration. Albino rats (40) were randomly distributed into eight experimental groups ($n = 5$). Group 1 served as the control (untreated, given 0.3 mL of the vehicle, tween 80); groups 2–8 were pretreated orally with levamisole (25 mg/kg b.w.) for group 2; 100, 250, and 500 mg/kg LMPGE for groups 3–5, respectively; and groups 6–8 were administered 100, 250, and 500 mg/kg LMPBE, respectively. The fluid obtained from the rat's peritoneum was washed twice with heparinized (5 U/mL) phosphate-buffered saline (hPBS), and total leukocyte counts (TLC) were determined in the Neubauer chamber. For differential leukocyte counting (DLC), a cell pellet was obtained by centrifugation and resuspended in 1 mL of hPBS containing 5% serum albumin. The cells were stained with Wright's stain, and the DLC was counted under light microscopy. The results were reported as the number of cells per cavity (Souza and Ferreira 1985).

Delayed-type hypersensitivity (DTH) response

The Naved et al. [27] procedure was employed to investigate the DTH response. The 40 albino rats used in the study were divided into eight groups, as reported above. The treatment was done 3 days before sensitization on day 0 with 0.02 mL of 109 cells/mL of antigen (SRBCs) and continued daily until challenge on day 5. The difference in paw size before and 24 h after the challenge was used to calculate the DTH response.

Hemagglutination antibody (HA) titre

The effects of the extracts on the humoral immune response were studied using the Sharma et al. [28] hemagglutination antibody (HA) titre assay. The antibody titer was expressed in a graded manner, with the minimum dilution (1/2) calculated as Log₂ of the dilution factor being ranked 1.

Cyclophosphamide-induced myelosuppression in rats

The Rasheed et al. [20] experimental procedure, with minor modifications, was used to assess cyclophosphamide-induced myelosuppression in rats. Myelosuppression was induced by intraperitoneal injection (i.p.) of cyclophosphamide (30 mg/kg b.w.). The rat total leukocyte counts (TLC) were determined on day 0 before suppression (TLC-A) and the seventh post-treatment day (TLC-B).

Statistical analysis

The generated data sets were analyzed with IBM Statistical Package for Service Solutions (SPSS) version 23 using one-way analysis of variance (ANOVA). In addition, Duncan's multiple comparisons and the T-test were employed to compare the significant differences between the groups at $p < 0.05$. The results are presented as the mean \pm standard deviation (SD) of replicated values.

Results

Qualitative and quantitative phytochemical composition of LMPGE and LMPBE

From the qualitative and quantitative phytochemical analysis, both extracts (LMPGE and LMPBE) contain steroids, tannins, soluble carbohydrates, cyanogenic glycosides, saponins, reducing sugars, glycosides, flavonoids, alkaloids, and terpenoids, while resins, fats, and oils were absent (Table 1). The LMPGE was found to contain significantly ($p < 0.05$) more amounts of tannins, cyanogenic glycosides, saponins, reducing sugars, glycosides, flavonoids, and alkaloids than LMPBE (Table 1). However, soluble carbohydrate was significantly ($p < 0.05$) more abundant in LMPBE than LMPGE.

Acute toxicity test (LD₅₀) of LMPGE and LMPBE

Both LMPGE and LMPBE showed no signs of acute toxicity in mice that received the extracts at doses from 10 to 2900 mg/kg b.w. However, administration of a higher dosage (5000 mg/kg b.w.) provoked drowsiness and weakness in the mice, although no death was recorded after 48 h (Table 2)

Effects of LMPGE and LMPBE on total and differential leukocyte counts

The rats treated with both LMPGE and LMPBE (experimental groups 3 to 8) prior to agar-induced in vivo leukocyte migration showed a significant ($P < 0.05$) increase in the total number of leukocytes as well as neutrophils and basophils when compared to the untreated group (group 1). Moreover, there were no significant differences ($p > 0.05$) in the lymphocyte count across the groups, and the number of eosinophils showed contrasting trends, as they decreased significantly in the some of treated groups compared to the untreated. Although the results did not follow a consistent dose-dependent pattern for all the immunological parameters examined (Fig. 1), it is interesting to note that both extracts (LMPGE and LMPBE) showed either significantly higher or similar outcomes compared to the experimental group (group 2) administered or treated with the standard drug (25 mg/kg b.w. of levamisole). Finally, from our results, LMPGE

Table 1 Qualitative and quantitative phytochemical composition of LMPGE and LMPBE

S/N	Phyto-chemicals	Qualitative analysis		Quantitative analysis				
		Assay	Observation	LMPGE	LMPBE	LMPGE (mg/100 g)	LMPBE (mg/100 g)	p-values
1	Steroid	Cone. H ₂ SO ₄	Reddish brown colouration	+	+	4.44 ± 0.003 ^a	4.43 ± 0.005 ^a	0.1971
2	Tannin	Braymer's test	Greenish brown precipitate	+	+	2.87 ± 0.003 ^b	1.97 ± 0.003 ^a	0.0163
3	Soluble carbohydrates	Molish's test	Purple interfacial ring	+	+	0.96 ± 0.004 ^a	1.43 ± 0.002 ^b	< 0.0001
4	Cyanogenic glycoside		Brown ring	+	+	0.07 ± 0.004 ^b	0.05 ± 0.002 ^a	0.0006
5	Saponins	Frothing's test	Persistence foaming	+	+	2.03 ± 0.004 ^b	1.22 ± 0.002 ^a	< 0.0001
6	Reducing sugars	Fehling's test	Brick red precipitate	+	+	56.52 ± 0.003 ^b	43.49 ± 0.003 ^a	< 0.0001
7	Glycoside	Sulphuric acid test	Brick red precipitate	+	+	1.19 ± 0.003 ^b	0.99 ± 0.002 ^a	< 0.0001
8	Flavonoids	Ethylacetate test	Intense yellow colouration	+	+	5.36 ± 0.003 ^b	4.79 ± 0.003 ^a	0.0306
9	Alkaloids	Wagner's test	Reddish-brown ppt	+	+	13.39 ± 0.009 ^b	11.35 ± 0.002 ^a	< 0.0001
10	Terpenoids		Grey coloration	+	+	0.49 ± 0.003 ^a	0.48 ± 0.001 ^a	0.8593
11	Resin	Acetic anhydride test	No orange-to-yellow coloration	-	-	-	-	-
12	Fats and oil	Spot test/ Stain test	No oil stain on the filter paper	-	-	-	-	-

