



# Immunomodulatory and anti-inflammatory activities of hydro-ethanolic extract of *Securidaca longipedunculata* Fresen leaves



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## ABSTRACT

**Introduction:** The use of *Securidaca longipedunculata* for solving health problems related to immunological disorders is long-standing. The immunomodulatory activity associated with the anti-inflammatory effect of *S. longipedunculata* has not yet been elucidated by scientific research. The aim of our study is to show that the leaves of *S. longipedunculata* can solve problems related to immunodeficiency and inflammation.

**Methods:** The phytochemical compounds of the plant were carried out by solubility tests. The anti-inflammatory activity of the plant was evaluated by the chicken egg albumin denaturation inhibition, membrane stabilization, and C-reactive protein (CRP) tests. The 2,2-diphenyl-1-picrylhydrazyl, total antioxidant capacity, and inhibition of lipoperoxidation tests were performed to evaluate the antioxidant activity of the plant. Immunosuppression was induced in rats by cyclophosphamide. The immunomodulatory activity of *S. longipedunculata* was studied by blood count. The lactate dehydrogenase (LDH) titration showed the effect of *S. longipedunculata* on the energy balance.

**Results:** The extract contained polyphenols, flavonoids, and tannins. The tests of albumin denaturation inhibition, membrane stabilisation, CRP, total antioxidant capacity, and 2,2-diphenyl-1-picrylhydrazyl revealed that the extract had anti-inflammatory and antioxidant effects. The blood count results revealed that the extract non-significantly increased the number of leukocytes except in the case of neutrophils and monocytes at a dose of 400 mg/kg ( $P < 0.05$ ). The extract also restored the energy balance in the rats according to the LDH results.

**Conclusion:** This study reveals that the hydro-ethanolic extract of *S. longipedunculata* leaves has potential immunostimulant and anti-inflammatory effects.

### Implication for health policy/practice/research/medical education:

The results of this study show that *Securidaca longipedunculata* leaves have pharmacological properties such as anti-inflammatory, antioxidant, and immunostimulant activities. Hence, *Securidaca longipedunculata* leaves might be used for drug production against these problems.

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## Introduction

Plant species are extremely rich in number and diversity. In addition to their role in the balance of the ecosystem, plants provide mankind with many natural resources that are essential for his survival and development (1). Traditional medicine provides relief for over 70% of Third World populations (2) and 80% of African populations

(3). Among the thousands of plants used in traditional medicine is *Securidaca longipedunculata*, a plant widely used in tropical Africa for medicinal purposes. In Togo, the powdered root bark is inhaled to cure headaches and migraines and is highly sternutatory. Among the Ifè and Ewe people of Togo, maceration of the plant root is used to treat sickle-cell anemia, aches and pains, amoebiasis,

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intestinal worms, and malaria.

Phytochemical screening by several researchers has revealed that *S. longipedunculata* is rich in polyphenols, flavonoids, saponins, tannins, anthraquinones, sterols, terpenes, elymoclavin, and dehydroelymoclavin (4-6).

Indeed, polyphenols and flavonoids are reputed to be responsible for most of the aforementioned biological activities derived from *S. longipedunculata*. However, no research has addressed the immunomodulatory and anti-inflammatory activities of the hydroethanol extract of *S. longipedunculata* leaves. The immune system is extremely important to human health, and most diseases are in some way linked to the immune system. At first glance, we might think of any microbial infection that it might be possible to avoid or sufficiently combat with the help of a strengthened immune system, but also of any pathology observed, from allergic disorders and autoimmune diseases to cancers, which are due to a deficient immune system. Good natural and acquired immunity is the key to future health and recovery (7,8).

Although efforts are made to manufacture immunostimulating drugs, it is clear that these drugs are not only expensive for poor populations but often have serious side effects such as allergic and skin reactions, fever, seizures, asthma, urticaria, bullous eruptions, malformations, iron deficiency, nasopharyngitis, otitis, angina, etc (7,8). It is therefore necessary to find alternatives at lower cost and accessible to all.

The present studies have revealed that it is possible to use *S. longipedunculata* leaves to treat immunodeficiency infections, which are responsible for other opportunistic diseases by proving the potential immunomodulatory and anti-inflammatory effects. To do this, *in vitro* and *in vivo* tests were carried out.

## Materials and Methods

### Chemicals

Polyvinylpyrrolidone (PVPP), rutin, gallic acid, Folin Ciocalteu reagent, ascorbic acid, aluminium chloride, Sodium acetate, 1, 1-diphenyl-2-picrylhydrazyl hydrate (DPPH), levamisole, aspirin, quercetin, ether, diclofenac, malondialdehyde (MDA), and cyclophosphamide were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Plant extracts preparation

