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Original Research Article

Selected Preclinical Studies on *Picralima nitida* Stem Bark Extract

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Abstract

Purpose: Traditional medicine has utilized *Picralima nitida* (Apocynaceae) to treat a variety of illnesses, including microbial and pain-induced infections, diabetes, inflammation, and malaria.
Methods: The phytochemical screening, haematological, and histological studies were carried out using already established methods.
Results: Alkaloids, saponins, tannins, proteins, carbohydrates flavonoids, reducing sugars, and terpenoids detected in the extract. The histology of the organ treated by the stem bark extract showed no form of organ damage and toxic damage when treated with the plant extract and when compared to the control. Haematological studies of the effects of the stem bark of *P. nitida* extract on the red blood cell and it's components; hamacrite (HCT), haemoglobin (HGB) and red blood cell (RBC) at 200, 400 and 800 mg/kg showed slight increase in RBC showing that *P. nitida* stem bark extract can increase the ability of the blood to transport oxygen and hence, could be effective in treating certain diseases like anaemia. All the mice that received the plant extract experienced an increase in WBC counts. White blood cells are involved in battling illness and the extracts may stimulate their production. The result of the white blood cell demonstrates that the *P. nitida* extract significantly affected the white blood cells (WBC), lymphocyte (LYM), and granulocyte (GR) counts.
Conclusion: The study revealed that *Picralima nitida* could serve as an intoxicating source of haematinic agent and as such; further studies should be carried out on the isolation of the active agents responsible for this potency

Keywords: *Picralima nitida*, Phytochemical screening, Haematology, Histology

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INTRODUCTION

Medicinal plants are often regarded as the primary source of medicine across all races and cultures.[1] They have been known to be endless sources of therapeutic agents over time, serving not only as a food source but also as a remedy for many diseases.[1,2] Because of the increasing need for therapies for various ailments, plants' relevance can be connected to the presence of metabolites found in these plants, which assist in treating numbers of sicknesses.

Many therapeutic plants have been studied using a range of scientific methodologies and have been used for the management of some sicknesses.[3] These curative herbs are used in botanical medicines to enhance public health and therapeutic advantages, and they serve as building blocks for the development of useful drugs.[4,5] Plants contain constituents which may be harmful in some particular doses and our forefathers ingest these medicinal plants to cure diseases without understanding their potential harm.[6]As the use of therapeutic plants and their products expands, so also does the concern about the potential toxicity..

The study of blood and its constituents is known as hematology.[7,8,9] It covers research on blood, blood-forming organs, and the proteins involved in coagulation.[7]A haematological test can be used to analyse a range of blood-related maladies. It can be used to diagnose inflammation, anemia, infection, haemophilia, blood coagulopathies, leukemia, and the reaction to chemotherapy. A complete blood count (CAC) test measures numerous blood components and characteristics. It may include measurements of white blood cells (WBC), red blood cells (RBC), haematocrit (HCT), hemoglobin (Hb), platelets, and so on.[7]

Red blood cells, white blood cells, mean corpuscular volume, and mean corpuscular hemoglobin concentration are haematological components that are useful for assessing the toxicity of xenobiotics or exogenous ligands.[8]Haemoglobin is carried by red blood cells or erythrocytes, and combines with the oxygen contained in the blood to produce oxyhaemoglobin.[10,11] Red blood cells provide the "support" needed for the transportation of oxygen and carbon dioxide. Blood is an essential, unique circulatory tissue that is required to maintain homeostasis.[7]Most vertebrates' red blood cells contain hemoglobin, an oxygen-transport metalloprotein that contains iron.[12] In order to release energy for other bodily functions, hemoglobin must carry carbon dioxide out of the body and oxygen to the tissues for the oxidation of food that has been consumed.[12, 13]

When evaluating circulatory erythrocytes, hemoglobin, mean corpuscular hemoglobin, and packed cell volume are crucial indicators. They are also useful pointers of the bone marrow's capacity to form red blood cells, similar to that of mammals, and are important in the diagnosis of anemia.[14]A lesser red blood cell count, therefore, means that less oxygen would otherwise reach the tissues and less carbon dioxide is exhaled back into the lungs.[12,13]

The primary roles of white bloods cells include combating infections, protecting the body through phagocytosis against foreign organisms, and producing or facilitating the transport and distribution of antibodies during immune response. As a result,low-white-blood-cell-count animals are more susceptible to infection, while high-white-blood-cell-count animals can produce antibodies during phagocytosis, exhibit high levels of disease resistance, and enhance their capacity to adapt to local environmental and disease-prevalent conditions. [12,14,15,] Blood platelets play a role in blood coagulation. In the event of an injury, a low platelet concentration suggests that the coagulation (blood clotting) process will be protracted, potentially resulting in excessive blood loss.