The key, +, indicates "the presence or availability of phytochemical" while -, indicate their absence (Qualitative results). The data of the quantitative analysis represent the mean ± SD of triple determination. Values with different superscripts in a row are significantly different, while mean values with the same letter as a superscript are not significantly different at $p < 0.05$. The results were analyzed with ANOVA, and post hoc multiple comparisons of each mean value were done with the T-test

Quantitative values with different superscripts (a or b) in a row are significantly different, while mean values with the same letter (a) as a superscript are not significantly different at $p < 0.05$

Table 2 The median lethal dose of the methanol extract of *Loranthus micranthus*

Dosages (mg/kg b. w.)	LMPGE		LMPBE	
	Mortality	BA	Mortality	BA
10	0/3	Nil	0/3	Nil
100	0/3	Nil	0/3	Nil
1000	0/3	Nil	0/3	Nil
1600	0/3	Nil	0/3	Nil
2900	0/3	Nil	0/3	Nil
5000	0/3	Drowsiness and weakness	0/3	Drowsiness and weakness

$n = 3$ mice in the groups, while BA stands for behavioural changes

showed better immunostimulatory activities than the LMPBE, as they fostered a greater increase in total leucocyte count as well as basophil number in the experimental group administered 250 and 500 mg/kg.

The values are presented as the mean ± SD of five rats. Values with a different alphabet in a given parameter are statistically significant at $p < 0.05$. However, mean values with the same lettered alphabet are not significantly different ($p < 0.05$). The results were analyzed with ANOVA, and post hoc multiple comparisons of each mean value were done with Duncan's multiple tests.

Effects of LMPGE and LMPBE on delayed-type hypersensitivity induced by SRBCs

Oral treatment with LMPGE and LMPBE significantly ($P < .05$) increased the paw sizes of the rats in a non-dose-dependent manner as compared to the untreated group. Interestingly, it was observed that the groups treated with 250 mg/kg b.w. of both extracts had larger paw size than what was recorded in levamisole-treated rats (Fig. 2).

The values are presented as the mean ± SD of five rats. Values with different alphabets are statistically significant at $p < 0.05$. However, mean values with the same lettered alphabet are not significantly different ($p < 0.05$). The results were analyzed with ANOVA, and post hoc multiple comparisons of each mean value were done with Duncan's multiple tests.

Effects of LMPGE and LMPBE on hemagglutination antibody (HA) titers

As shown in Fig. 3, groups treated with the extracts after SRBC immunization and challenge had significant ($p < 0.05$) elevation in primary and secondary antibody titers relative to the negative control (group 1). The increase in the primary titre values was highest in groups 4 and 5 treated with 100 and 250 mg/kg b.w. of LMPGE, respectively. Moreover, the primary and secondary antibody titers of the extract-treated groups were found to