The leaves of *S. longipedunculata* were collected in June 2022 at Lodji, a village located 15 km west of Anié town. The plant was identified and a specimen was deposited under number TOGO06917 in the herbarium of the Faculty of Sciences of the University of Lomé. Plant organs were washed and dried at laboratory temperature, and protected from light and humidity. After drying, *S. longipedunculata* leaves were powdered. Then, 500 g of *S. longipedunculata* leaf powder was macerated in 5 L of an equal volume (50/50, v/v) mixture of water and

ethanol for 72 hours. The preparation was filtered twice, first with cotton and then with Whatman N°1 paper. The extracts were evaporated to dryness using a rotavapor. The extracts obtained were weighed and stored in tubes at 4 °C, protected from light until use. The extraction yield was determined by the following formula:

$$\text{Yield} = 100 \times (\text{Mass of dry residue of evaporated extract}) / (\text{Mass of dry plant material powder})$$

### Animals

Sprague-Dawley rats (male and female) were used in this study. They were selected according to age (8 to 10 weeks) and weight (150 to 180 g). Breeding was carried out at the Animal Physiology Department of the University of Lomé. Experimental animals were kept at room temperature, 27±2 °C, and a 12-h/12-h light/dark cycle, with free access to drinking water and food. All tests using rats, blood, and eggs were performed with the approval of the ethical committee of the Department of Animal Physiology of the University of Lomé, a branch of the ethical committee for the control and supervision of animal experiments and the use of blood, Ref n° 006/2020 / BC-BPA / FDS-UL.

### Phytochemical analysis

The hydroethanol extract of *S. longipedunculata* was dissolved in distilled water and then filtered. The filtrate was used to search for certain chemical compounds such as flavonoids, tannins, polyphenols, saponosides, alkaloids, and carbohydrates using standard staining tests (9).

### Quantitative determination of phytochemicals

#### Determination of total flavonoids

Flavonoids were determined by the calorimetric method using aluminum chloride. This is based on the properties of flavonoids to form aluminum chelates with aluminum chloride (5). Two milliliters aluminum chloride (2%) and 6 mL sodium acetate (50 mg/mL) were added to 2 mL extract (1 mg/mL) or rutin (1 mg/mL). The blank was made with 2 mL ethanol in place of the sample. Optical density (OD) reading was taken at 440 nm after 30 minutes. The total flavonoid content of *S. longipedunculata* extract was determined from the linear regression equation of the calibration range established with rutin (5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL) and expressed as µg rutin equivalent per milligram dry extract (µg EQ/mg extract). Rutin was used as the reference. Tests were repeated three times.

#### Determination of phenols and tannins

The total phenol content of the extract was determined by the calorimetric method using the Folin-Ciocalteu reagent after tannin fixation by PVPP (10). This method involved two steps:

Step 1: 500 µL of the extract (stock solution at 1 mg/mL) was added to a tube, which contained 10 mg PVPP and methanol. Then, it was incubated on ice for 30 minutes and centrifuged. Then, 200 µL of the supernatant was transferred to dry a tube for assay with Folin-Ciocalteu reagent. The blank was prepared with 1 mL methanol instead of extract.

Step 2: To 200 µL of the extract solution (stock solution at 1 mg/mL) or 200 µL of the gallic acid solutions (50, 25, 12.5, 6.25, and 0 µg/mL) or 200 µL of the solution obtained in step 1 (extract + PVPP), 200 µL of 10% Folin-Ciocalteu reagent (10-fold diluted in distilled water) was added. After 10 minutes incubation at room temperature, 750 µL sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) (60 g/L) was added. OD was read on a spectrometer at 725 nm against a blank (5). Total phenol was expressed in terms of mg gallic acid equivalent/g extract. The total amount of tannin was calculated using the following formula:

$$ODT = ODE - ODE + PVPP$$

ODT = DO tannins; ODE = OD extract; ODE + PVPP = OD extract + PVPP

#### *In vitro* anti-inflammatory test

##### *Egg albumin denaturation inhibition*

The reaction mixture (5 mL) composed of 0.2 mL egg albumin from fresh chicken's egg, 2.8 mL phosphate-buffered saline (PBS; pH 6.4), and 2 mL extract or reference drug (Diclofenac sodium) at different concentrations (500, 250, 125, 62.5, and 0 µg/mL). Control and test samples were incubated at 37 °C for 25 minutes, then at 70 °C for 5 minutes. After cooling to 37 °C, the OD of each sample was measured at 660 nm (11), and the percentage inhibition of protein denaturation that determined anti-inflammatory activity was calculated according to the formula:

$$\text{Anti-inflammatory activity (\%)} = (1 - (At/Ac)) \times 100$$

which Ac = absorbance of the negative control and At = absorbance of the test performed

##### *Membrane stabilization test*

First, the rats were anesthetized with ether, then 5 mL of blood was drawn from the retro-orbital sinus into heparin tubes. The blood was centrifuged at 1500 rpm for 10 minutes to separate erythrocytes from plasma and buffy coat. The erythrocytes were then washed three times with the same normal saline solution. The resulting erythrocyte pellet was then suspended in 10 volumes of normal saline (12).

