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Acute Toxicity, Immunomodulation Activities, and Phytochemical Profiles of Sapium ellipticum Stem Bark Aqueous Extract

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Abstract

Background: The stem bark of *Sapium ellipticum* is a medicinal plant frequently utilized in traditional medicine for treating a range of ailments. Despite its widespread use, there is limited data regarding the safety and immunomodulatory potential of its extract.

Objective: This investigation was conducted to assess the phytochemical profile, acute toxicity, and immunomodulatory activity of the aqueous extract of *S. ellipticum* stem bark.

Methods: Acute toxicity was assessed using the Organization for Economic Cooperation and Development (OECD) guidelines at doses of 123, 300, and 2000 mg/kg. Immunomodulatory activity was evaluated using real-time quantitative polymerase chain reaction (RT-qPCR) to measure the expression levels of IL-10, IL-6, IL-1Ra, and IL-1β. Phytochemical profiling was carried out using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: The aqueous extract of *S. ellipticum* stem bark did not produce any hematological or biochemical signs of toxicity, indicating that the lethal dose was greater than 2000 mg/kg. The extract showed potential as an immunomodulator, with downregulation of IL-6 and IL-10 and upregulation of IL-1Ra and IL-1β. Phytochemical analysis revealed the presence of anti-inflammatory compounds, such as Fraxetin and L-carnitine. However, the extract also showed upregulation of the pro-inflammatory cytokine IL-1β, with no inflammatory compounds identified.

Conclusion: Findings from the present study suggest aqueous extract of *S. ellipticum* stem bark is safe up to a dose of 2000 mg/kg, and shows immunomodulatory potential through the presence of anti-inflammatory compounds. Furthermore, provides a foundation for future research on the potential medicinal uses of this extract.

Keywords: *Sapium ellipticum*, toxicity, stem bark, immunomodulation, cytokines

Introduction

Medicinal plants have been advocated as alternative drugs for immunomodulation activities. *Sapium ellipticum* (Hochst.) Pax is a plant species in the family Euphorbiaceae (Ighodaro & Akinloye, 2017). This plant species is distributed in different areas of the world such as America, China, Vietnam, India, Malaysia and Western, Central and East Africa (Ighodaro & Akinloye, 2017). *Sapium ellipticum* length ranges from 10 to 15 meters high, is edible and has medicinal use in the treatment of bacterial infections (Ighodaro & Akinloye, 2017). However, it is worth noting that the medicinal properties and uses of this plant may vary depending on the region and culture.

In Africa, *S. ellipticum* has been utilized for a variety of therapeutic purposes. For instance, in Nigeria, it has been used to manage fever, inflammation, and skin infections (Al Muqarrabun et

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al., 2014). In Cameroon, it has been employed as an antidiarrheal agent (Wansi et al., 2014; Merveille et al., 2017; Njouendou et al., 2018). In Kenya, the root decoction of *S. ellipticum* has been used for treating coughs (Ochwang'i et al., 2014), while in Burundi and Zambia, the stem bark decoctions have been used to treat fever, anaemia, elephantiasis, rheumatic problems, and guinea worms (Al Muqarrabun et al., 2014). Similarly, in Ethiopia, the leaves of the plant have been used to manage mumps (Bekele-Tesemma et al., 1993). In Tanzania, the powder of the stem has been applied to wounds, swellings, head and chest to reduce pain (Kisangau et al., 2009; Mpinda et al., 2018; Masalu et al., 2020). Moreover, the leaves of *S. ellipticum* have been used to alleviate abdominal swelling and treat eye diseases (Kisangau et al., 2009). Some communities in Tanzania even use the root decoction of this plant species to treat malaria (Wilfred et al., 2006; Moshi et al., 2010).

Numerous *in vitro* and *in vivo* studies have confirmed the traditional belief in the medicinal benefits of *S. ellipticum* (Evans et al., 2015; Mpinda et al., 2018; Njouendou et al., 2018; Masalu et al., 2020;). The plant's stem bark and leaf extracts have been reported to exhibit antimicrobial properties to a range of species causing bacterial and fungal infections (Ighodaro & Akinloye, 2017; Mpinda et al., 2018; Masalu et al., 2020). These have confirmed the traditional use of the plant in the management of opportunistic infections in Uganda and Tanzania (Kisangau et al., 2009; Ighodaro & Akinloye, 2017; Masalu et al., 2020).

On the other hand, *S. ellipticum* crude extracts have been reported to possess antioxidant activities, thus consumption of extracts from this plant could prevent cancer development, slow ageing and free the body from oxidative stress (Adesegun et al., 2008; Nana et al., 2013; Ochwang'i et al., 2014; Evans et al., 2015). Moreover, *S. ellipticum* has been found to have hypoglycemic effects, thus, showing potential in managing diabetes (Ighodaro & Akinloye, 2017). Methanolic crude extracts of *S. ellipticum* stem bark have been reported to exhibit a hepatoprotective effect against liver damage induced by carbon tetrachloride (CCl_4) in rats (Njouendou et al., 2018). Therefore, *S. ellipticum* is a plant species with a rich history and diverse medicinal uses, warranting the need for ongoing research to enhance scientific understanding, uncover new benefits, and ensure the safety of the communities utilizing its different products for medical purposes.

Evidence has shown that conventional immunomodulating drugs present significant challenges (Crane et al., 2005; Bascones-Martinez et al., 2014), prompting the exploration of alternative options such as plant-based products (Jantan et al., 2015; Nair et al., 2019). *S. ellipticum*, with its rich traditional history and promising *in vitro* evidence, holds the potential as a valuable candidate in this regard. However, there remain substantial scientific gaps, particularly regarding its in vivo toxicity and immunomodulatory activities.

Recently there has been a growing interest in investigating the immunomodulatory potential of medicinal plants (Gupta et al., 2016; Yin et al., 2019; Jeon et al., 2022). This interest has been promoted by studies demonstrating the capacity of plant-based polysaccharides to effectively modulate the immune system through various mechanisms (Yin et al., 2019; Jeon et al., 2022). Plant-based polysaccharides have been associated with the ability to activate immune cells, complements, and cytokines, presenting a unique mechanism for immune system control (Gupta et al., 2016; Yin et al., 2019; Jeon et al., 2022). Therefore, understanding the molecular mechanisms through which these phytochemicals activate or inhibit the expression of genes linked to the enhancement or suppression of immune cells, complements, and cytokines is a new and interesting area of study.