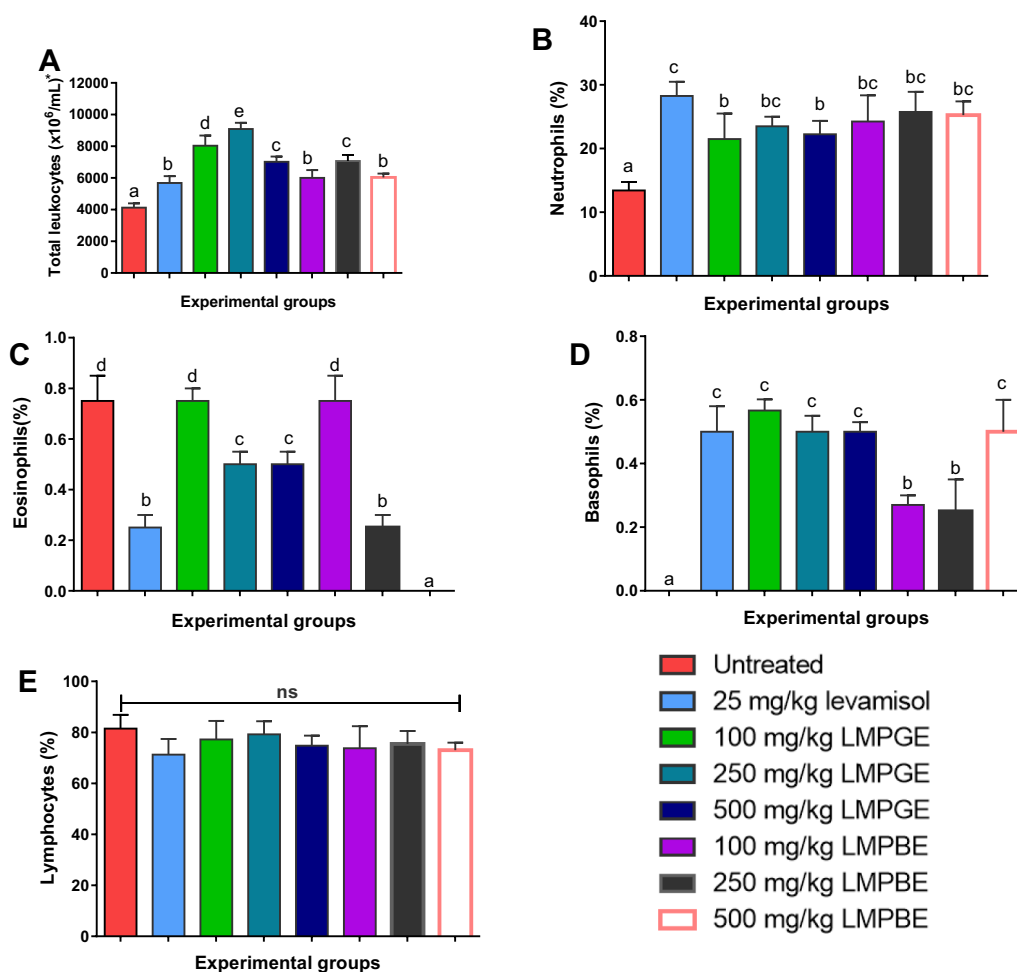


Fig. 1 The effects of LMPGE and LMPBE on total and differential leukocyte counts

be significantly ($p < 0.05$) higher than those of group 2, treated with levamisole, with the exception of the group administered 50 mg/kg b.w. of LMPBE.

The values are presented as the mean \pm SD of five rats. Values with a different alphabet in a given parameter are statistically significant at $p < 0.05$. However, mean values with the same lettered alphabet are not significantly different ($p < 0.05$). The results were analyzed with ANOVA, and post hoc multiple comparisons of each mean value were done with Duncan’s multiple tests.

Effects of LMPGE and LMPBE on cyclophosphamide-induced myelosuppression

The rats treated with both LMPGE and LMPBE extracts (experimental groups 3 to 8) after cyclophosphamide induction showed a significant ($p < 0.05$) dose-dependent increase in the TLC, with LMPGE having a higher potency. Furthermore, it was also observed that the extracts increased TLC more than levamisole, except for the group treated with 50 mg/kg b.w. of LMPBE (Table 3).

The values are presented as the mean \pm SD of five rats. TLC A and TLC B stand for TLC before suppression and after suppression, respectively. Values with asterisks differed significantly at $p < 0.05$ compared with the untreated group (negative control). The results were analyzed with ANOVA, and post hoc multiple comparisons of each mean value were done with Duncan’s multiple tests.

Discussion

The immunomodulatory activity of natural products that stimulate or suppress the immune system offers excellent therapeutic benefits in regulating immune-mediated diseases [1, 6]. In the current study, the phytochemical screening showed that the extracts are rich in reducing sugars, alkaloids, flavonoids, steroids, and tannins, whereas saponins were present in low amounts. These results agree with the work of Govindappa et al. [29], who also recorded a high abundance of alkaloids, flavonoids, phenols, and tannins

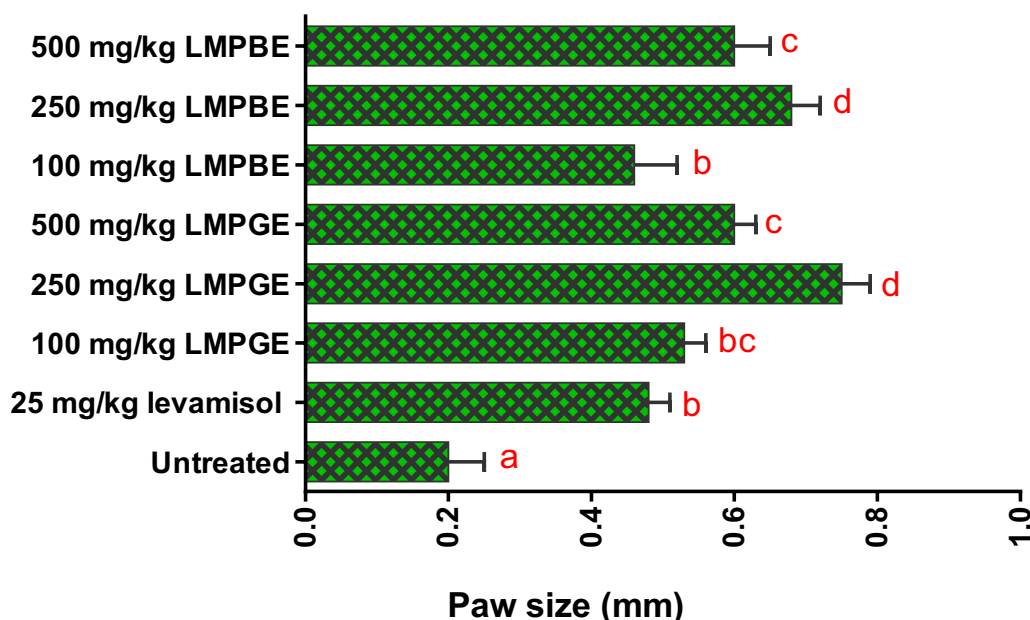


Fig. 2 Effects of LMPGE and LMPBE on delayed-type hypersensitivity induced by SRBCs