The study of tissues and organs under a microscope through staining, sectioning, and microscopic examination is known as histology. Histology, also known as microscopic anatomy and histochemistry, allows one to see the structure of tissues as well as any noticeable alterations they may have experienced. It is used in scientific research, medical diagnostics, autopsies, and forensic investigations.

Picralima nitida is a member of the *apocynaceae* family. It is a glabrous tree or shrub of between 9 to 75 feet high. The terminal inflorescences are generally heavily constricted and contain white to yellow flowers. The ovary has 70 to 130 ovules, as opposed to *hunteria umbellata*, popularly known as Abere, which has just 1-30 ovules. The Yoruba call it Abere, and it's known as Osu in Edo. *P. nitida* stem bark is one among the plants used to produce the antimalarial medication at home. The stem bark is especially rich in alkaloids, the fruit, and seeds also contain some alkaloids. Medicinal inquiries showed that the natural extract or isolated chemicals from this plant had analgesic, anti-inflammatory, hypoglycemic, hypotensive, antiplasmodial, antibacterial, antiulcer, antioxidant, antimicrobial, antidiarrheal, antihelmintic, antiallergic, antiviral and anti-tumorigenic properties.[16,17,18] Traditional therapies use formulations from many different parts of the plant to treat and prevent malaria, abscesses,

hepatitis, diabetes, hypertension, pneumonia, and other chest-related conditions.[19]

Plants create phytochemicals, which are biologically active substances. They can be obtained from a diversity of sources such as whole grains, vegetables, fruits, herbs, and nuts, and over a thousand phytochemicals have been identified to date. Alkaloids, phenolic, tannins, phytosterols, isoprenoids, dietary fibers, saponins, and some polysaccharides are some of the most essential phytochemicals obtained from *P. nitida*.[20,21] Additionally, they influence transcription of the gene, improve immunity, boost gap junction communication, and give protection against prostate and lung cancer.[22]

Using mice, the study sought to examine specific preclinical activities on the methanol extract of *P. nitida* stem bark

MATERIALS AND METHODS

Collection of Sample

In January 2024, fresh stem bark of *Picralima nitida* were collected in Useh community Benin City, Nigeria. Prof. Akinnibosun, H. A. of the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, identified the plant with a voucher specimen number UBH-P424, deposited.

Preparation of Extract

P. nitida stem bark was allowed to air dry at room temperature, using a mechanical grinder. The sample was ground into powder using Wokocha and Okereke's method, [23] with a net weight of 250 g. The powdered stem bark was stored in a separate sealed air-tight container marked with the date of preparation and kept for subsequent investigation. 200 g of *P. nitida* dry stem powder was macerated using 1.5 L of methanol. The mixture was shaken for 72 hours before being filtered through a funnel and a filter paper. The percentage yield was computed and the extract was kept in a fridge at 4^{o}C until further analysis.

$$Percentage\ Yield\ (\%) = \frac{A}{B}$$

A= quantity of extract recovered from solvent
B= total quantity of plant material.

Phytochemical Screening

The phytochemicals were assessed using established methods by Thangaraj,[28] using acetone, ethyl acetate and water as the extract solvent.

Experimental Animals

Twelve (12) healthy Swiss mice of 9-12 weeks old and weighing between 18-20g were utilized in this study. They were acquired from the Animal House Unit of the Department of Pharmacology at the University of Benin's Faculty of Pharmacy in Benin City, Nigeria. The Swiss mice were kept in the Animal House of the Department of Biochemistry at the University of Benin in Benin City, in steel cages. The animals were given unrestricted access to food and water for fourteen (14) days prior to the start of the study. A natural light-dark cycle of 12 hours was maintained in the animal room, which was well-ventilated and maintained at a room temperature of 25 ± 1 0C and a relative humidity of 45–55%. Good hygiene was maintained by regular cleaning and changing of the bedding daily. All animals were handled following ethical guidelines on animal care and handling during the research study. The experiment followed the rules specified by the National Institute of Health's (NIH) Guide for the Care and Use of Laboratory Animals.[25,26]

Haematological and Histological Assessment Experimental protocol

The safety evaluation of the extract of *Picralimanitida*was carried out on Swiss mice by analyzing the histological and hematological parameters.Twelve mice in all were split into four groups at random, each consisting of three mice (n = 3). Group 4 was the control group. Group 4's animals were given only distilled water.