##### *Hemolysis induced by hypotonic solution*

Reference drug (aspirin) at different concentrations (25-200 µg/mL) or 1 mL *S. longipedunculata* extract and 2 mL hyposaline solution (0.36%) were added to 1 mL

erythrocyte suspension. After 30 minutes of incubation at 37 °C, the mixtures were centrifuged at 3000 rpm for 15 minutes, then the OD was read at 560 nm. All measurements were repeated 3 times. The percentage of membrane stabilization reflecting anti-inflammatory activity was determined according to the following formula:

$$\text{Anti-inflammatory activity (\%)} = (1 - (At/Ac)) \times 100$$

which Ac = absorbance of the negative control and At = absorbance of the test performed.

##### *Heat-induced hemolysis*

To 1 mL erythrocyte suspension was added 1 mL extract or reference drug (aspirin) at different concentrations (25-200 µg/mL). The mixture was then incubated at 56 °C in a water bath for 30 minutes. After cooling, the solutions were centrifuged at 2500 rpm and the absorbance of the supernatant was read at 560 nm. The percentage inhibition was calculated according to the following formula:

$$\text{Anti-inflammatory activity (\%)} = (1 - (At/Ac)) \times 100$$

which Ac = absorbance of the negative control and At = absorbance of the test performed

##### *Antioxidant activity in vitro*

##### *Total antioxidant capacity (phosphomolybdenum assay)*

The test is based on the reduction of molybdenum Mo (VI) present as molybdate ions MoO<sub>4</sub><sup>2-</sup> to molybdenum Mo (V) MoO<sup>2+</sup> in the presence of the extract or an antioxidant agent. This reduction results in a greenish complex (phosphate/Mo(V)) at an acid pH (13). The increase in color of the molybdenum (VI) complex was measured in the presence of antioxidants.

The method consisted of introducing into a tube 1000 µg/mL of *S. longipedunculata* extract mixed with 3 mL of a reagent composed of H<sub>2</sub>SO<sub>4</sub> (0.6M), Na<sub>2</sub>PO<sub>4</sub> (28mM), and ammonium molybdate (4mM). The tube was then tightly closed and incubated at 95 °C for 90 minutes. After cooling, the absorbance was measured at 695 nm. The control consisted of 100 µL ethanol mixed with 1000 µL of the above-mentioned reagent. The samples and controls were incubated under the same conditions.

##### *Evaluation of anti-free radical activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical method*

The DPPH method is based on the reduction of the stable radical species, DPPH, in the presence of a hydrogen-donating antioxidant (AH), resulting in the formation of a non-radical form, DPPH-H (diphenyl picrylhydrazine). In the presence of free-radical scavengers, the violet DPPH is reduced to yellow DPPH.H. DPPH free radical reduction can be monitored by UV visible spectrometry, measuring the decrease in absorbance at 517 nm (14).

Procedure: One ml of freshly prepared ethanolic solution

of DPPH (0.1 mM) was added to 3 ml of extract or standard solution of ascorbic acid at different concentrations (3.125 µg/mL to 100 µg/mL). After incubation in the dark for 30 minutes at room temperature, the absorbance readings were taken at 517 nm using a spectrophotometer.

The percentage inhibition was calculated using the following formula:

$$I (\%) = (1 - (At/Ac)) \times 100$$

which Ac = absorbance of the negative control and At = absorbance of the test performed.

#### *Inhibition of lipoperoxidation induced by FeCl<sub>2</sub>-ascorbic acid on bone marrow homogenate (Malondialdehyde assay)*

Malondialdehyde (MDA) level is a marker of lipid peroxidation. It reacts with a chromogenic reagent to give a stable chromophore compound with a maximum absorbance at 586 nm. Bone marrow tissue was rapidly removed from sacrificed rats. Two grams of bone marrow was homogenized with buffer KCl Tris HCl (10 mL; 150 mM; pH 7.4). The mixture consisted of 500 µL of organ homogenate, 100 µL of ascorbic acid (0.1 mM), 200 µL of buffer KCl Tris HCl (150 mM; pH 7.4), 100 µL of FeCl<sub>2</sub> (4 mM), and 100 µL of various concentrations of standard or *S. longipedunculata* extract. The mixture was incubated for 1 hour at 37 °C in closed tubes. 650 µL of 1-methyl-2-phenyl-indole (10.3 mM) in acetonitrile was diluted with methanol containing 32 mM FeCl<sub>3</sub> (3: 1). The mixture was added to 250 µL sample and vortexed. Then, 150 µL of 37% (v/v) HCl was added, tightly sealed with leakproof caps, and incubated at 45 °C for 1 hour. After cooling, it was centrifuged at 4000 rpm for 10 minutes and its absorbance was measured at 586 nm. A standard curve of 1,1,3,3-tetra-methoxypropane was run for the MDA dosage (15).

#### *Total protein determination in bone marrow tissue*

The protein content of experimental samples was measured by the Bradford method using crystalline bovine serum albumin (BSA) as a standard. To 15 µL of homogenate or BSA at different concentrations, 750 µL of Bradford reagent was added, and the absorbance was read 5 min later at 595 nm (15).