Therefore, this study aimed to bridge these knowledge gaps by conducting the *in vivo* evaluations of acute toxicity and immunomodulatory properties using mice model organisms. Furthermore, the study evaluated the phytochemical profiling to find out the compounds associated with the medicinal properties of the plant species. The results from the study provide

additional and new evidence on the medicinal potential of *S. ellipticum*, which may contribute to further research and clinical trials.

Materials and Methods

Sample collection

The stem bark of *S. ellipticum* was collected in October 2018 from the Bukoba rural district in the Kagera region. To ensure the accuracy of the plant identity, taxonomic identification was conducted by a knowledgeable plant taxonomist. The voucher specimen, with the identification number SN03, was then deposited at the Botany Department of the University of Dar es Salaam (UDSM).

Preparation of crude extract

To obtain aqueous crude extracts from the stem bark of *S. ellipticum*, a total of 500 g of ground plant material was weighed and soaked in 1500 mL of distilled water. The mixture was then left to soak for two days with shaking every 12 hours. After the soaking period, the mixture was decanted and filtered using cloth gauze, and then further filtered using Whatman filter paper number 4 with a pore size of 25 µm. This ensured that any solid particles present in the mixture were removed, leaving only the liquid extract. The filtered extracts were then concentrated using a freeze dryer (ScanVac Coolsafe, LaboGene™) at the Institute of Traditional Medicine located at the Muhimbili University of Health and Allied Sciences (MUHAS). The resulting extract was then stored at 4°C until further analysis was conducted.

Test animals

A total of 24 male Swiss albino mice, aged between 90 to 150 days were procured from the Zoology Department at the University of Dar es Salaam. Male Swiss albino mice were chosen over females to minimize random variations attributed to oestrous or menstrual cycles, which can lead to hormonal fluctuations and introduce additional variables that could complicate the study and necessitate a larger number of research animals. The mice were acclimatized to the laboratory conditions for seven days before the commencement of the experiments. During this period, they were housed in clean, well-ventilated cages, with a 12-hour light and dark cycle. They were also provided with standard rodent pellet feed and water. The mice were monitored daily to ensure that they remained healthy and free from any infections or diseases.

Toxicity evaluation

The acute toxicity of *S. ellipticum* stem bark was evaluated by the Organization for Economic Co-operation and Development (OECD) guideline number 425 for acute toxicity (Organisation for Economic Co-operation and Development, 2002). A total of 12 male mice were used for the study, weighing between 20 g to 35 g, and were divided into three groups to test different doses of the extract: 123 mg/kg, 300 mg/kg, and 2000 mg/kg, with the fourth administered distilled water served as a negative control. The chosen concentrations of the extracts were based on the antibacterial minimum inhibitory concentration of the drug (123 mg/kg) and the recommended concentration for acute toxicity by OECD (2002), *Test no. 423* (300, and 2000 mg/kg). Each group was restricted to three animals, and the experimental conditions were carefully refined to minimize suffering, and to advocate the principles of animal welfare, particularly the three R's (Replacement, Reduction, and Refinement).

The mice fasted for three hours but had free access to water before the extract was administered via oral gavage, based on their body weight. The behaviour (writhing, grooming, convulsion, and alertness), body weight, and rectal temperature of the mice were closely monitored. They were starved for one hour after administration and then given food, after which

their behaviour was observed for four hours and continuously monitored for fourteen days. After the completion of the study, the mice were humanely euthanized, and blood samples were collected using cardiac puncture with a sterile needle and a 1 ml insulin syringe. The blood was stored in tubes containing ethylenediaminetetraacetic acid (EDTA) for biochemical analysis, including alanine transaminase (ALT), aspartate transaminase (AST), total and conjugated bilirubin, and haematology tests, such as red blood cell count (RBC), haemoglobin (HB), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, white blood cell count (WBC), lymphocytes, neutrophils, basophils, and monocytes.

Immunomodulation assay

Nine male mice weighing between 25 g to 40 g were utilized to evaluate the immunomodulation activity. The mice were distributed into three groups, with each group consisting of three mice. To induce inflammation, each mouse in each group was intraperitoneally challenged with LPS of 1 mg/kg. After three hours, the treatment group was administered with 2000 mg/kg of extract, while the positive control group was given 1 mg/kg of ibuprofen. The negative control group, on the other hand, received only LPS and water, without any extract or ibuprofen treatment. The mice were monitored for four hours following the administration of extract or ibuprofen. After 24 hours, the mice were humanely euthanized, and 200µl of whole blood was taken for RNA extraction.

RNA extraction

RNA was extracted from whole blood using Quick-RNA MiniPrep Plus (Catalog Nos. R1057 & R1058) (https://zymoresearch.eu/products/quick-rna-miniprep-plus-kit) by following the manufacturer's instructions. First, 200 µL of 2X concentrated DNA/RNA Shield™ was added to a 200 µL frozen blood sample and thoroughly combined. Then, 400 µL of 8 U/µL Proteinase K was added to the reagent/blood mixture and well incorporated. The mixture was then incubated at 30 °C for 30 minutes. Isopropanol (408 µL) was added to the mixture in an equal proportion and vortexed. Next, the mixture was transferred to a Spin-Away™ Filter in a collection tube and centrifuged to eliminate genomic DNA. The flow-through was discarded, and 1 mL of 95% ethanol was added to the filter and stirred thoroughly. The mixture was then transferred to a Zymo-SpinTM III CG Column in a collection tube and spun at 10000 RCF for 30 seconds using a Hettrich Zentrifugen MIKRO 220 centrifuge machine. The flow-through was discarded, and the column was washed with 400 µL RNA wash buffer, centrifuged, and the flow-through was discarded again.