Group 1 Mice were given 200 mg/kg of *P. nitida* methanol extract and were given a regular diet.

Group 2 mice were given 400 mg/kg of the methanol extract of *P. nitida* and were fed with standard diet.

Group 3 mice were given 800 mg/kg of the methanol extract of*P.nitida* and were fed with standard diets for 14 days.

Ethics approval

The University of Benin's Faculty of Life Sciences' Ethical Review Board gave its approval to all experiment procedures (LS23026).

Collection of Blood and Organ samples

After 14 days, the mice in their individual groups were killed, and blood samples were extracted from the abdominal aorta vein while the mice were mildly sedated with chloroform. The kidney and liver were surgically removed and placed in a sample bottle for evaluation after the blood was placed into ethylene diamine tetra acetic acid (EDTA) bottles and centrifuged for 15 minutes at 3,500 rpm. The serum was then collected and kept in the refrigerator for later use.

Histological Assessment

It was neutral buffered formalin that was used to fix the liver and kidney. After being completely dehydrated with 99.9% ethanol, 70% ethanol, and 96% ethanol, the attached organs were cleaned with

distilled water. Haematoxylin-eosin dye was used to stain the prepared 4µm sections. The Leica MC170 HD optical photomicroscope (Leica Biosystems, Germany) was used to view the stained organ at a magnification of x400.

Hematological Analysis

The human-automated hematology system analyzer's chamber was filled with EDTA bottles containing blood samples that had been diluted with an isotonic saline solution. Red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume, white blood cell count, platelet count, and differential white blood cell count were among the indicators that were examined.[26]

Statistical Analysis

Using one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test ($p < 0.05$), the results were analyzed and presented as Mean ± S.E.M. Data analysis was done using Graph Pad Prism version 9.

RESULT AND DISCUSSION

Percentage Yield of the Extract

The percentage yield of *P. nitida* stem bark extract was determined to be 7.605%.

Phytochemical Evaluation on the Stem Bark of *P. nitida*

Alkaloids, tannins, reducing sugars, protein, and carbohydrates were detected in *P. nitida* stem bark through qualitative phytochemical screening using ethyl acetate, acetone, methanol and water, for all solvents used, as indicated in Table 1. The polarity differences could be the reason for the varied phytochemicals in the different solvents.[16,17] Biological actions are primarily due to these bioactive compounds present in the plant.[27,28]Alkaloids have been known over the years for their anti-malarial activities,[16,18] and cytotoxicity. They have also been used for antimicrobial, antidiarrheal and anthelmintic.[16] A previous study reported the activity of saponin against *Escherichia coli*, *Staphylococcus aureus*, *Neisseria gonorrhea*, and *streptococcus pneumonia*, [8]The anti-inflammatory and erythrocyte hemolysis properties of saponins are well known.[29,30] Flavonoids, alkaloids, phenols, and tannins' have been reported to have anti-inflammatory, antibacterial, antioxidant, antithrombotic, antiallergic, antimutagenic, antineoplastic, and antiviral activities.[16, 29] Tannins have also been known to be effective in wound healing and anti-parasitic activity.[31] The presence of these phytochemicals in *Picralima nitida*a could be the reason for its first-class medicinal properties.[1]

Table 1: Phytochemical screening of Acetone, Water, Ethyl- Acetate and Methanol Extracts of *P. nitida* Stem bark

Phytochemicals	Acetone	Water	Ethyl-Acetate	Methanol
Alkaloids	+	+	+	+
Saponins	+	+	-	+
Terpenoids	+	-	+	-
Flavonoids	+	-	+	-
Phenolic	-	+	-	+
Compounds	+	+	+	+
Reducing Sugars	+	+	+	+
Tannins	-	-	-	_
Glycosides	+	+	+	+
Protein	+	+	+	+
Carbohydrates				