#### *Immunomodulatory and anti-inflammatory activity in vivo*

##### *Conception of animals' groups*

The animals were given no food prior to treatment. They were divided into 5 groups of 5 rats, each as follows:

- Control group: distilled water 10 mg/kg (day 0- day 13) + 1 mL/kg NaCl 0.9% (day 11-day 13)
- Cyclophosphamide group: distilled water (day 0- day 13) + cyclo 30 mg/kg (day 11-day 13)
- Extract 200 group: extract 200 mg/kg (day 0-day 13) + cyclo 30 mg/kg (day 11-day 13)

- Extract 400 group: extract 400 mg/kg (day 0-day 13) + cyclo 30 mg/kg (day 11-day 13)
- Levamisole group: levamisole 30 mg/kg (day 0- day 13) + cyclo 30 mg/kg (day 11-day 13)

#### *Sampling*

After treatment, the animal was sacrificed, and the blood was collected in dry tubes and tubes containing EDTA. The collected blood was centrifuged at 3500 rpm for 10 minutes. The supernatant (serum) was used to determine biochemical and enzymatic parameters. Whole blood from EDTA tubes was used to count the numerical blood count.

#### *Blood count*

The blood count was carried out using an automated system based on flow cytometry.

*Principle:* The cells suspended in a liquid flow were passed one by one through a laser beam. Physical scattering of the light emitted by the light source depends on cell size and granularity (granule content, more or less segmented structure of the nucleus). Scattering in the direction of the light source (forward scatter, FSC) provided size information, while scattering at 90 °C (side scatter, SSC) provided information on granularity or structure. An electronic system converted optical signals (photons) into electronic signals. The signals were collected by photomultipliers, amplified, digitized, and stored in a computer. A computer system visualized the signals.

#### *Determination of C-reactive protein (CRP) or Pantraxin 1*

CRP is an early, sensitive and specific marker of the inflammatory reaction, and is proportional to its intensity. CRP was measured in rat serum using an automated system.

#### *Assessment of lactate dehydrogenase (LDH) enzyme activity*

LDH is a ubiquitous intracellular enzyme. The highest concentrations of LDH are found in liver, heart, kidney, skeletal muscle, and erythrocytes. It catalyzes the reversible conversion of pyruvate to lactate in the presence of NAD<sup>+</sup>/NADH. The determination of LDH in rat serum was also carried out using an automated system.

#### *Data analysis*

Results are presented as mean ± SEM (standard error of the mean). They are processed using Graph Pad Prism 8.0.1 software, which was also used to construct histograms. Tukey's multiple comparison test was used to compare data means. Differences between results were considered significant at 5% threshold (*P* value < 0.05).

#### **Results**

##### *Extract yield*

The *S. longipedunculata* leaves, characterized by their



viscous texture and brown color, showed a yield of 15.05%.

**Qualitative tests**

Table 1 shows the results of phytochemical tests on the hydro-ethanolic extract (50/50, v/v) of *S. longipedunculata* leaves.

**Determination of flavonoids, total polyphenols and tannins**

The total quantity of flavonoids, total phenols, and tannins present in the extract of *S. longipedunculata* leaves was expressed in milligrams of rutin equivalent for flavonoids and gallic acid equivalent for phenolics and tannins per gram of extract (Table 2).

**Egg albumin denaturation inhibition test**

IC<sub>50</sub> values for extract and diclofenac in the albumin denaturation inhibition test were as follows: the *S. longipedunculata* extract exhibited an IC<sub>50</sub> of 99.87 ± 0.26 µg/mL, while diclofenac had a lower IC<sub>50</sub> value of 49.54 ± 0.04 µg/mL, indicating its stronger inhibitory effect.

**Membrane stabilization tests**

IC<sub>50</sub> values for extract and standard (Aspirin) are shown in Table 3.

**In vitro antioxidant tests**

**TAC (total antioxidant capacity) and DPPH (2,2-diphenyl-1-picrylhydrazyl)**

The antioxidant capacity of the extract and ascorbic acid, determined by phosphomolybdenum reduction, and the percentage DPPH scavenging effect of the extract and ascorbic acid are shown in Table 4.

**Table 1.** Chemical groups in hydro-ethanolic extract of *Securidaca longipedunculata* leaves

Chemical groups	<i>S. longipedunculata</i> extract
Flavonoids	+
Tannins	+
Polyphenols	+
Saponosides	+
Alkaloids	+
Carbohydrates	+

+ Present

**Table 2.** Quantification of phenols, flavonoids, and tannins in hydro-ethanolic extract of *Securidaca longipedunculata* leaves

Groups	Quantity
Flavonoids	50.820 ± 1.0250 mg/RE/g
Total phenols	72.3820 ± 0.040 mg/GAE/g
Tannins	22.5587 ± 1.801 mg/GAE/g

GAE, gallic acid equivalent; RE, rutin equivalent. The results represent mean ± SEM (n = 3).

**Liperoxidation inhibition**

The liperoxidation inhibition results by hydro-ethanolic extract of *S. longipedunculata* leaves extract are recorded in Figure 1.