To remove any residual DNA, a mixture of 5 µL DNase I (1 U/µL) and 75 µL DNA digestion buffer was added directly to the column matrix and the column was incubated at 30 °C for 15 minutes. Then, 400 µL RNA prep buffer was added, the column was centrifuged at 10000 RCF for 30 seconds, and the flow-through was discarded. 700 µL RNA wash buffer was added to the column, followed by centrifugation, and the flow-through was discarded. Another 400 µL RNA wash buffer was added, followed by centrifugation for 2 minutes to ensure complete removal of the wash buffer. The column was then carefully transferred to an RNase-free tube, and 100 µL DNase/RNase-Free Water was added directly to the column matrix and centrifuged. The eluted RNA was immediately stored at -70 °C.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) Assay

To determine cytokine gene expression, the Luna® Universal One-Step RT-qPCR Kit (NEB #E3005S/L/X/E) (https://www.neb-online.de/literatur/pdf/NEB_Luna_qPCR.pdf) was utilized following the manufacturer's protocol with modifications. Specifically, 5 µL of Luna Universal

One-Step Reaction Mix (1X) was mixed with 0.5 µL of Luna WarmStart RT Enzyme Mix (1X), 0.4 µL of each forward and reverse primers at a concentration of 0.4 µM, and 2 µL of 1 µg template RNA (prepared sample). The total volume was adjusted to 10 µL by adding 1.7 µL of nuclease-free water. RT-qPCR was conducted using an Applied Biosystems 7500 Fast Instrument, with operating conditions detailed in Table 1. The primers used for the targeted cytokines are presented in Table 2.

Table 1: RT qPCR reaction condition of the assay

Cycle Step	Temperature	Time	Cycles
Reverse Transcription	55°C	10 minutes	1
Initial Denaturation	95°C	1 minute	1
Denaturation	95°C	10 seconds	40-45 s for extension and denaturation
Extension	60°C	60 seconds	

Table 2: Primer of cytokines, IL – 1β, IL – 6, IL – 1Ra and IL – 10

Cytokine	Primer 5' – 3'	Sequence	Accession number
IL – 1β	Forward	CAC CTC TCA AGC AGA GCA CAG	M98820
	Reverse	GGG TTC CAT GGT GAA GTC AAC	NW_047658
IL – 6	Forward	TCCTACCCCAACTTCCAATGCTC	E02522
	Reverse	TTGGATGGTCTTGGTCCTTAGCC	M26745
IL – 1Ra	Forward	AAGACCTTCTACCTGAGGAACAACC	C M63101
	Reverse	GCCCAAGAACACATTCCGAAAGTC	NW_047651
IL – 10	Forward	CGGGAAGACAATAACTGCACCC	BC120612
	Reverse	CG GTT AGC AGT ATG TTG TCC AGC	BC137844

LC-MS/MS Profiling of Crude Extracts

Liquid chromatography Mass spectrometry analysis was conducted using Thermo Scientific's Hypersil GOLD Q Mass Spectrometer (X. Wang et al., 2014). The samples were separated using the UltiMate 3000 RSLCnano System and injected in a volume of 10 L into a C18 column (LC column 100 2.1 mm) containing 1.9 mm-sized particles. Chromatographic separation was carried out at a flow rate of 0.3 mL/min using a gradient elution program. The program started with 95% eluent A (water with 0.1 per cent formic acid, v/v) and gradually changed to 5% eluent B (100% acetonitrile acidified with 0.1% formic acid (v/v)) and back over 30 minutes. The exact gradient parameters were 95% eluent A/5% B for 2 min, gradually changing to 95 % A and 5 % B over 13 min, 0 % A/100 % B for 10 min, then returning to 95 % eluent A/5 % B over 5 min.

Mass spectrometry measurements were performed using the Q Exactive and Orbitrap (Thermo Fisher). The system operating parameters were; sheath gas flow rate of 48 arbitrary units, auxiliary gas flow rate of 11 arbitrary units, HESI voltage of 4.0 kV, capillary voltage of 3.5 kV, and capillary temperature of 320 °C. Samples were introduced into the MS through electrospray ionization, and the acquired spectra were scanned with a resolution of 140000 spanning a mass/charge number range of 150–2000 m/z. Data processing and acquisition were done using XCalibur (Version 4.1.31.9).

Statistical Analysis

The results were expressed as the mean value ± standard deviation (SD) of three independent measurements. To evaluate the difference between the controls and extract groups, a one-way analysis of variance (ANOVA) was performed. A p-value less than 0.05 was considered statistically significant for determining the significance of the results.

Results

Behavioural and body weight

The behavioural changes including writhing, grooming, convulsion, and alertness were observed in mice after administering different doses of *S. ellipticum* extract, which were 123 mg/kg, 300 mg/kg, and 2000 mg/kg. The results showed that there were no significant alterations in the behaviour of the mice at any of the administered doses. This indicates that the extract did not induce any observable changes in the behaviour of the mice at the tested doses, suggesting that it is safe and well-tolerated.

Hematological Toxicity

To determine the safety of the crude extracts, haematological analysis results obtained from mice fed with the extracts were compared to those obtained from mice fed with water. The results were also compared to reference ranges established by Serfilippi et al., (2003), as presented in Table 3. The findings revealed that mice fed with extracts at doses of 123 mg/kg and 300 mg/kg exhibited low neutrophil counts, which were within acceptable limits at a dose of 2000 mg/kg. MCHC levels were below the reference range, but the difference was not statistically significant ($p = 0.7744$).

Overall, haematological parameters including RBC, haemoglobin, hematocrit, MCV, MCH, platelet count, WCB, lymphocytes, and monocytes were within the normal range. Statistical analysis using One Way ANOVA showed no significant difference between the experimental group and the control group at 123 mg/kg, 300 mg/kg, and 2000 mg/kg ($p = 0.7053$, 0.9466, and 0.9841, respectively).

Table 3: Hematology parameter of Swiss albino at different doses

Plant crude Extract	123 (mg/kg)	300 (mg/kg)	2000 (mg/kg)	Control	Reference (Serfilippi et al., 2003).
RBC(10^{12}/L)	8.54±0.15	9.56±0.08	9.03±0.18	9.53±0.3	6.66-11.18
Haemoglobin (g/dL)	12.95±0.55	13.95±0.25	13.33±0.55	13.97±0.34	11.0-18.3
Hematocrit %	40.5±1.5	47±0	44.33±2.5	44±1.2	33.1–48.8
MCV(fl)	47.5±0.9	49.15±0.45	49.3±1.9	46.83±0.33	41.2-51.4
MCH (pg)	15.15±0.35	14.6±0.4	14.77±0.35	14.67±0.25	14.1-17.8
MCHC (g/dl)	31.9±0.2	29.7±0.5	29.97±0.55	31.3±0.36	32.2-36.8
Platelet Count (K/µL)	859±148	892.5±552	1000.3±60.5	1336.33±163	784–1808
WBC (K/µL)	3.68±0.97	4.74±1.2	10.75±1.4	8.26±3.4	2.69-18.33
Neutrophils (x 10^9/l)	0.17±0.095	0.23±0.06	4.98±0.86	0.83±1.6	0.58-8.80
Lymphocytes (x 10^9/l)	2.66±0.4	3±0.67	4.89±0.23	4.34±2.02	1.83–15.58
Monocytes (x 10^9/l)	0.085±0.04	0.54±0.25	0.70±0.29	0.18±0.1	0–0.82
Eosinophil (x 10^9/l)	0±0	0±0	0.11±0.03	0.23±0.3	0–0.89
Basophils (x 10^9/l)	0.77±0.4	0.97±0.32	0.06±0.03	1.25±0.69	0–0.11

Liver function indices of plant crude extracts

Table 4 displays the liver function indices of mice that were administered with crude extract at doses of 123 mg/kg, 300 mg/kg, and 2000 mg/kg. Upon performing statistical analysis using one-way ANOVA, no statistically significant difference was found between the experimental group and the control group ($p = 0.9977$).