+= Present. - = Not detected

Hematology Evaluation on the Stem Bark of Picralimanitida

It is necessary to perform haematological evaluations of medicinal plant extracts because the presence of medicinal plants in the system could impact some physiological changes on the total blood counts; such as red and white blood cells, platelets, hemoglobin, and hemocrit, which can harm vital organs or tissues and could cause kidney and liver disease.[26]

Statistical Result Of Haematological Studies Of Red Blood Cell (Rbc)

In electron transport and oxygen transfer for hydroxylation processes, hemoglobin—which is measured as the total volume in the erythrocytes and has a value of roughly 10 to 17 g/dL in mice—is crucial.[22]An examination of the relationship between erythrocyte size and hemoglobin concentration is made possible by the data obtained in the study, such data include, RBC, MCV, MCH, HCT, and MCHC. This analysis is essential for determining the different levels of anemia. MCV is used to determine the level of anisocytosis (erythrocyte size) and to categorize anemia among normocytic, microcytic, and macrocytic erythrocytes.[32]The estimation of MCH is made possible by the relationship between the mean intracellular hemoglobin concentration and the erythrocyte count, which in mice can range from 13 to 17 pg (picograms). MCHC measures the amount of hemoglobin in erythrocytes; in mice, the results range from 30 to 38 g/dL. RDW assesses the range of variation in erythrocyte size in the blood sample by using a histogram of the erythrocyte distribution

according to volume. Various disorders have the potential to either raise or lower these parameters.[33] The result of the methanol stem bark of *P. nitida* extract on the red blood cell after 14 days of administration with different doses in mice showed no significant impact on the following red blood cell characteristics which include haemoglobin (HGB), haematocrit (HCT) and red blood cell (RBC) at 200, 400 and 800 mg/kg doses when compared with the control group as shown in Table 2. However, there was a decrease in RDW-SD at 200mg/Kg when compared with the control and other groups; although, this decrease was not significant ($p>0.05$). Research on this plant's seed extract found that the overall WBC counts decreased.[36,29] Table 3 shows a slight increase in the WBC and it shows that *P. nitida* extract can increase the blood's ability to carry oxygen and hence, it is possible that *P. nitida* stem bark extract will be effective in treating certain diseases like anaemia and other disorder that impact the generation of red blood cell.

Table 2: Effects of *P. nitida* stem bark extract on Red blood cell (RBC) and it's component indexes in mice

Parameters	Control	200mg/kg	400mg/kg	800 mg/kg
RBC $\times10^6$/µL	8.26±0.21	7.85±0.24	8.13±0.35	8.30±0.17
HGB.g/dl	15.0±0.41	13.7±1.03	15.1±0.27	15.5±0.44
HCT%	42.2±1.18	38.2±1.90	40.9±1.87	42.7±0.51
MCV.fl	50.9±0.54	48.7±1.11	50.4±0.09	51.8±0.77
MCH.pg	18.9±0.20	17.3±0.80	18.6±0.50	18.7±0.20
MCHC.g/dl	37.2±0.12	35.7±1.05	37.1±1.07	36.3±0.83
RDW-SD fL	43.4±0.59	39.2±2.53	41.3±0.56	43.4±1.53
RDW-CV %	19.0±0.38	17.7±0.77	18.2±0.27	18.7±0.48

Red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW) CV(Coefficient of variation), SD(Standard deviation)

Table 3: Effects of *P. nitida* stem bark extract on white blood cell and its component indexes in mice White blood cells (WBC), lymphocytes (Ly),

Parameters	Control	200mg/kg	400mg/kg	800mg/kg
WBC $\times10^3$/µL	7.63±0.56	8.1±0.35	7.4±O.36	13.4±2.56
LYM $\times10^3$/µL	92.1±1.07[a]	94.2±0.49[a]	94.2±0.35[a]	91.6±1.22[a]
MID $\times10^3$/µL	4.6±0.66	3.7±0.46	3.6±0.36	4.8±0.73
GR $\times10^3$/µL	3.2±0.49	2.0±0.66	2.1±0.13	3.5±0.92
LYM%	7.0±0.34	7.6±0.34	7.0±0.32	12.2±2.28
MID%	0.3±0.66	0.3±0.44	0.3±0.44	O.7±0.24
GR%	0.2±0.77	8.3±0.35	0.1±0.00	0.4±0.99

monocytes (MO), granulocytes (GR).