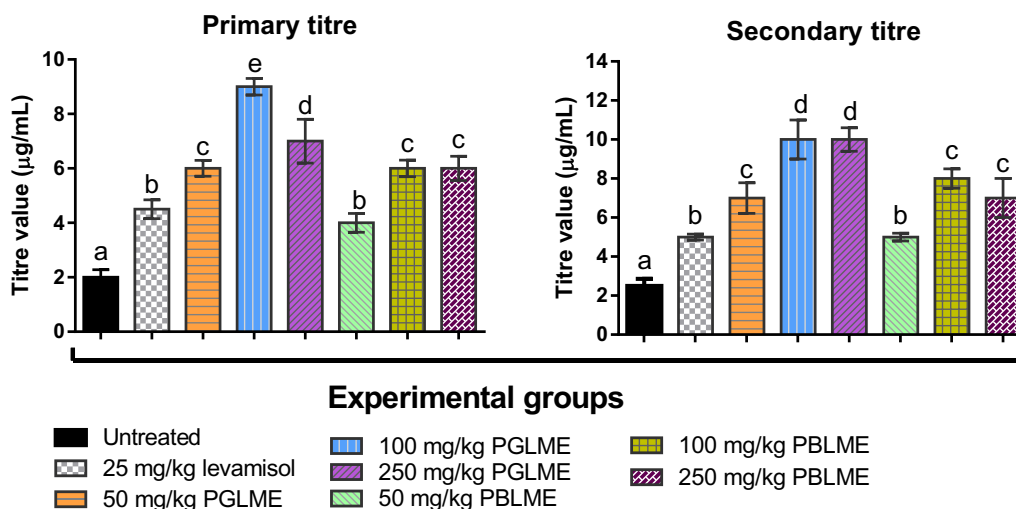


Fig. 3 Primary and secondary antibody titres of rats challenged with SRBCs

in methanol extracts of *L. micranthus* growing on *Azadirachta indica* except that saponins were not identified from their n-hexane and methanol extracts. The possible reason for this slight difference in the phytochemical composition of the plants collected from different hosts and locations is supported by the fact that the phytochemical contents are influenced by the host plant, habitat, and environmental factors [30]. According to ethnopharmacological studies, these identified phytoconstituents have sound therapeutic effects [31–33]. Among the identified phytochemicals, saponins

have cytotoxic, antiallergic, antiviral, molluscicidal, and immunomodulating activities [34]. Flavonoids and tannin compounds contain multiple hydroxyl groups required to neutralize free radicals, chelate metal ions, decompose peroxides, and quench singlet oxygen [35]. According to Yahfouf et al. [36], polyphenols regulate the immune system through their regulatory actions on signaling pathways that are not limited to the mitogen-activated protein kinases (MAPk), Janus kinases-signal transducer and activator of transcription proteins (JAK/STAT), nuclear factor-κB (NF-κB), and

Table 3 Effects of LMPGE and LMPBE on cyclophosphamide-induced myelosuppression

Experimental groups	Treatments (mg/kg b.w.)	TLC-A ($\times 10^9$ /L)	TLC-B ($\times 10^9$ /L)
Negative control	–	11,950.00 \pm 30.00	3425.00 \pm 25.00
Levamisole	25	11,000 \pm 23.17	5212.50 \pm 10.00*
LMPGE	50	12,110 \pm 17.29	6200.00 \pm 30.00*
	100	12,200 \pm 15.01	7812.50 \pm 192.00*
	250	1275 \pm 15.00	9712.50 \pm 178.00*
LMPBE	50	11,950 \pm 47.00	5225.10 \pm 195.00*
	100	12,175 \pm 132.00	7137.50 \pm 165.00*
	250	11,475 \pm 170.00	8000.00 \pm 105.00*

TLC-B vValues with asterisks (*) differed significantly at $p < 0.05$ compared with the untreated group (negative control)

arachidonic acid pathways. Notably, the high amounts of alkaloids in the extracts evoke much research interest due to their biological activity and therapeutic potential. Alkaloids have anticancer, antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties [32, 37]. Collectively, the presence of these phytochemicals with immune modulatory effects suggests that the extracts could have promising potential in treating immune-related diseases.

Medicinal plants are considered an alternative therapy against the severe adverse effects of synthetic immunomodulatory drugs [38]. However, there is no inclusive technical information regarding their safety [2]. Undoubtedly, toxicological evaluation of medicinal plants is necessary to ascertain their safety for human use [22]. The acute toxicity study results of the extracts showed no observable signs of toxicity or death after 48 h of administration of 2900 mg/kg b.w. of both extracts. The drowsiness and weakness observed after oral administration of 5000 mg/kg b.w. show that *L. micranthus* should not be administered at this dose to avert these symptoms. According to Porwal et al. [39], scientific knowledge of acute toxicity is vital to identify doses that could be used in animal studies and clinical trials and to reveal any possible clinical signs provoked by drugs under investigation. The safety profile of *L. micranthus* has previously been reported by Ani et al. [40], who did not record any organ damage in the histological architecture of major organs after sub-acute toxicity studies in animals administered *L. micranthus* extract. Likewise, our result corroborated that of Jimoh et al. [41], who did not identify any adverse biochemical changes or organ toxicity after mistletoe leaf meal-supplemented diets. Based on the acute toxicity test findings, the extracts were considered suitable for usage as medicine, and all these results above served as a basis

for choosing the actual dose selection for in vivo immunological studies.