Table 4: Biochemistry parameters of mice at different concentrations

Biochemistry parameters	123 (mg/kg)	300 (mg/kg)	2000 (mg/kg)	Control (H_2O)	Reference (Serfilippi et al. 2003)
Total bilirubin (µmol/L)	10.65±1.25	1±0	30±5	4.5±1.7	3.42-25.65
Conjugated bilirubin (µmol/L)	4.3±1.9	0.55±0.15	12.4±5.1	0	1.7-22.23
Aspartate aminotransferase (1U/L)	180.5±28.5	262.5±0.5	220±40	294±48.4	35–185
Alanine aminotransferase (1U/L)	25.5± 2.5	56.5±6.5	38.5±8.5	66±20.6	19-166

Immunomodulation activity of the crude extracts

Table 5 shows the RT-qPCR cycles of expression of genes for anti-inflammatory cytokines (IL 10 and IL-1RA) for different treatments. The results indicate that treatment with *S. ellipticum* extract led to higher expression levels of both IL-10 (31.02±0.09 cycles) and IL-1RA (36.15±1.95 cycles) compared to the LPS (negative control) and ibuprofen (positive control) treatment. This suggests that *S. ellipticum* extract may have an immunomodulatory effect by increasing the expression of anti-inflammatory cytokines.

Table 5: RT-qPCR cycles of expression of the gene for anti-inflammatory cytokines

Extracts	IL 10	IL-1RA
	RT-PCR cycles	
S. ellipticum	31.02±0.09	36.15±1.95
LPS (-Ve)	30.83±0.64	38.94±1.32
Ibuprofen(+Ve)	30.38±0.51	37.62±1.53

Table 6 shows the RT-qPCR cycles of expression of genes for pro-inflammatory cytokines (IL 6 and IL-1β) for different treatments. The results indicate that treatment with *S. ellipticum* extract resulted in higher expression levels of IL-6 (37.12±3.84) compared to the LPS (-VE) control (34.17±1.44 cycles) and ibuprofen (+VE) treatment (38.55±3.77 cycles). However, the expression of

IL-1β was lower in the *S. ellipticum* extract treatment (23.74±2.61 cycles) compared to the LPS (-VE) control (26.91±2.86 cycles) and ibuprofen (+VE) treatment (27.89±1.61cycles). These results suggest that *S. ellipticum* extract may have a dual effect on the immune system, with both anti-inflammatory and pro-inflammatory effects.

Table 6: RT-qPCR cycles of expression of the gene for pro-inflammatory cytokines

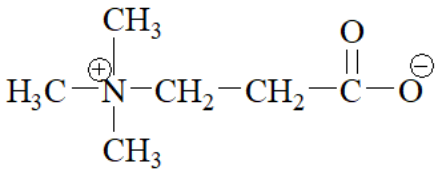
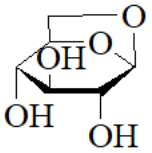
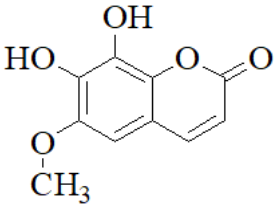
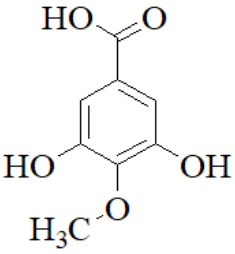
Extracts	**IL 6**	**IL 1β**
	RT-PCR cycles	
S. ellipticum	37.12±3.84	23.74±2.61
LPS (-Ve)	34.17±1.44	26.91±2.86
Ibuprofen(+Ve)	38.55±3.77	27.89±1.61

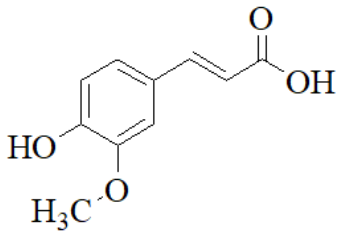
Phytochemical Profile

LC-MS profile of Aqueous Crude Extract

Five (5) phytochemical compounds identified from *S. ellipticum* crude extract are presented in Table 7. The compounds identified are known to have nutritive and medicinal values.

Table 7: LC-MS/MS profile of Aqueous Crude Extract *S. ellipticum*

S/N	Compound name	Retention Time	Chemical structure
1	L-Carnitine	**2.67**	
2	1,6-anhydro-β-D-Glucopyranose	**4.03**	
3	Fratexin	**10.29**	
4	5-Hydroxyisovanillic acid	**18.67**	

5	Trans ferulic acid	**0.68**	

Discussion

Acute Toxicity

Haematology analysis

According to the results, there were variations in the blood parameters of the test and control groups. The low neutrophil counts detected in the experimental groups may be attributed to the effects of the extract at lower doses, which could either suppress cell differentiation processes or act directly on cells, affecting the innate immune response in the mice. Neutrophils play a crucial role in the innate immune system. The findings of the present study were consistent with those of Etame et al., (2017), who used a dose of 200 mg/kg, and observed that neutrophil counts returned to normal upon increasing the extract concentration.

In the current investigation, the mean cell haemoglobin concentrations (MCHC) and basophil levels of all the experimental groups deviated from the reference range. Although the MCHC levels were below the reference range, the low levels could be related to dietary factors such as low iron levels in the mice's food (Ventrella et al., 2016). However, the effects of plant crude extracts on erythropoiesis or polycythemia could only be established if other metrics such as RBC and hematocrit deviated from the reference range.