The result obtained from the analysis of the white blood cells parameters as shown in Table 3 revealed that the groups administered with 200, 400 and 800 mg/kg, did not significantly affect the white blood cell (WBC) compared to the control. *P. nitida* stem bark extract significantly affected the white blood cells (WBC), granulocyte (GR), and lymphocyte (LYM) counts. There was an increase in WBC counts at 800 mg/kg. White blood cells are involved in battling illness and the extracts may stimulate their production. This suggest that the *P. nitida* stem bark extract helps to boost the immune system of swiss mice which could help in protecting the animal from infections. Table 4 presents the hematology report on the platelets of Swiss mice at different concentrations of *P. nitida* stem bark extract. The platelet count (PLT) showed a significant increase at the highest concentration of 800 mg/ml compared to the control group, signifying a dose-dependent consequence of the extract on platelet levels. Specifically, the platelet count was 1431.00±393.10 in the 800 mg/ml group compared to 990.70±151.70 in the control group.

Mean platelet volume (MPV) exhibited a slight decrease in all treatment groups compared to the control, although the differences are not statistically significant. This suggests that the extract may not have a significant impact on the platelets.

Platelet distribution width (PDW) showed a notable increase at the 400 mg/ml and a further increase at 800 mg/ml, indicating a potential dose-dependent effect on platelet distribution width.

Platelet crit (PCT) levels remained relatively stable across all treatment groups, with a slight increase observed at the 400 mg/ml concentration.

Platelet-large cell ratio (P-LCR) demonstrated a substantial increase in the 400 and 800 mg/ml groups compared to the control, suggesting a

potential effect of the extract on the proportion of larger platelets. Overall, the hematology report indicates that *P. nitida* stem bark extract may influence platelet parameters in mice, with significant changes observed in platelet count and distribution width at higher concentrations.

Table 4: Hematology report on the platelets of Swiss mice

Parameters	Control	200mg/ml	400mg/ml	800mg\ml
PLT	990.70 ±151.70	941.00 ±217.30	937.00 ±183.20	1431.00 ±393.10
MPV	10.40± 1.30	9.70±0. 60	10.20± 0.90	9.60±0. 60
PDW	11.10± 1.80	11.00± 0.60	12.60± 1.40	10.90±0 .90
PCT	1.00±0. 400	0.90±0. 20	1.50±0. 50	1.00±0. 60
P-LCR	6.80±1 3.300	16.10± 4.80	22.90± 8.40	17.40±6 .10

PLT= platelet count MPV= Mean platelet volume
PDW= Platelet distribution width PCT= Plateletcrit
P-LCR= Platelet-large cell ratio

Histological Analysis

The histological changes in the kidney section of the different group of mice (normal/treated with extract) are shown in plate 1. In the kidney of every experimental animal, the renal corpuscle and interstitial space are visible, along with a mononuclear infiltrate (arrow).

The change in the liver sections of the different groups of mice (normal control/treated with extract) revealed centriole with no inflamed cells around the liver cells and therefore, there is no form of autoimmune hepatitis as a result of the plant extract. The histology showed no form of organ damage and toxic damage when treated with the methanol extract of *P. nitida* stem bark and when compared to the control.

Plate 1 revealed normal prominent renal cells for both control and treated groups. The kidney reveals visible renal corpuscle and interstitials space and tubules at normal control and with no tubules distortion with extract. *P. nitida* stem bark extract (800 mg/kg) revealed focal tubular necrosis, long and short arrow, mild congestion. *P. nitida* stem bark extract (400 mg/kg) revealed normal glomerulus long and short arrow, tubules. *P. nitida* stem bark extract (200 mg/kg) revealed normal tubules, long arrow. Normal control revealed normal tubules, long arrow (Haematoxylinans Eosin x 100).