**Immunomodulatory and anti-inflammatory activity of hydro-ethanolic extract of *S. longipedunculata* leaves in vivo**

**Blood count**

Table 5 shows the results of the blood count.

**CRP assay**

The dosage of CRP to show the *in vivo* anti-inflammatory effect of *S. longipedunculata* is displayed in Figure 2.

**Results of LDH**

The results of LDH are illustrated in Figure 3 to demonstrate the effect of *S. longipedunculata* on energy balance.

**Discussion**

The work carried out by Odeblyi (16) showed the absence of flavonoids in *S. longipedunculata* leaves. Our study, on the other hand, revealed the presence of flavonoids in *S. longipedunculata* leaves. This may be due to the plant's geographical origin. This study confirms the immunosuppressive effect of cyclophosphamide administered on days 11, 12, and 13 to rats in the cyclophosphamide group: leukopenia ( $P < 0.001$ ), lymphopenia ( $P < 0.001$ ), neutropenia ( $P < 0.0001$ ), hypobasophilia ( $P < 0.001$ ), and monocytopenia ( $P < 0.05$ ) as already proved by some previous researchers (17,18).

The 200 mg/kg and 400 mg/kg doses of *S. longipedunculata* extract increased the number of

**Table 3.** Anti-inflammatory effect of hydro-ethanolic extract of *Securidaca longipedunculata* leaves on red blood cell membrane stabilization

Substances	IC <sub>50</sub> (µg/mL)	
	Hypotonic	Heat
<i>S. longipedunculata</i> extract	305.657 ± 0.133	471.750 ± 0.096
Aspirin	246.117 ± 1.055	368.743 ± 0.632

The results are expressed as mean ± SEM (n = 3).

**Table 4.** Antioxidant activity of hydro-ethanolic extract of *Securidaca longipedunculata* leaves by TAC (total antioxidant capacity) and DPPH (2,2-diphenyl-1-picrylhydrazyl) tests

Substances	TAC	DPPH
	(mg GAE/g)	IC <sub>50</sub> (µg/mL)
<i>S. longipedunculata</i> extract	97.83 ± 1.29	76.22 ± 0.02
Ascorbic acid	-	38.5 ± 0.04

GAE, gallic acid equivalent.

The results are expressed as mean ± SEM (n = 3).

**Table 5.** Effect of hydro-ethanolic extract of *S. longipedunculata* leaves on the number of leukocytes per microliter of blood

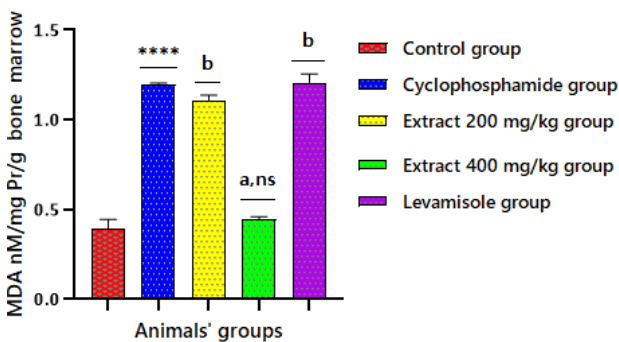
Cells	Control	Cyclo-phosphamide	Extract 200 mg/kg	Extract 400 mg/kg	Levamisole
Leukocytes	7280±168	794±140.7	876±175.3	950±169.9	1062± 274.2*
Lymphocytes	3506±100.5	476±67.72	518±130	580±70.85	678 ± 150*
Neutrophils	1738±458	218±55.80	244±54.92	316±82.79*	160 ± 44.12
Basophils	366±62.82	76±28.74	90±25.50	96±32.19	88 ± 4
Monocytes	324±13.02	10±3.016	20±10.49	60±41.47*	52 ± 2

The results are expressed as Mean ± SEM (n = 3). \* Significant compared with the control group).

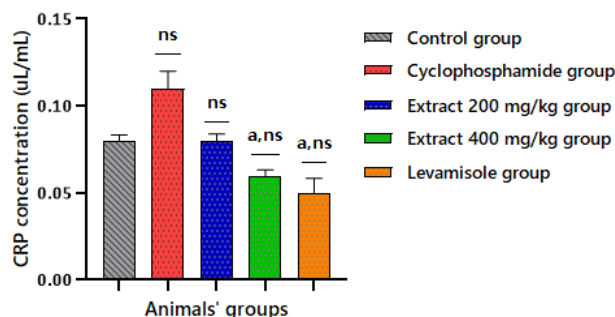
immune cells insignificantly ( $P < 0.05$ ), except in the case of neutrophils and monocytes at doses of 400 mg/kg. Levamisole is a drug used or cited by several authors in their studies as an immunostimulant reference (18-20). Our analyses showed no difference between the results of the groups of rats treated with extract and the group treated with levamisole revealing the immunostimulant effect of the extract similar to that of levamisole. It would be preferable to continue by administering the extract alone, one or two weeks longer to the rats after

cyclophosphamide gavage to detect the difference between the values of the measured parameters of different treated groups.