Basophils are white blood cells that respond to allergens and other antigenic stimuli by producing significant amounts of IL-4 (Rhiouani et al., 2008). Higher basophil counts may indicate the presence of dietary allergens, while low basophil levels suggest the absence of allergens in the diet. In the current study, all the mice used in the experiments had higher basophil levels, suggesting that the food may have contained unidentified allergens (Rhiouani et al., 2008).

The haematological analysis results showed that RBC, haemoglobin, hematocrit, MCV, MCH, platelet count, WCB, lymphocytes, and monocytes were generally within the normal range for the mice fed with crude extract concentrations equivalent to 123, 300, and 2000 mg/kg compared to the control group and reference levels. This indicates that the extracts did not have any toxic effects on the blood parameters, similar to the findings reported by Tom et al., (2018), who observed no changes in blood parameters after toxicity evaluation of *Harungana madagascariensis* at a dose of 600 mg/kg.

Biochemical analysis

When assessing the safety of extracts in animal models such as mice, variations in the liver's biochemical characteristics serve as crucial risk assessment tools (Shittu et al., 2020). Serum aspartate transaminase (AST) and alanine transaminase (ALT) enzymes are clinically significant markers used to identify liver injury, whether inflammation or necrosis. ALT is more concentrated in the liver, and an increase in its serum levels beyond the normal range indicates liver tissue injury. In contrast, aspartate transaminase is less selective than ALT as a biomarker of liver impairment since it is also present in other organs' tissues, such as the kidney, heart, and muscles. However, the higher serum levels of these two enzymes are a sign of liver tissue injury and altered cell membrane permeability (Ramaiah, 2007).

In the current investigation, mice administered with crude extract concentrations equal to 123 mg/kg, 300 mg/kg, and 2000 mg/kg had higher levels of AST than ALT. Mice with elevated serum AST concentrations compared to ALT levels may have had tissue damage in organs other than the liver since ALT levels in the serum are usually higher than AST levels, indicating liver

tissue damage (Ajibade, 2017). This indicates that the liver was not affected by the aqueous stem bark crude extracts of *S. ellipticum* in the current study when doses up to 2000 mg/kg were administered.

According to the results, testing for bilirubin levels in all the mice showed no liver injury. This finding provides more evidence in support of the transaminase test results (ALT and AST). A normal bilirubin level indicates that the liver is functioning correctly by eliminating bilirubin from the body without any hepatic damage (Abou Seif, 2016). Therefore, the current study's results justify the traditional use of *S. ellipticum* stem bark aqueous crude extracts.

Immunomodulation activity of the crude extracts

The anti-inflammatory cytokine IL-10 helps regulate the immune system during infection to minimize harm to the host (Wu et al., 2022). However, downregulation of IL-10 during infection can worsen the condition, as observed in mice treated with an aqueous crude extract of *S. ellipticum,* indicating possible pro-inflammatory activity (Sasaki et al., 2000). On the other hand, interleukin-1 receptor antagonists (IL-1Ra) can prevent prolonged inflammation that may damage the body by antagonizing IL-1α and IL-1β (Yazdi & Ghoreschi, 2016). In the present study, *S. ellipticum* showed relatively strong upregulation of the IL-1Ra gene, which inhibited excessive production of cytokines IL-1α and IL-1β, demonstrating anti-inflammatory properties (T. Wang & He, 2018). Similar results were observed in a study on orange peel extract and *Phleobodium decumanum* (Punzón et al., 2003).

On the other hand, IL-6, a pro-inflammatory cytokine associated with the cytokine storm during infection, has been linked to various inflammatory disorders (Hirano, 2021). In the current study, *S. ellipticum's* aqueous crude extract downregulated cytokine (injected with LPS but no drug provided) when compared to the control group, demonstrating anti-inflammatory properties (de Araújo Moreira et al., 2020). Similar results were reported in studies on ethanolic bark extract of *Terminalia argentea* and *Phleobodium decumanum* (Punzón et al., 2003).

Interleukin-1β (IL-1β) is a key cytokine involved in the stimulation of pro-inflammatory signalling pathways in the brain and peripheral tissues (Goto et al., 2016). The current study demonstrated that *S. ellipticum's* extract had pro-inflammatory activity by upregulating IL-1β when compared to the negative control group (consisting of only LPS-infected cells). Similar results were reported in a study on loquat leaf extract (*Eriobotrya japonica)* by Hoseinifar et al., (2018).

Phytochemical Profile

L-carnitine is an amino acid derivative that plays a critical role in transporting fatty acids into the mitochondria for energy production (Matera et al., 2003). In plants, L-carnitine biosynthesis occurs through the modification of lysine, which is converted into trimethyllysine and then into L-carnitine with the help of enzymes such as trimethyllysine dioxygenase (Jacques et al., 2018; Seline & Johein, 2007). L-carnitine has several potential medicinal uses, such as improving lipid metabolism, reducing inflammation, and boosting protein synthesis (Kraemer et al., 2008). It may also affect erythropoietin (rHuEPO) requirements, decrease pro-inflammatory cytokines, and increase nitrogen balance (Golper et al., 2003). In the context of the present study, the presence of L-carnitine in the stem bark crude extract could be linked to the observed anti-inflammatory properties.

1,6-Anhydro-β-D-Glucopyranose is a rare sugar that was revealed to be present in the stem bark crude extract o*f S. ellipticum* in the present study. It is derived from glucose through the action of enzymes such as 1,6-anhydro-beta-D-glucopyranose synthase. In plants, it is involved in the biosynthesis of cell wall components such as hemicellulose (Liu et al., 2008). The potential medicinal uses of 1,6-anhydro-β-D-Glucopyranose are not well-known, but it has been reported to have some antibacterial properties (Procter et al., 1990). Its presence in the stem bark crude extract of *S. ellipticum* may contribute to its reported antimicrobial activity.

Fraxetin is a flavonoid found in several plant species, including *S. ellipticum*. It is biosynthesized from the precursor molecule naringenin through the action of enzymes such as flavone synthase II (Wang et al., 2014). Fraxetin has several potential medicinal uses, such as antioxidant, antimicrobial, anti-inflammatory, and anti-fibrotic properties (H. Wang et al., 2014). It has also been shown to have the ability to suppress apoptosis triggered by interleukin 1β. In the context of the present study, the presence of fraxetin in the stem bark crude extract can be attributed to its reported antimicrobial activity and anti-inflammatory and immunomodulatory properties.