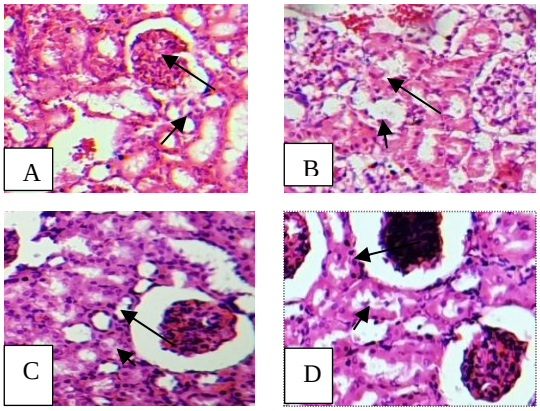

Plate 1: Effects of extracts on kidney cells **A.** Normal control: Kidney reveals visible renal corpuscle and interstitial space and tubules. **B.** 50 mg/kg extract: Kidney reveals visible atrophied renal corpuscle with no tubules distortion. **C. 2**00 mg/kg extract: Kidney reveals visible atrophied renal corpuscle withtubules having mononuclear infiltrates. **D.** 800 mg/kg extract: Kidney had a visible renal corpuscle and interstitial space with mononuclear infiltrate.

Plate 2 showed normal hepatocytes (liver cells) for both the control and the extract. However the liver histology in normal control revealed congested centriole with hepatocytes.

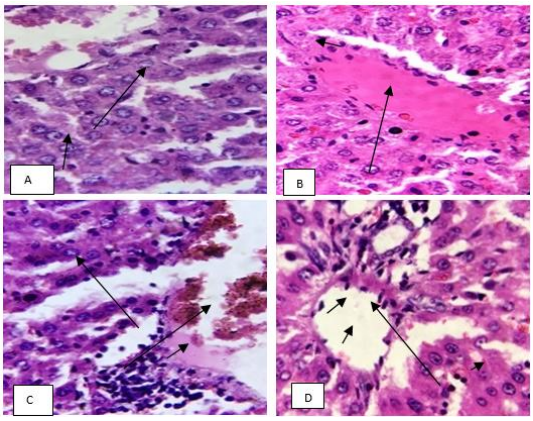

Plate 2: Effects of extract on hepatic cells. **A.** Normal control: Liver histology revealed congested centriole, with hepatocytes. **B**. 200 mg/kg extract: Liver revealed centriole surrounded with thickened wall. **C.** 400 mg/kg extract: Liver revealed centriole with no inflammed cells around the liver cell. **D.** 800 mg/kg extract: Liver revealed centriole with thickened wall, surrounding by hepatocytes prominent blood vessles

In 800 mg/kg, the stem bark extract of *P. nitida* exhibited moderate kupffer cell activation, short

arrow, long arrow, and mid-arrow vascular congestion. Two long arrows with mild vascular congestion and short arrows with mild kupffer cell activation were observed when 400 mg/kg of *P. nitida* stem bark extract was administered. At 200 mg/kg, the stem bark extract of *P. nitida* exhibited short arrow, moderate kupffer cell activation and mild vascular congestion, long arrow. Mild kupffer cell activation, short and long arrow, mild vascular congestion were observed in the normal control (Haematoxylinans Eosin x 100).

Certain components of the plant, such as flavonoids, which block the synthesis of inflammatory mediators and, in conjunction with saponins, improve microcirculation into the liver and kidney due to their surfactant properties.[35, 36]

CONCLUSION

The phytochemical study revealed that the methanol extract of *Picralima nitida* stem bark contains phytochemicals which include alkaloids, tannins, reducing sugars, proteins, carbohydrates for all four solvent which shows potential medicinal actions of therapeutic plants.When compared to the control, the histology revealed no signs of organ damage following treatment with the plant extract. The fact that the red blood cells and their constituents have not changed significantly suggests that the methanol extract from the stem bark of *Picralima nitida* will be useful in the treatment of certain illnesses, such as anemia. When compared to the control and other groups, the stem bark extract from *Picralima nitida* increased WBC and LYM levels, indicating a strong immune system. Further research should be conducted to also evaluate more outstanding medicinal actions of *Picralima nitida* stem bark.

Conflict of interest

The authors declare no conflict of interest.