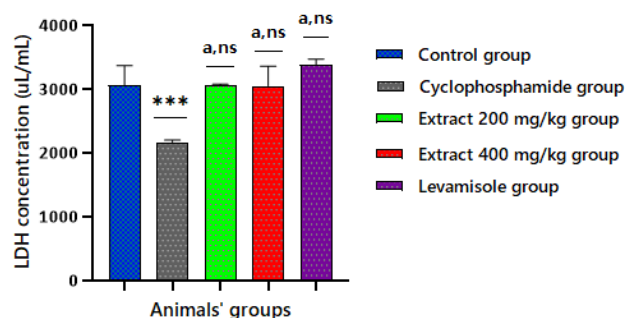
Indeed, cyclophosphamide induces immunosuppression and inflammation as side effects (21,22). In our study, the *S. longipedunculata* extract played an anti-inflammatory activity. The results of the chicken egg albumin denaturation inhibition and membrane stabilization tests, supported by those of CRP, which is a good marker of the acute phase of inflammation (23,24), made the anti-inflammatory activity of *S. longipedunculata* coupled with its immunomodulatory effect more certain. Inflammation is an integral part of immunity, and our study is further proof of this. CRP results showed that *S. longipedunculata* extract reduced inflammation caused by cyclophosphamide. This was reflected by a reduction in CRP concentration from  $0.11 \pm 0.01$  ul/mL (G. Cyclo) to  $0.08 \pm 0.01$  ul/mL (200 mg/kg group) and  $0.06 \pm 0.04$  uL/mL (400 mg/kg group). These results suggest that *S. longipedunculata* has stimulated the bone marrow to produce leukocytes, and at the same time, it has protected the cells against chemical and enzymatic modifications induced by the immunosuppressive agent. The extract limited the inflammatory response by curbing auto-antigen production, which alleviated the organ damage caused by inflammatory processes (25). During



**Figure 1.** Effect of hydro-ethanolic extract *Securidaca longipedunculata* on malondialdehyde (MDA) level in bone marrow. Results are expressed as mean ± standard error of the mean. (\*\*\*\* $P < 0.0001$  significant compared with the control group; ns: not significant compared with the control group; a: significant compared with the cyclophosphamide (Cyclo) group ( $P < 0.001$ ); b: not significant compared with the Cyclo group).



**Figure 2.** Influence of hydro-ethanolic extract of *Securidaca longipedunculata* on C-Reactive Protein (CRP) titer. Results are expressed as mean ± standard error of the mean. (ns: not significant compared with control group  $P > 0.05$ ; a: significant compared with cyclophosphamide group ( $P < 0.05$ )).



**Figure 3.** Influence of hydro-ethanolic extract of *Securidaca longipedunculata* on the enzymatic activity of lactate dehydrogenase (LDH). Results are expressed as mean ± standard error of the mean. (\*\*\* $P < 0.001$  significant compared with the control group; ns: not significant compared with the control group ( $P > 0.05$ ); a: significant compared with the cyclophosphamide group ( $P < 0.001$ )).

inflammation, lysosomal enzymes, such as bactericidal enzymes and proteases are released and several typical alterations can occur (12). Lysosomal membrane stabilization is important for limiting the inflammatory response by preventing the release of lysosomal constituents from activated neutrophils that cause further tissue inflammation upon their extracellular release. The membranes of erythrocytes and lysosomes are similar, so the membrane stabilization of red blood cells could be extrapolated to the membrane stabilization of lysosomes (26,27). The anti-inflammatory activity of levamisole was also revealed by this study, as the difference between the CRP values of the cyclophosphamide and levamisole groups was significant ( $P < 0.05$ ). Relying on this research that the extract at the dose of 400 mg/kg and levamisole at the dose of 30 mg/kg would have been sufficient to significantly prevent hepatocytes from releasing CRP, whose biological functions are to activate the complement system, stimulate phagocytosis and opsonization.

The extract also had antioxidant activity. A significant ( $P < 0.0001$ ) increase of MDA was found in the rats receiving cyclophosphamide and untreated ( $0.3923 \pm 0.1110$  nM/mg Pr/g of bone marrow) compared with the normal control group ( $1.1979 \pm 0.1101$  nM/mg Pr/g of bone marrow). The extract administered at a dose of 200 mg/kg did not significantly reduce the MDA value compared with the group of rats treated only with cyclophosphamide. Furthermore, no significant variation of MDA was observed between rats receiving cyclophosphamide and pretreated with extract at the dose of 400 mg/kg ( $0.4457 \pm 0.02289$  nM/mg Pr/g of bone marrow) and those of normal control ( $P < 0.05$ ). These results, supported by those of the TAC and DPPH tests, probably explain the protection of the animals by *S. longipedunculata* extract against oxidative stress. It could be that cyclophosphamide, which is an alkylating agent, has produced free radicals that interacted directly with the DNA of bone marrow cells in G0 phase, forming covalent bonds with the nucleophilic substrate and causing the destruction of cells by the inhibition of DNA transcription and replication. Briefly, cyclophosphamide has caused bone marrow aplasia. This could prevent the rapid proliferation of hematopoietic stem cells and the immunostimulant effect of *S. longipedunculata* extract.