Leukocyte migration is paramount for transporting cellular components of the immune system to eliminate pathogenic stimuli. The decrease in TLC and DLC, major biomarkers of immune function, in the negative control group could be a pointer to a poor immune response. Interestingly, although the lymphocyte counts remained relatively constant, we recorded an increase in TLC and the number of immune cells, such as neutrophils and basophils. The increment in TLC and DLC in the extracts-treated groups in a manner comparable with the levamisole-treated groups (positive control) suggests possible immune stimulation. This finding agrees with those of Ogbue et al. [42] and Dogan [43], who also recorded an increase in leucocyte counts after treatment with *Musanga cecropioides* and levamisole, respectively. Dogan [43] hypothesized that the imidazole and sulfur structures of levamisole have stimulant effects on T lymphocytes. Remarkably, empirical studies have reported that pharmaceutical agents that enhance the TLC and DLC could lower the risk of opportunistic infections and avert the risk of mortality and morbidity due to compromised immune status [42]. The reported immune-enhancing effects of the extracts may be underpinned by the high abundance of polyphenols, especially flavonoids and tannins, already known as active immunostimulatory agents [42]. As Yahfouf et al. [36] described, polyphenols regulate the immune system by increasing the activity of T-cells, interferons (IFNs), cytokines, macrophages, and B-lymphocytes. Notably, the synergistic effects of these phytochemicals might have elicited the mobilization of leucocytes, which are an integral part of cell-mediated and humoral immunity [11], thereby unveiling the immunomodulatory action of the extracts.

The DTH response is an antigenic-cell-mediated immune-inflammatory reaction modulated by T lymphocytes, macrophages, and their products [44]. From literature, antigens sensitize T cells to release proinflammatory mediators leading to increased DTH reaction in animals, as shown by paw edema [45]. Thus, we investigated the paw sizes of the SRBC-induced antigenic rats to establish a scientific basis for developing immune inflammation. According to our findings, treatment with the extracts significantly ($p < 0.05$) increased the paw size of the SRBC-induced antigenic rats compared with the negative control, with maximal effects recorded at 250 mg/kg b.w. Currently, we cannot pinpoint the precise cause of the 500 mg/kg body weight dose-related decrease in effect. Nevertheless, the reduced effect may have resulted from an inhibition brought on by an excessive dosage of each extract present at the site of action. Furthermore, the paw size diminution at 500 mg/kg b.w.

might indicate that the extracts also exhibit anti-inflammatory properties at that dosage. This is another possible thrust for future research with the extracts. Remarkably, the decrease in paw size at the higher doses is consistent with the findings of Osadebe and Omeje [21], who also obtained a reduction in the DTH response at higher doses of Nigerian mistletoe epiphytic on *Kola acuminata*, *Persia americana*, and *Pentaclothra macrophylla*. This is a pointer that the extracts should be administered at low concentrations to achieve optimal immune stimulatory effects. Interestingly, our findings corroborate Pollard and Bijker's hypothesis [44] that immunostimulants such as vaccines must be administered at relatively low doses to achieve optimal effects. The fact that the positive control increased the paw volume corresponds with former reports that levamisole increases the number of T cells by stimulating phagocytes, enhancing sensitivity to antigens and mitogens [43]. Moreover, the increase in paw size after treatment with the extracts suggests that the extracts may activate adaptive immune responses since research studies have established that an increment in DTH reaction in the SRBC-induced antigenic rats after treatment shows activation of adaptive immunity [3, 20]. This potential might be through the increased secretion and activation of lymphocytes and pro-inflammatory mediators involved in the DTH reaction [20]. Generally, the activated cytokines and mediators increase vascular permeability, exuding immune cells and activating macrophage cells in the challenged region, which prompt phagocytosis and successfully eliminate the antigen or infection [30]. Imperatively, the increase in paw size by the extracts may be attributed to the immune-stimulating effects of its active phytoconstituents.

Antibody production involves several immune actions, such as antigen processing, presentation, antigen recognition by pathogen-recognition receptors (PRRs), and cytokine activation of the memory B-cell response [46]. Thus, measuring primary and secondary antibody titers is valuable for evaluating humoral immunity. An increase in primary and secondary antibody titers was recorded in groups treated with levamisole and graded doses of the extracts, with LMPGE having higher potency. This result is consistent with those of El-Sawy et al. [47], who discovered that levamisole supplementation provoked a marked increase in antibody titer against Newcastle disease. The increased antibody titre in the treated groups might be due to the mobilized leucocytes, which have stimulatory effects on B and T cells involved in antibody production. At the same time, the increase in the secondary titre connotes the presence of memory cells. This result supports the hypothesis that subsequent antigenic stimulation enhances the responsiveness of macrophages and

lymphocytes required for antibody synthesis because there is now an expanded clone of cells with a memory of the original antigen available to proliferate into the mature cell [48]. Previous studies have reported that an increase in antibody titre increases humoral response, preventing disease progression and microbial dissemination and enhancing microbial clearance [42]. The reported increase in antibody titre by the extracts might be due to the synergistic effects of their phytochemicals, especially flavonoids and tannins, already known as active immunostimulatory agents. Behl et al. [37] pointed out that these phytochemicals modulate signaling pathways involved in cytokine synthesis, enhance antigen recognition, and stimulate immune cells. The abundance of these phytochemicals in LMPGE may account for its higher immunomodulatory action. This concurs with the findings of Shabbir et al. [49], who reported high immunomodulatory effects of *Psidium guajava*. Thus, the difference in the humoral immunity of *L. micranthus* extracts collected from the two host trees supports the possibility of metabolic exchange between the mistletoe and the host tree. These findings agree with what has been established in several studies in literature: that several factors, including the host and species of mistletoe, play essential roles in the pharmacological activities of mistletoe [16, 29].