Authors Declaration

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

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REFERENCES

1. Ogbeide OK., Akhigbe IU, Unuigbe CA, Erharuyi O, Oseghale I, Imieje V, Iheanacho C, Ikeke, K, Ayeni B, Irabor E, Owolabi JB, and Falodun A.. Isolation, characterisation and in vivo anti-malarial investigation of pulcherrimin A from Caesalpiniapulcherrima stem bark. GSC Biological and Pharmaceutical Sciences 2020;12(2), 056-063.
2. Azwanida NN,. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med. Aromat. Plants2015*;* 4, 196.
3. Ogbeide OK, Ogbonnaya, CJ, Asakitikpi E, Uyi, DO, Aluge BO, Nosakhare O, Otahagwa WO, Ehigie CA, Omoruyi U, Aifuwa, OS, Gabriel BO. . Analgesic and Anti-inflammatory Activities of the Stem Bark of Yellow Flamboyant (Peltophorum pterocarpum),' J Appl. Sci. Environ. Manage. 2019*;* 23(7), p. 13-15. https://doi.org/10.4314/jasem.v23i7.19
4. Fagbohun ED.. Chemical composition and antimicrobial activities of Urenalobata L. (Malvaceae).J Med. Plants Res. (2012); 6(12).
5. Purves WK, Sadava D, Orians, GH, and Heller, HC.. *Life:* The science of Biology 2003; (7th ed., p.954). Sinauer Associates and W. H. Freeman.
6. Lisa CA 2019. Haematology*:* Total blood count.International Animal

Medicine(2nded.). USA: Mosby Publishers.
7. Peters SO, Gunn HH, Imumorin IG, Agaviezor BO, Ikeobi CO. (. Haematological studies on frizzled and naked neck genotypes of Nigerian native chickens. Tropical Animal Health Production, 2011; 43(3), 631-638.
8. Weiss DJ and Wardrop KJ. *Schalm's Veterinary Hematology*. 6thed Ames, IA: Wiley Blackwell, 2010
9. Oyawoye BM, and Ogunkunle, HN. (Biochemical and haematological reference Values in normal experimental animals 2004; (p. 212-218).
10. Johnston JK, and Morris DD. (1996). Alterations in blood proteins. In B. P. Smith (Ed.),
11. Sidell B, and Kristin OB.). "When bad things happen to good fish: the loss of hemoglobin and myoglobin expression in Antarctic icefishes". J Exp. Biol. 2006; 209 (Pt 10): 1791–802.
12. Ugwuene MC. Effect of Dietary Palm Kernel Meal for Maize on the Haematological and Serum Chemistry of Broiler Turkey. Nig. J of Animal Sci. (2011); 13, 93-103.
13. Francis U, Isaac, Z, Yakubu A, Enosakhare A, Felix, E..Haematological parameters of malaria infected patients in the University of Calabar Teaching Hospital, Calabar, Nigeria. JHTD 2014; 2(6), 1-4.
14. Soetan KO, Akinrinde, S, and Ajibade TO.. Preliminary studies on the haematological Parameters of cockerels fed raw and processed guinea corn (*Sorghum bicolor)*Proceedings of 38th Annual Conference of Nigerian Society for Animal Production 2013; (p. 49-52).
15. Kabir M, Akpa GN, Nwagu, BI, Adeyinka, IA, and Bello UI. (2011). Sexual Dimorphism, breed and age characteristics of rabbits in Zaria, Nigeria(p.133-137). Proceedings of the 16th Annual Conference of Animal Science Association of Nigeria
16. Ogbeide O.K,,Sajere, O, Obukuru, IS, Akedayi, P, Omorefe, NO, Unachukwu, CI, Mbonu, R, Aghedo, NO, Iyekowa1, Uadia, JO. Comparative Studies on the Bioactive Chemical Composition, Antioxidant and Antimalarial potentials of *Picralimanitida* Leaves and Stem Bark Extracts. Benin J of Phy Sci. 2024; 1(1):231-250
17. Awodi S, Ayo, JO.,Atodo AD, Dzende T.. Some haematological parameters and the erythrocyte osomotic fragility in the laughing dove (Streptopella senegalensis) and the Village weaner bird(*Ploceuscucullatus*) Proceedings of the 10th Annual Conference of Animal Science Association of Nigeria 2005; (p.384-387).
18. Ifijen IH, Aghedo, ON, Odiachi, IJ, Ogbeide OK, Maliki, M., Okereke, OC.. Investigation of the Anti-malaria Potency and Chemical Constituents of the Bark Extracts of Ficuselastica in Plasmodium berghei Infected Mice.Chemistry Africa 2020; 3 (4): 1045-1051
19. Figueiredo E, and Smith GF.). Plants of Angola, Strelitzia.National Botanical Institute, Pretoria 2008*;* 22: 271-279.
20. Sharma BR, Kumar V, Gat Y, Kumar N, Parashar A, Pinakin DJ.. Microbial maceration: A sustainable approach for phytochemical extraction. *3 Biotech*. 2018;8:401.
21. Shikov AN., Mikhailovskaya IY, Narkevich IA, Flisyuk EV, Pozharitskaya ON. Evidence-Based Validation of Herbal Medicine. Elsevier; Amsterdam, The Netherlands*:*. Methods of extraction of medicinal plants 2022; pp. 771–796.
22. Rowles JL, 3rd, Erdman JW. Carotenoids and their role in cancer prevention. Biochimicaet Biophysica Acta. Mol. Cell Biol. Lipids 2020;1865:158613.
23. Wokocha RC, and Okereke VC. Fungitoxicactivity of extracts of some medicinal plants onsclerotiumrolfsii, the causal organism of the basal stem rot disease of tomato. Nig. J. of plant prot. (2005); 22:106-111
24. Thangaraj P. Pharmacological Assays of Plant-Based Natural Products. Springer, Geneva.Pandey, A. and Tripathi, S.Concept of Standardization, Extraction and Pre-Phytochemical Screening Strategies for Herbal Drug. J Pharmacogn Phytochem. 2016*;* 2, 115-119.