5-Hydroxyisovanillic acid is a phenolic acid found in the stem bark crude extract of *S. ellipticum*. It is biosynthesized from tyrosine through the action of enzymes such as tyrosine decarboxylase and tyrosine hydroxylase (Khammar & Djeddi, 2012). The potential medicinal uses of 5-Hydroxyisovanillic acid are not well-known, but it has been reported to have some antioxidant properties. Its presence in the stem bark crude extract of *S. ellipticum* could potentially contribute to its reported antioxidant activity.

Trans-ferulic acid is a phenolic acid found in several plant species, including *S. ellipticum*. It is biosynthesized from phenylalanine through the action of enzymes such as phenylalanine ammonia-lyase (Wang et al., 2017). Trans-ferulic acid has several potential medicinal uses, such as anti-inflammatory, antioxidant, and anticancer properties. It has also been shown to have beneficial effects on oxidative stress, inflammation, vascular endothelial damage, fibrosis, apoptosis, and platelet aggregation (Wang et al., 2017). Its presence in the stem bark crude extract of *S. ellipticum* can be attributed to the revealed anti-inflammatory and immunomodulatory properties.

Conclusion

This study evaluated the safety and potential medicinal value of the aqueous extract of *Sapium ellipticum* stem bark. The results demonstrated that the extract was not toxic at doses up to 2000 mg/kg, suggesting a high safety profile. The immunomodulatory activity of the extract was also investigated and showed significant downregulation of the pro-inflammatory cytokines IL-6 and IL-10, as well as upregulation of the anti-inflammatory cytokines IL-1Ra and IL-1β, which may have therapeutic implications in the treatment of inflammatory diseases. Additionally, the phytochemical profiling of the extract using LC-MS/MS revealed the presence of compounds with anti-inflammatory properties, such as Fraxetin and L-carnitine. The upregulation of IL-1β, a pro-inflammatory cytokine, in the extract, suggests that it may have potential as a treatment for certain conditions where increased inflammation is needed. Overall, the results of this study provide important information on the safety and potential therapeutic effects of *S. ellipticum* stem bark extract, and further research is warranted to explore its full potential.

Authors' contributions

The authors of this study contributed equally to the conception, design, writing, review and approval of the manuscript.

Conflict of Interest

No conflict of interest

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References

Abou Seif, H. S. (2016). Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. *Beni-Suef University Journal of Basic and Applied Sciences*, *5*(2), 134–146. https://doi.org/10.1016/j.bjbas.2016.03.004

Adesegun, S. A., Elechi, N. A., & Coker, H. A. B. (2008). Antioxidant activities of methanolic extract of Sapium ellipticum. *Pakistan Journal of Biological Sciences*, *11*(3), 453–457. https://doi.org/10.3923/pjbs.2008.453.457

Ajibade, S. (2017). *Harungana madagascariensis Lam ex poir (Hypericaceae) Fruits Oil Extract: Photochemistry and Acute Toxicity Evaluation*.(8),3341-3346. DOI: 10.13040/IJPSR.0975-8232.8(8).3341-46

Al Muqarrabun, L. M. R., Ahmat, N., & Aris, S. R. S. (2014). A review of the medicinal uses, phytochemistry and pharmacology of the genus Sapium. *Journal of Ethnopharmacology*, *155*(1), 9–20. https://doi.org/10.1016/j.jep.2014.05.028

Bascones-Martinez, A., Mattila, R., Gomez-Font, R., & Meurman, J. H. (2014). Immunomodulatory drugs: Oral and systemic adverse effects. *Medicina oral, patologia oral y cirugia bucal*, *19*(1), e24. https://doi.org/10.4317%2Fmedoral.19087

Bekele-Tesemma, A., Birnie, A., & Tengnas, B. (1993). Useful trees and shrubs for Ethiopia. Identification, Propagation and Management of Agricultural and Pastoral Communities. Regional Soil Conservation Unit (RSCU), Swedish International Development Authority, Nairobi, Kenya.

Crane, E., & List, A. (2005). Immunomodulatory drugs. *Cancer investigation*, *23*(7), 625-634. https://doi.org/10.1080/07357900500283101.

de Araújo Moreira, M. dos R., Sales-Campos, H., Fontanari, C., Galvão Meireles, A. F., Borges Prado, M. K., Zoccal, K. F., Sorgi, C. A., Tefé da Silva, C., Groppo, M., & Faccioli, L. H. (2020). The ethanolic extract of Terminalia argentea Mart. & Zucc. Bark reduces inflammation through the modulation of cytokines and nitric oxide mediated by the downregulation of NF-κB. *Journal of Ethnopharmacology*, *261*, 113150. https://doi.org/10.1016/j.jep.2020.113150

Etame, R. M. E., Mouokeu, R. S., Ngane, R. A. N., Assam, J. P. A., Masoohe, A. M., Tientcheu, R., Hopogap, M. L., & Etoa, F. X. (2017). Acute and sub-acute toxicity of Harungana madagascariensis LAM (Hypericaceae) stem bark methanol extract. *Journal of Applied Pharmaceutical Science*, *7*(3), 160–167. http://dx.doi.org/10.7324/JAPS.2017.70326

Evans, E., Witabouna, K., Honora, T., & Adama, B. (2015). Iron Reducing and Radical Scavenging Activities of 13 Medicinal Plants From Côte d'Ivoire. *Pharmacognosy Journal*, *7*(5), 266–270. https://doi.org/10.5530/pj.2015.5.2

Golper, T. A., Goral, S., Becker, B. N., & Langman, C. B. (2003). L-carnitine treatment of anaemia. *American Journal of Kidney Diseases*, *41*, S27–S34. https://doi.org/10.1016/S0272-6386(03)00114-8

Goto, T., Naknukool, S., Yoshitake, R., Hanafusa, Y., Tokiwa, S., Li, Y., Sakamoto, T., Nitta, T., Kim, M., Takahashi, N., Yu, R., Daiyasu, H., Seno, S., Matsuda, H., & Kawada, T. (2016). Proinflammatory cytokine interleukin-1β suppresses cold-induced thermogenesis in adipocytes. *Cytokine*, *77*, 107–114. https://doi.org/10.1016/j.cyto.2015.11.001

Gupta, V. K., Kumar, M. M., Bisht, D., & Kaushik, A. (2017). Plants in our combating strategies against Mycobacterium tuberculosis: progress made and obstacles met. *Pharmaceutical biology*, *55*(1), 1536-1544. https://doi.org/10.1080/13880209.2017.1309440