The MDA value obtained in rats receiving cyclophosphamide and pretreated with levamisole ( $1.2072 \pm 0.05364$  nM/mg Pr/g of bone marrow) was not significantly different from the MDA value of the rats treated only with cyclophosphamide. This proves that levamisole probably did not inhibit lipid peroxidation, and it is clear that the immunostimulant mechanism of levamisole was distinct from that of our extract.

The antioxidant effect is due to natural compounds, notably the polyphenols and flavonoids present in the extract. Polyphenols exert their antioxidant activity

through various mechanisms, the main ones are direct scavenging of ROS, chelation of metal ions that initiate ROS production, and direct inhibition of enzymes involved in oxidative stress or their transcription (28).

The LDH enzyme assay, which averaged  $3060 \pm 169.9$  IU/mL in the normal rats and  $2167 \pm 287.7$  IU/mL in the rats treated with cyclophosphamide, proved that cyclophosphamide reduced cellular energetic activity. Nevertheless, the LDH results obtained in the groups treated with the extract (200 mg/kg and 400 mg/kg) whose values are respectively  $3052 \pm 613.3$  IU/mL and  $3049 \pm 293.9$  IU/mL are not significantly different in comparison with the value obtained for the normal group. This shows that *S. longipedunculata* extract restored the lactate/pyruvate (L/P) ratio. Therefore, the extract ensures energy balance in the cells. The extract would have a cytoprotective effect. Not only *S. longipedunculata* extract has boosted leukocyte production, but also these cells would have the energy they need to vigorously carry out their role in defending the body.

Overall, our research proves that *S. longipedunculata* leaves' extract has immunostimulation and anti-inflammation activities. There is hope that *S. longipedunculata* leaves can be used against immunodeficiency and inflammation problems.

## Conclusion

The hydroalcoholic extract *S. longipedunculata* leaves had immunostimulant, anti-inflammatory, antioxidant, and cytoprotective activities; the results may encourage scientists to deepen immunomodulatory research on *S. longipedunculata* to save mankind who is constantly confronted diseases linked to immunological disorders. Thus, we envisage the following perspectives: continue treating animals with the extract until day 21 or 28; take blood samples before and just after gavage of the animals with cyclophosphamide (days 10 and 14), and at day 21 or 28; use doses higher than 400 mg/kg; use extracts from maceration of different mixtures: Volume-water/Volume-alcohol (ethanol, methanol, butanol).

## Authors' contributions

Conception: Kporvie Atsu Kodjo George, Kindji Kpoyizoun Pascaline;

Design, data collection, and processing: Kporvie Atsu Kodjo George, Dermane Affo;

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Review and final revision approval: all authors.

## Conflict of interests

The authors declare that there aren't conflicts of interest in this work.

**Data availability statement**

All data are included in this manuscript; however, additional information may be obtained from the corresponding author upon request.

**Ethical considerations**

The protocol of this study was approved by the Ethical Committee of the Department of Animal Physiology of the University of Lomé, a branch of the ethical committee for the control and supervision of animal experiments and the use of blood (Ref: 006/2020/BC-BPA/FDS-UL). Ethical issues, including plagiarism, double publication, and data fabrication have been completely observed by the authors.