25. NIH, Guide for the care and use of laboratory animals. U.S. Department of Health and Human Services) NIH Publication 1985; 86:1-83.
26. Ogbeide, OK, Akhigbe I.U, Unuigbe C.A, Erharuyi O, Imieje V, Benjamin GO, Ikeke K, Ayeni B, Irabor E, Owolabi JB, Falodun A. Haematological and Biochemical Examination of *Pulcherrimin A* isolated from *Caesalpiniapulcherrim*astem bark. Trop J Nat Prod Res 2021; 5(11):2011-2015.
27. Ighodaro I, Anegbe B, Ogbeide KO, and Onaiwu, GE. The phytochemical and chemotherapeutic effect of three indigenous Africa plant used in asthma therapy. JPharmacognos and Phytochem 2015; 3(6), 244–247.
28. Ogbeide OK, Okhomina OK, Omoregie G, Unuigbe CA, Ighodaro A, Akhigbe IU, Iheanacho CM, Akubuiro PC, Solomon A, Irabor EEI, Owolabi BJ, Falodun A.Antimalarial, ferric reducing antioxidant power and elemental analysis of Caesalpiniapulcherrima leaf extract. J. ChemSoc Nig. 2020; 45(4):704-711
29. Aghedo ON., Owolabi JB. andOgbeide, OK, Chemical Composition and Antimicrobial Activities of *Picralimanitida* Stem Bark Extracts.ChemSearch J 2021; 12(2): 55 – 63.
30. Ogbeide OK, Alao E, Jonathan EM.. Phytochemical investigation and anti-inflammatory activity of stem bark of pride of barbados (Caesalpiniapulcherrima). J ChemSoc Nig. 2020; 45(3):492-498.
31. Okwu DE. andOkwu ME.Chemical composition of Spondiasmombinlinn. plant parts. *J Sustain* Agric Environ 2007; 6(2):140-147.
32. Shahsavaani D, Kazerani HR, Kaveh S, Gholipour-Kanani H. Determination of some normal serum parameters in starry sturgeon (Acipenserstellatus Pallas, 1771) during spring season, 2010 Comp. Clin. Pathol. 2010; 19(1):57–61. doi:10.1007/s00580-009-0899-3.
33. Silva M, and Counto NMR, Evaluatiopn of RDW as an indicator of the iron deficiency in patients with chronic renal failure under hemodialysis. Rev Bras Anal Clin. 2016; 48:211-215.
34. Akinlye OA, Samade OT, Akindele AS, Adelabu KB, Elijah FT, Adewumi OJ. Anticlasstogenic and Hepatoprotective properties of ginger (*Zingiberofficinale*) extract against nitrobenzene-induced toxicity in rat.Rom. J. Biochem 2014; 51(1), 3 -15
35. Wan L and Jiang JG.. Protective effects of plant – derived flavonoids on; 2018: 44:283-291
36. Alturkistani HA, Tashkandi FM, Mohammedsaleh ZM. Histological Stains: A Literature Review and Case Study. Glob J Health Sci 2015; 25;8(3):72-90.