He, Q., Zhang, L., Li, T., Li, C., Song, H., & Fan, P. (2021). Genus Sapium (Euphorbiaceae): A review on traditional uses, phytochemistry, and pharmacology. *Journal of Ethnopharmacology*, *277*, 114206. https://doi.org/10.1016/j.jep.2021.114206

Hirano, T. (2021). IL-6 in inflammation, autoimmunity and cancer. *International Immunology*, *33*(3), 127–148. https://doi.org/10.1093/intimm/dxaa078

Hoseinifar, S. H., Zou, H. K., Van Doan, H., Kolangi Miandare, H., & Hoseini, S. M. (2018). Evaluation of some intestinal cytokines genes expression and serum innate immune parameters in common carp (Cyprinus carpio) fed dietary loquat (Eriobotrya japonica) leaf extract. *Aquaculture Research*, *49*(1), 120–127. https://doi.org/10.1111/are.13440

Ighodaro, O. M., & Akinloye, O. A. (2017). The anti-diabetic potential of Sapium ellipticum (Hochst) Pax leaf extract in Streptozotocin(STZ)-induced diabetic Wistar rats. *BMC Complementary and Alternative Medicine*, *17*(1), Article 1. https://doi.org/10.1186/s12906-017-2013-8

Jacques, F., Rippa, S., & Perrin, Y. (2018). Physiology of L-carnitine in plants in light of the knowledge of animals and microorganisms. *Plant Science*, *274*, 432–440. https://doi.org/10.1016/j.plantsci.2018.06.020

Jantan, I., Ahmad, W., & Bukhari, S. N. A. (2015). Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. *Frontiers in plant science*, *6*, 655. https://doi.org/10.3389/fpls.2015.00655

Jeon, H., Oh, S., Kum, E., Seo, S., Park, Y., & Kim, G. (2022). Immunomodulatory effects of an aqueous extract of black radish on mouse macrophages via the TLR2/4-mediated signalling pathway. *Pharmaceuticals*, *15*(11), 1376. https://doi.org/10.3390/ph15111376

Khammar, A., & Djeddi, S. (2012). *Pharmacological and Biological Properties of some Centaurea Species*. *European Journal of Scientific Research*. 84(3), 398-416.

Kisangau, D. P., Hosea, K. M., Lyaruu, H. V. M., Joseph, C. C., Mbwambo, Z. H., Masimba, P. J., Gwandu, C. B., Bruno, L. N., Devkota, K. P., & Sewald, N. (2009). Screening of traditionally used Tanzanian medicinal plants for antifungal activity. *Pharmaceutical Biology*, *47*(8), 708–716. https://doi.org/10.1080/13880200902933039

Kraemer, W. J., Volek, J. S., & Dunn-Lewis, C. (2008). L-Carnitine Supplementation: Influence upon Physiological Function. *Current Sports Medicine Reports*, *7*(4), 218. https://doi.org/10.1249/JSR.0b013e318180735c

Liu, H., Cheng, S., Liu, J., Du, Y., Bai, Z., & Du, Y. (2008). Synthesis of Pentasaccharide and Heptasaccharide Derivatives and Their Effects on Plant Growth. *Journal of Agricultural and Food Chemistry*, *56*(14), 5634–5638. https://doi.org/10.1021/jf800326r

Masalu, R., Ngassa, S., Kinunda, G., & Mpinda, C. (2020). Antibacterial and Anti-HIV-1 Reverse Transcriptase Activities of Selected Medicinal Plants and Their Synthesized Zinc Oxide Nanoparticles. *Tanzania Journal of Science*, *46*(3), Article 3. https://doi.org/10.4314/tjs.v46i3

Matera, M., Bellinghieri, G., Costantino, G., Santoro, D., Calvani, M., & Savica, V. (2003). History of L-carnitine: Implications for renal disease. *Journal of Renal Nutrition*, *13*(1), 2–14. https://doi.org/10.1053/jren.2003.50010

Merveille, T. O., Denis, Z., Abdel, N. J., Ursula, A. A., Adie, A., Smith, B. B., & Clement, A. (2017). Antimicrobial Activities of Some Medicinal Plants against Multiresistants Microorganisms Causing Urogenital Tract Infections in Cameroon. *Journal of Diseases and Medicinal Plants*, *3*(2), 33–41. doi: 10.11648/j.jdmp.20170302.12

Moshi, M. J., Otieno, D. F., Mbabazi, P. K., & Weisheit, A. (2010). Ethnomedicine of the Kagera Region, northwestern Tanzania. Part 2: The medicinal plants used in Katoro Ward, Bukoba district. *Journal of Ethnobiology and Ethnomedicine*, *6*(1), 1–5. DOI: https://doi.org/10.1186/1746-4269-8-14

Mpinda, C., Meyer, D., & Lyantagaye, S. (2018). In vitro antimicrobial activity of extracts of some plant species used in the management of HIV/AIDS in Tanzania. *Tanzania Journal of Science*, *44*(1), Article 1. https://doi.org/10.4314/tjs.v44i1

Nair, A., Chattopadhyay, D., & Saha, B. (2019). Plant-derived immunomodulators. In *New look to phytomedicine* (pp. 435-499). Academic Press. https://doi.org/10.1016/B978-0-12-814619-4.00018-5

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Ngaoundere, Cameroon, Ngassoum, M. B., & Department of Biomedical Sciences, Faculty of Science, University of Ngaoundere, P. O. Box 454 Ngaoundere, Cameroon. (2013). Phytochemical screening, antioxidant and antiplasmodial activities of extracts from Trichilia roka and Sapium ellipticum. *The Journal of Phytopharmacology*, *2*(4), 22–29. https://doi.org/10.31254/phyto.2013.2404

Njouendou, A. J., Assob, J. C. N., Tabouguia, M. O., Nde-Fon, P., Beng, V. P., Badami, S., Wanji, S., & Nkeng-Efouet, A. P. (2018). *Hepatoprotective activities of Autranella congolensis and Sapium elliptical against carbon tetrachloride-induced liver injuries in experimental rats.* Investigational Medicinal Chemistry and Pharmacology 2018 1(1):8.