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**References**

- Salhi S, Fadli M, Zidane L, Douira A. Floristic and ethnobotanical study of medicinal plants of Kénitra (Maroc). *Lazaroa*. 2010;31:133-46. doi: 10.5209/rev\_LAZA.2010.v31.9.
- Malaisse F. The management of edible wild products. *Défis-Sud*. 1992;7:18-9.
- Jiofack T, Fokunang C, Guedje N, Kemeuze V, Fongnzossie E, Nkongmeneck BA, et al. Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. *Int J Med Med Sci*. 2010;2(3):60-79.
- Lenz W. Study of the interior of the root of *Securidaca longipedunculata*. *Pharm Inst D Univ Berlin*. 1993;10:177-80.
- Kola P, Metowogo K, Manjula SN, Katawa G, Elkhenany H, Mruthunjaya KM, et al. Ethnopharmacological evaluation of antioxidant, anti-angiogenic, and anti-inflammatory activity of some traditional medicinal plants used for treatment of cancer in Togo/Africa. *J Ethnopharmacol*. 2022;283:114673. doi: 10.1016/j.jep.2021.114673.
- Costa C, Bertazzo A, Allegri G, Curcuruto O, Traldi P. Indole alkaloids from the roots of an African plant *Securidaca longipedunculata*. I. Isolation by column chromatography and preliminary structural characterization by mass spectrometry. *J Heterocycl Chem*. 1992;29(6):1641-7. doi: 10.1002/jhet.5570290647.
- Anderson LA. Common side effects for antibiotics, allergies and reactions. *J Med Reviews*. 2023. <https://www.sciencedirect.com/science/article/pii/B978145570891800066X>.
- Felippe MJ. Immunotherapy. In: *Equine Infectious Diseases*. 2nd ed. WB Saunders; 2014. p. 584-97. doi: 10.1016/b978-1-4557-0891-8.00066-x.
- Kpoyizoun PK, Metowogo K, Kantati YT, Missebukpo A, Dare T, Lawson-Evi P, et al. Anti-inflammatory and antioxidant evaluation of *Maytenus senegalensis* hydroalcoholic roots extract fractions in allergic asthma. *J Phytopharmacol*. 2020;9(4):252-7. doi: 10.31254/phyto.2020.9407.
- Dosseh K, Kpatcha T, Adjrah Y, Idoh K, Agbonon A, Gbéassor M. Anti-inflammatory effect of *Byrsocarpus coccineus* Schum. and Thonn. (Connaraceae) root. *World J Pharm Res*. 2014;3(3):3585-98.
- Saleem A, Saleem M, Akhtar MF. Antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: an ethnomedicinal plant of Moringaceae family. *S Afr J Bot*. 2020;128:246-56. doi: 10.1016/j.sajb.2019.11.023.
- Javed F, Jabeen Q, Aslam N, Awan AM. Pharmacological evaluation of analgesic, anti-inflammatory and antipyretic activities of ethanolic extract of *Indigofera argentea* Burm. f. *J Ethnopharmacol*. 2020;259:112966. doi: 10.1016/j.jep.2020.112966.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999;269(2):337-41. doi: 10.1006/abio.1999.4019.
- Athamena S, Chalghem I, Kassah-Laouar A, Laroui S, Khebri S. Antioxidant and antimicrobial activity of *Cuminum cyminum* L. extracts. *Leban Sci J*. 2010;11(1):69-81.
- Kpemissi M, Eklü-Gadegbeku K, Veerapur VP, Potârniche AV, Adi K, Vijayakumar S, et al. Antioxidant and nephroprotection activities of *Combretum micranthum*: a phytochemical, in-vitro and ex-vivo studies. *Heliyon*. 2019;5(3):e01365. doi: 10.1016/j.heliyon.2019.e01365.
- Odeblyi OO. Preliminary phytochemical and antimicrobial examinations of leaves of *Securidaca longipedunculata*. *Niger J Pharm*. 1978;9(6):29-30.
- Bah S, Jäger AK, Adersen A, Diallo D, Paulsen BS. Antiplasmodial and GABA(A)-benzodiazepine receptor binding activities of five plants used in traditional medicine in Mali, West Africa. *J Ethnopharmacol*. 2007;110(3):451-7. doi: 10.1016/j.jep.2006.10.019.
- Guo Z, Xu HY, Xu L, Wang SS, Zhang XM. In vivo and in vitro immunomodulatory and anti-inflammatory effects of total flavonoids of *Astragalus*. *Afr J Tradit Complement Altern Med*. 2016;13(4):60-73. doi: 10.21010/ajtcam.v13i4.10.
- Libeau G, Pinder M. [Deleterious effect of levamisole on experimental trypanosomiasis in mice]. *Rev Elev Med Vet Pays Trop*. 1981;34(4):399-404. doi: 10.19182/remvt.8248. [French].
- Tibitondwa J, Ikwap K, Tamale A, Tumwine G, Kateregga J, Wamala SP, et al. Immunomodulatory activity of the *Chenopodium opulifolium* total crude extract in Wister albino rats. *Afr J Tradit Complement Altern Med*. 2018;15(2):96-102. doi: 10.21010/ajtcamv15i2.12.
- Sevko A, Sade-Feldman M, Kanterman J, Michels T, Falk CS, Umansky L, et al. Cyclophosphamide promotes chronic inflammation-dependent immunosuppression and prevents antitumor response in melanoma. *J Invest Dermatol*. 2013;133(6):1610-9. doi: 10.1038/jid.2012.444.
- Agence Nationale de Sécurité du Médicament (ANSM). Summary Product Features. 2017. Available from: <http://agence-prd.ansm.santé.fr>.
- Engler R. Protéines de la réaction inflammatoire. *Revue Française des Laboratoires*. 1995;1995(276):93-9. doi: 10.1016/s0338-9898(95)80365-3.



24. Ernest W, Jean-Luc D, Siggy R, Bernard W. The biological markers of inflammation. Short version 2008.
25. Woolbright BL. Inflammation: cause or consequence of chronic cholestatic liver injury. *Food Chem Toxicol.* 2020;137:111133. doi: 10.1016/j.fct.2020.111133.
26. Agarwal H, Nakara A, Shanmugam VK. Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: a review. *Biomed Pharmacother.* 2019;109:2561-72. doi: 10.1016/j.biopha.2018.11.116.
27. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). *Daru.* 2012;20(1):76. doi: 10.1186/2008-2231-20-76.
28. Migdal C, Serres M. [Reactive oxygen species and oxidative stress]. *Med Sci (Paris).* 2011;27(4):405-12. doi: 10.1051/medsci/2011274017. [French].