Cyclophosphamide (CP) induces immunosuppression by interfering with DNA and RNA functions, inhibiting DNA synthesis, and cross-linking DNA, ultimately hindering B cell proliferation and lymphocyte proliferation [50]. To investigate the effects of the extracts on the immunosuppressed state, we generated a myelosuppressive rat model using CP. We discovered that, although CP administration decreased the TLC, consistent with prior reports by Parveen et al. [6] and Noh et al. [11], treatment with both extracts significantly ($p < 0.05$) increased TLC in a dose-dependent manner. These results suggest that the extracts could attenuate the myelosuppressive effects of CP. Restoration of the TLC in the treated rats, which was more pronounced in LMPGE, may be due to its higher phytochemical contents. Previously, studies have reported that phytochemicals exert complementary and synergistic effects to increase the release and activation of immune cells and mediators involved in activating cell-mediated and humoral immune responses [6]. The increase in TLC is a pointer to the protective effects of the extracts against CP-induced suppression of T and B cell proliferation. Studies have shown that an increase in TLC increases host defense against the invasion of foreign agents and is also a possible means of inhibiting disease progression in humans without eliciting harmful effects [51]. Thus, the extracts may be helpful as immune stimulants.

Conclusions

Our preliminary experiments identified a high abundance of pharmacologically relevant phytochemicals, which were higher in LMPGE extract. Furthermore, this study provides suggestive clues towards the immunomodulatory potential of the extracts, as evidenced by activation in leucocyte mobilisation, paw odema, antibody titre values, and TLC in rats after immune suppression. However, we recommend further studies regarding the characterization, isolation, and elucidation of the detailed molecular mechanism(s) responsible for the immunomodulatory potential of the extracts to fully harness their health-promoting properties.

Abbreviations

Ab	Antibody
CP	Cyclophosphamide
DTH	Delayed-type hypersensitivity
DLC	Differential leukocyte counts
HA	Hemagglutination antibody
Ig	Immunoglobulin;
IFN	Interferon
IL-4	interleukins-4
JAK/STAT	Janus kinases -signal transducer and activator of transcription proteins
LD ₅₀	Lethal Dose
<i>L. micranthus</i>	<i>Loranthus micranthus</i>
LMPBE	<i>L. micranthus</i> epiphytic on <i>Parkia biglobosa</i>
LMPGE	<i>L. micranthus</i> extracts epiphytic on <i>Psidium guajava</i>
MAPk	Mitogen-activated protein kinases
NF-κB	Nuclear factor-κB
SRBCs	Sheep red blood cells
TLC	Total leukocyte counts
TNF-α	tumour necrosis factor-α.

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Authors' contributions

Conceptualization: N.D.I., and O.F.C.N.; Investigation, N.D.I. and SEM; Methodology, NDI, OFCN, PEJ, IFC, WFA, NHA and FNN; Data curation, SEM, IFC and PEJ; Formal analysis, IFC and PEJ; Writing-original draft preparation, NDI, FNN, WFA, NHA and SEM; Writing-review and editing, OFCN, PEJ, IFC and M.D.W., Fund acquisition was the responsibility of WFA, NHA and MDW who also participated in data curation and the revision of the manuscript. The final draft was read and approved by all the authors.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Loranthus micranthus leaves were collected from different wild host trees, *Psidium guajava* and *Parkia biglobosa*, as stipulated in the University of Nigeria, Nsukka Institutional Guidelines for Plant Collection. A plant taxonomist, Mr. Alfred Ozioko, collected the plant. The farmers who owned the various plant fields gave permission to collect the plants. All the studies involving experimental animals were carried out as specified in the ARRIVE guidelines and

revised National Institute of Health Guide for Laboratory Animal Care and Use (Pub No. 85–23, revised 1985) together with the ethical procedure of the Ethics and Biosafety Committee of the Faculty of Biological Sciences, University of Nigeria, Nigeria (ethical approval number: UNN/FBS/EC/1075).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Jantan I, Ahmad W, Nasir S, Bukhari A. Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. *Front Plant Sci.* 2015;6:655.
- Sunitha VS, Midhun SJ, Sunil MA, Radhakrishnan EK, Mathew J. Valencene-rich fraction from *Vetiveria zizanioides* exerts immunostimulatory effects in vitro and in mice. *Asian Pacific J Trop Biomed J.* 2021;11:335–43.
- Ekwueme FN, Nwodo OFC, Eluka PE, Nkwocha C, Ezugwu AL, Odo I. Evaluating the immunomodulatory potential of the aqueous leaf extract of *Sennamosoides* in Wistar albino rats. *J Pharm Biol Sci.* 2015;10:83–9.
- Vonmoos M, Hirsiger S, Preller KH, Baumgartner MR, Quednow BB. Cognitive and neuroanatomical impairments associated with chronic exposure to levamisole-contaminated cocaine. *Transl Psychiatry.* 2018;8:235–46.
- Gartz A, Pawlik E, Eckhardt J, Ritz-timme S, Huhn R, Mayer F. Effects of cocaine and levamisole (as adulterant) on the isolated perfused Langendorff heart. *Int J Legal Med.* 2020;134:1741–52.
- Parveen A, Zahiruddin S, Agarwal N, Akhtar M, Husain S, Ahmad S. Modulating effects of the synergistic combination of extracts of herbal drugs on cyclophosphamide-induced immunosuppressed mice. *Saudi J Biol Sci.* 2021;28:6178–90.
- Aziz M, Ahmad S, Khurshid U, Pervaiz I, Lodhi AH, Jan N, et al. Comprehensive biological potential, phytochemical profiling using GC-MS and LC-ESI-MS, and in-Silico assessment of *Strobilanthes glutinosus* Nees: an important medicinal plant. *Molecules.* 2022;27:6885–903.
- Hussen EM, Endalew SA. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Complement Med Ther.* 2023;23:146–57.
- Chukwuma IF, Apeh VO, Nworah FN, Nkwocha CC, Emaimo J, Ezeanyika LU, et al. Inhibition of phospholipase A2 and prostaglandin synthase activities as possible mechanistic insight into the anti-inflammatory activity of *Brenania briei* methanol and chloroform fractions. *Thai J Pharm Sci.* 2022;46:75–84.
- Harun NH, Septima AW, Ahmad, W. A. N. W. Suppian R. Immunomodulatory effects and structure-activity relationship of botanical pentacyclic triterpenes: a review. *Chinese Herb Med.* 2020;12:118–124.
- Noh E, Kim J, Lee HY, Song H, Joung SO, Yang HJ, et al. Immuno-enhancement effects of *Platycodon grandiflorum* extracts in splenocytes and a cyclophosphamide-induced immunosuppressed rat model. *BMC Complement Altern Med.* 2019;19:322–34.
- Alhazmi HA, Najmi A, Javed SA, Sultana S. Medicinal plants and isolated molecules demonstrating immunomodulation activity as potential alternative therapies for viral diseases including COVID-19. *Front Immunol.* 2021;12:637553.