Ochwang'i, D. O., Kimwele, C. N., Oduma, J. A., Gathumbi, P. K., Mbaria, J. M., & Kiama, S. G. (2014). Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. *Journal of Ethnopharmacology*, *151*(3), 1040–1055. https://doi.org/10.1016/j.jep.2013.11.051

Organisation for Economic Co-operation and Development. (2002). *Test no. 423: Acute oral toxicity-acute toxic class method*. OECD publishing.

Procter, G., Genin, D., & Challenger, S. (1990). 1,6-Anhydro-β-d-glucopyranose in organic synthesis: Preparation of a fragment for the synthesis of rosaramycin. *Carbohydrate Research*, *202*, 81–92. https://doi.org/10.1016/0008-6215(90)84072-3

Punzón, C., Alcaide, A., & Fresno, M. (2003). In vitro anti-inflammatory activity of Phlebodium decumanum. Modulation of tumour necrosis factor and soluble TNF receptors. *International Immunopharmacology*, *3*(9), 1293–1299. https://doi.org/10.1016/S1567-5769(03)00117-6

Ramaiah, S. K. (2007). A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food and Chemical Toxicology*, *45*(9), 1551–1557. https://doi.org/10.1016/j.fct.2007.06.007

Rhiouani, H., El-Hilaly, J., Israili, Z. H., & Lyoussi, B. (2008). Acute and sub-chronic toxicity of an aqueous extract of the leaves of Herniaria glabra in rodents. *Journal of Ethnopharmacology*, *118*(3), 378–386. https://doi.org/10.1016/j.jep.2008.05.009

Sasaki, H., Hou, L., Belani, A., Wang, C.-Y., Uchiyama, T., Müller, R., & Stashenko, P. (2000). IL-10, but not IL-4, suppresses infection-stimulated bone resorption in vivo. *The Journal of Immunology*, *165*(7), 3626–3630. https://doi.org/10.4049/jimmunol.165.7.3541

Seline, K.-G., & Johein, H. (2007). The determination of l-carnitine in several food samples. *Food Chemistry*, *105*(2), 793–804. https://doi.org/10.1016/j.foodchem.2007.01.058

Serfilippi, L. M., Pallman, D. R. S., & Russell, B. (2003). Serum clinical chemistry and hematology reference values in outbred stocks of albino mice from three commonly used vendors and two inbred strains of albino mice. *Contemporary Topics in Laboratory Animal Science*, *42*(3), 46–52.

Shittu, O., Opeyemi, O. A., Salawu, M. K., Ashiru, A. A., Medaiyese, S. A., Asogwa, N., Sheba, K. F., Kadir, R. A., Aremu, O. I., & Ajibaye, O. (2020). Alterations in histological, biochemical and hematological parameters in Plasmodium berghei NK-65 infected balb/c mice treated with Bridelia ferruginea stem bark extract. *Journal of Complementary and Integrative Medicine*, *18*(1), 93–105. https://doi.org/10.1515/jcim-2018-0219

Tom, E. N. L., Nankia, F. D., Nyunaï, N., Girard-Thernier, C., Demougeot, C., & Dimo, T. (2018). Myocardial potency of aqueous extract of Harungana madagascariensis stem bark against isoproterenol-induced myocardial damage in rats. *Univ J Pharm Res*, *3*(1), 17–24. https://doi.org/10.1186/s12917-017-0946-2

Ventrella, D., Dondi, F., Barone, F., Serafini, F., Elmi, A., Giunti, M., Romagnoli, N., Forni, M., & Bacci, M. L. (2016). The biomedical piglet: Establishing reference intervals for haematology and clinical chemistry parameters of two age groups with and without iron supplementation. *BMC Veterinary Research*, *13*(1), 1–8. https://doi.org/10.1186/s12917-017-0946-2

Wang, H., Zou, D., Xie, K., & Xie, M. (2014). Antibacterial mechanism of fraxetin against Staphylococcus aureus. *Molecular Medicine Reports*, *10*(5), 2341–2345. https://doi.org/10.3892/mmr.2014.2529

Wang, T., & He, C. (2018). Pro-inflammatory cytokines: The link between obesity and osteoarthritis. *Cytokine & Growth Factor Reviews*, *44*, 38–50.

Wang, X., Zhao, P., Liu, X., Chen, J., Xu, J., Chen, H., & Yan, X. (2014). Quantitative profiling method for phytohormones and betaines in algae by liquid chromatography-electrospray ionization tandem mass spectrometry. *Biomedical Chromatography*, *28*(2), 275–280. https://doi.org/10.1016/j.cytogfr.2018.10.002

Wang, Z., Xie, D., Gan, X., Zeng, S., Zhang, A., Yin, L., Song, B., Jin, L., & Hu, D. (2017). Synthesis, antiviral activity, and molecular docking study of trans-ferulic acid derivatives containing acyl hydrazone moiety. *Bioorganic & Medicinal Chemistry Letters*, *27*(17), 4096–4100. https://doi.org/10.1016/j.bmcl.2017.07.038

Wansi, S. L., Nguelefack-Mbuyo, E. P., Nchouwet, M. L., Miaffo, D., Nyadjeu, P., Wabo, J. P., Mbiantcha, M., NKeng-Efouet, P. A., Nguelefack, T. B., & Kamanyi, A. (2014). Antidiarrheal Activity of Aqueous Extract of the Stem Bark of Sapium Ellipticum (Euphorbiaceae). *Tropical Journal of Pharmaceutical Research*, *13*(6), Article 6. https://doi.org/10.4314/tjpr.v13i6.15

Wilfred, P., Madoffe, S. S., & Luoga, E. J. (2006). Indigenous plant uses and use values in Uluguru mountains, Morogoro, Tanzania. *Journal of East African Natural History*, *95*(2), 235–240. https://doi.org/10.2982/0012-8317(2006)95[235:IPUAUV]2.0.CO;2

Wu, Y., Hsing, C., Chiu, C., Huang, H., & Hsu, Y. (2022). Roles of IL-1 and IL-10 family cytokines in the progression of systemic lupus erythematosus: Friends or foes? *IUBMB Life*, *74*(2), 143–156. https://doi.org/10.1002/iub.2568

Yazdi, A. S., & Ghoreschi, K. (2016). The interleukin-1 family. *Regulation of Cytokine Gene Expression in Immunity and Diseases*, 21–29.

Yin, M., Zhang, Y., & Li, H. (2019). Advances in research on immunoregulation of macrophages by plant polysaccharides. *Frontiers in immunology*, *10*, 145. https://doi.org/10.3389/fimmu.2019.00145