13. Olaoye AB, Ologunde CA, Molehin OR, Nwankwo I. Comparative antioxidant analysis of *Moringa oleifera* leaf extracts from South Western states in Nigeria. *Futur J Pharm Sci*. 2021;7:1–15.
14. Sarfraz M, Griffin S, Sad TG, Alhasan R, Nasim MJ, Masood MI, et al. Milling the Mistletoe : Nanotechnological conversion of African mistletoe (*Loranthus micranthus*) Intoantimicrobial materials. *Antioxidants*. 2018;7:60–9.
15. Idoko ND, Chukwuma IF, Nworah FN, Joshua PE, Nwodo OFC. Elucidation of immunomodulatory potential of methanol extracts of *Loranthus micranthus* leaves from two host plants: *Guajava* and *Parkia biglobosa*. *7th Int Electron Conf Med Chem*. 2021; <https://doi.org/10.3390/ECMC2021-11423>.
16. Moghadamtousi SZ, Hajrezaei M, Kadir HA, Zandi K. *Loranthus micranthus* Linn.: Biological Activities and Phytochemistry. Evidence-Based Complement Altern Med. 2013;2013:273712.
17. Osadebe PO, Johnson-Ajinwo OR. An investigation into the seasonal variation of the phytochemical and anti-diabetic properties of the eastern Nigerian specie of African mistletoe (*Loranthus micranthus*) sourced from *Kola acuminata*. *Diabetes Complicat*. 2021;5:1–5.
18. Ebokaiwe AP, Osawe S, Griffin S, Keck CM, Olusanya O, Ehiri RC. *Loranthus micranthus* nanoparticles abates streptozotocin-instigated testicular dysfunction in Wistar rats: involvement of glucose metabolism enzymes, oxido-inflammatory stress, steroidogenic enzymes/protein and Nrf2 pathway. *Andrologia*. 2020;52(10):e13749.
19. AVMA. Guidelines for the Euthanasia of Animals: 2020 Edition. 1931 N. Meacham Road Schaumburg, IL 60173; 2020.
20. Rasheed HMF, Rasheed F, Qureshi AW, Jabeen Q. Immunostimulant activities of the aqueous methanolic extract of *Leptadenia pyrotechnica*, a plant from Cholistan desert. *J Ethnopharmacol*. 2016;186:244–50.
21. Osadebe PO, Omeje EO. Comparative acute toxicities and immunomodulatory potentials of five eastern Nigeria mistletoes. *J Ethnopharmacol*. 2009;126:287–93.
22. Tabassum S, Ahmad S, Khan R, Ali B, Usman F, Jabeen Q, et al. Chemical profiling and evaluation of toxicological, antioxidant, anti-inflammatory, anti-nociceptive and Tyrosinase inhibitory potential of *Portulacaria afra* using in-vitro, in-vivo and in-silico studies. *Arab J Chem*. 2023;16(6):104784.
23. Harborne JB. *Phytochemicals methods: a guide to modern technique of plant analysis*. 2nd ed. New York: Chapman and Hall; 1998.
24. Trease GE, Evans W. *Pharmacognosy*. 11th ed. London: Macmillan Publishers; 1989.
25. Lorke D. Determination of acute toxicity. *Arch Toxicol*. 1983;53:275–87.
26. Ribeiro RA, Flores CA, Conha FQ, Ferreira SH. IL-8 causes in vivo neutrophil migration by a cell dependent mechanism. *Immunology*. 1999;73:472–7.
27. Naved T, Siddiqui JI, Ansari SH, Ansari AA, Mukhtar HM. Immunomodulatory activity of *Magnifera indica* L. Fruits (cv. Neelam). *J Nat Remedies*. 2005;5:137–40.
28. Sharma ML, Singh B, Chandan BK, Khajuria A, Kaul A, Bani S, et al. Actions of some flavonoids on specific and nonspecific immune mechanisms. *Phytomedicine*. 1996;3:191–5.
29. Govindappa C, Sadananda TS, Chandrappa CP. Phytochemical analysis of *Loranthus micranthus* extracts and their in vitro antioxidant and antibacterial activities. *J Biol Act Prod from Nat*. 2014;4:303–15.
30. Kumolosasi E, Ibrahim SNA, Shukri SMA, Ahmad W. Immunostimulant activity of standardised extracts of *Mangifera indica* leaf and *Curcuma domestica* rhizome in mice. *Trop J Pharm Res*. 2018;17:77–84.
31. Chukwuma IF, Nkwocha CC, Ezeanyika LUS, Ogugua VN. Phytochemical investigation and in vitro antioxidant potency of root bark of *brenania brieyi* fractions. *Trop J Nat Prod Res*. 2020;4:970–5.
32. Nkwocha CC, Ogugofor MO, Chukwuma IF, Njoku OU. Identification and characterization of phytochemicals and constituents in *Desmodium velutinum* stem using high-performance liquid chromatography (HPLC). *Pharmacol Res - Mod Chinese Med*. 2022;3:100090.
33. Nworah F, Chukwuma I, Osuji D, Ezeako E. Assessment of the aqueous methanol extract and ethyl acetate fraction of *Tapinanthus globiferus* leaves and their effects on ulceration and leukocyte mobilization in Wistar albino rats. *Lett Appl Nanosci*. 2023;12:1–15.
34. Ekwueme FN, Nwodo OFC, Nkwocha PE, Eluca PE. Qualitative and quantitative phytochemical screening of the aqueous leaf extract of *Senna mimosoides*: its effect in in vivo leukocyte mobilization induced by inflammatory stimulus. *Int J Curr Microbiol Appl Sci*. 2015;4:1176–88.
35. Gashaye MB, Birhan YS. Phytochemical constituents, antioxidant and anti-bacterial activities of *Plectocephalus varians* (a. rich.) C. Jeffrey ex Cufod root extracts. *BMC Complement Med Ther*. 2023;23:135–44.
36. Yahfouf N, Alsadi A, Jambi M, Matar C. The immunomodulatory and anti-inflammatory role of polyphenols. *Nutrients*. 2018;10:1618.
37. Behl T, Kumar K, Brisc C, Rus M, Nistor-Cseppento DC, Bustea C, et al. Exploring the multifocal role of phytochemicals as immunomodulators. *Biomed Pharmacother*. 2021;133:110959.
38. Apeh VO, Chukwuma FI, Nwora FN, Njoku OU, Nwodo FO. Significance of crude and degummed *Citrullus lanatus* seed oil on inflammatory cytokines in experimental infection induced by *Candida albicans*. *Acta Pharm Sci*. 2021;59:363–83.
39. Porwal M, Khan NA, Maheshwari KK. Evaluation of acute and subacute Oral toxicity induced by Ethanolic extract of *Marsdenia tenacissima* leaves in experimental rats. *Sci Pharm*. 2017;85:29–39.
40. Ani ON, Okwudili A, Okwuosa C. Toxicological study of leaf extracts of *Loranthus micranthus* Linn using albino Wistar rats. *Int J Biochem Res Rev*. 2020;29:9–16.
41. Jimoh OA, Ihejirika UG, Balogun AS, Adelani SA, Okanlawon OO. Antioxidant status and serology of laying pullets fed diets supplemented with mistletoe leaf meal. *Niger J Anim Sci*. 2018;20:52–60.
42. Ogbue CO, Onyegbule FA, Ezugwu CO, Nchekwube IHM, Ajaghaku AA. Immunostimulatory and Immunorestorative effects of leaf extract and fractions of *Musanga cecropioides* on immunocompetent and experimentally immunocompromised mice. *Clin Complement Med Pharmacol*. 2023;3:100075.
43. Doğan E. Effects of levamisole application on immunity system in Anthrax-vaccinated. *Kocatepe Vet J*. 2022;15:348–54.
44. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol*. 2021;21:83–100.
45. Rasheed HMF, Jabeen Q. Pharmacological Role of *Capparis decidua* (Forssk.) Edgew in Preventing Cyclophosphamide-induced Myelosuppression and Modulating Innate and Adaptive Immune Response. *Drug-Response*. 2022;20(3):1–16.
46. Utaiwat S, Senawong G, Khongsukwiwat K, Woranam K, Prompipak J, Sattayasai J. Stimulation of humoral and cell-mediated immunities in healthy and cyclophosphamide-induced immunosuppressed rats by the lyophilized *Houttuynia cordata* fermented drink. *Food Agric Immunol*. 2021;32:798–819.
47. El-sawy AEF, El-maddawy ZK, Awad AA. The growth promoting and immunostimulant effects of levamisole hydrochloride on broiler chicks. *Egypt J Basic Appl Sci*. 2022;9:462–76.
48. Tagne MAF, Noubissi PA, Gaffo EF, Fankem GO, Mukam JN, Kamgang R, et al. Effects of aqueous extract of *Anogeissus leiocarpus* (D.C.) Guill. et Perr. (Combretaceae) leaves on acetic acid-induced ulcerative colitis in rats. *Adv Tradit Med*. 2021;22:631–40. <https://doi.org/10.1007/s13596-021-00572-9>.
49. Shabbir A, Butt HI, Shahzad M, Arshad HM, Waheed I. Immunostimulatory effect of methanolic leaves extract of *Psidium guajava* (guava) on humoral and cell-mediated immunity in mice. *J Anim Plant Sci*. 2016;26:1492–500.
50. Chen L, Qi Y, Qi Z, Gao K, Gong R, Shao Z. A comparative study on the effects of different parts of *Panax ginseng* on the immune activity of. *Molecules*. 2019;24:1096–111.
51. Chulanetra M, Chaicumpa W. Revisiting the mechanisms of immune evasion employed by human parasites. *Front Cell Infect Microbiol*. 2021;11:702125.

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