In Vivo Anti-Anemic Effect of an Aqueous Root Extract of Phyllanthus Muellerianus (Kuntze) Exell in Model Rats.

James Nyirenda (✉ nyirendaj@unza.zm)  
The University of Zambia  https://orcid.org/0000-0001-7122-3189

Gershom B. Lwanga  
University of Zambia - Ridgeway Campus: University of Zambia School of Medicine

Kaampwe M. Muzandu  
University of Zambia School of Veterinary Medicine

David K. Chuba  
University of Zambia School of Natural Sciences

Gibson M. Sijumbila  
Mulungushi University

Research

Keywords: Hematological indices, Phyllanthus muellerianus, Phytochemicals, Indigenous Traditional Knowledge Systems.

DOI: https://doi.org/10.21203/rs.3.rs-743554/v1

License: ☑️ ☑️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Ethnopharmacological relevance

Anemia is a very serious condition in Zambia. One of the plants that has been used traditionally is Phyllanthus muellerianus where different parts of shrub are used to treat a number of diseases in Zambian folklore medicine. Earlier studies have investigated medicinal properties of its aqueous root extracts. This study evaluated the effect of P. muellerianus roots on the hematological indices of albino rats and determined its phytochemical profile.

Aim of the study

To carry out phytochemical screening of the root extract and assess the ant-anemic effect of the aqueous extract on laboratory rats with tail-bled induced anemia

Materials and Methods

Thirty-six male albino rats placed in six groups were used for the study. The groups comprised the 100, 200, and 400 mg/kg plant extract, Ranferon (200 mg/kg) positive control, anemic non treated control and a normal (non-anemic) control. Anemia, induced through bleeding of the rats, was defined as hemoglobin (Hb) levels less than 12 g/dL. The anti-anemic potential of the plant was determined by comparing its effect on the hematological parameters of rats on treatment to that of the control group.

Results

After treatment, rats on the 400 mg/kg plant extract dose showed the greatest increase in the mean values for Hb, Packed cell volume (PCV) and RBC count were 43.3±1.2%, 15.4±0.3 g/dL and 6.3±0.3 x10^6 /mL respectively, when compared to the negative control group (P < 0.05). Phytochemical screening revealed positive results for alkaloids, flavonoids, saponins, glycosides, steroids, triterpenoids and tannins with varying amounts.

Conclusions.

The aqueous root extract of P. muellerianus was efficacious against anemia in a dose-dependent manner. The phytochemical compositions seem to be responsible for its hematopoietic properties. Thus, the root decoction of P. muellerianus is useful in alleviating anemia and the results lend credence to its use in traditional medicine in the management of anemia.

1. Introduction

Anemia is a condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs 1–3. The cut off points for diagnosis of anemia have remained largely unchanged since 1968 with the exception that the original age group of
children 5–14 years of age was split, and a cut-off of 0.5 g/dL lower was applied to children 5–11 years of age to reflect findings among non-iron deficient children in the USA. The cut offs range from children 6–59 months (10-10.9 g/dL), children 5–11 years (11-11.4 g/dL), children 12–14 years (11-11.9 g/dL), non-pregnant women 15 years and above (11-11.9 g/dL), pregnant women (10-10.9 g/dL) and men 15 years and above (11-12.9 g/dL). It is a common blood disorder that affects people of all ethnicity and ages; although the elderly, young women of child bearing age and infants at greater risk. The causes of anemia are patho-physiologically diverse and multifactorial. Thus, there are more than 400 types, some are mild while others are severe or even life threatening if not treated. In rodents, symptoms include; rapid or labored respiration, anorexia, immobility, abnormal appearance or posture periocular and nasal porphyrin discharge. Treatment varies depending on the type of Anemia. Anemia associated with a serious disease is treated by treating the underlying disorder. Additional medications that boost RBCs may be prescribed when symptoms persist or worsen. These include; iron tablets, supplements, fortifications, erythropoietin injections, blood transfusions, removal of the spleen, plant products such as groundnuts, tomatoes and spinach, animal products such as liver and red meat. Interventions to prevent or treat anemia are insufficient in Zambia because of; inadequate qualified human resource, high disease burden, inadequate emergency facilities, the diet of majority Zambians is mainly composed of cereals (maize) and starchy roots with little micronutrient-dense foods such as animal products and fruits. Further, the prevalence of anemia is 46 % which is a severe public health problem based on the World Health Organization (WHO) standards. This implies that there is a great loss of man hours of healthy adults who find themselves off work to nurse anemic patients. It is also among the top 10 causes of morbidity and mortality. In Africa and most Asian countries, anemia is treated using herbs such as; - Khaya senegalensis, Justicia secunda, and Amaranthus spinosus. The study investigated the effect of Phyllanthus muellerianus (Kuntze) Excell aqueous root extract on hematological indices of albino rats. P. muellerianus is one of the plants used to treat anemia by local people of the Northern part of Zambia. However, its efficacy had not been scientifically established. Local names for P. muellerianus in Zambia include: Chewa-Mkuzandola, Tumbuka-Kapikanduzi, Icibemba-Umupetwalupe, Kaonde-Mulembalemba, Mambwe-Mupetwandupe. It belongs to the family Phyllanthaceae consisting of approximately 1000 species which are widely distributed in tropical and subtropical areas of Africa, Asia, America and Australia. It is an evergreen scendent shrub with numerous stems from the base or a small tree up to 12 meters tall. The branches are arched and pendulous almost to the ground. It naturally occurs in riverine forest and wooded grasslands on deep and well-drained soils. It is widely distributed and easy to access in Zambia. Additionally P. muellerianus grows easily from seed and hence has potential to contribute sustainably towards medical solutions coming from local flora. P. muellerianus has many medicinal uses such as the treatment of wounds, menstrual disorders, fevers, inflammation, intestinal problems, kidney and urinary bladder problems, diabetes and hepatitis B, body pain and as an antiseptic.
Agyare et al 21 studied *P. muellerianus* leaf extracts for the stimulatory effect of ellagitannins on cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. Earlier studies on the leaf extract by Boakye et al 22 have shown that *P. muellerianus* has anti-inflammatory activity. The review by Calixto and friends 23 showed that the phyllanthus species have a number of metabolites with pharmacological potential isolated and characterised from all the parts of the plant, leaves, roots, stem and bark. Other studies investigated the antimicrobial properties of the stem and bark parts of 24, 25. This study investigates the ethnobotany and ethnopharmacological effect of *P. muellerianus* roots on hematological indices of albino rats.

2. Materials And Methods

2.1 Plant Collection and Identification

Ethnobotanical authentication and annotation was done at the University of Zambia Herbarium (UZL). The specimen was identified as *Phyllanthus muellerianus* (Kuntze) Exell belonging to the family Phyllanthaceae and voucher specimen/accession number 22287. It has also been verified on the http://www.theplantlist.org website.

2.2 Preparation of the aqueous root extract of the plant

The roots of the plant were harvested in October 2018 from Kaunda Square area of Lusaka Province (Latitude 15°21'33.1"S and Longitude 28°21'57.9"E), Zambia. They were thoroughly washed to remove debris and all soil material. After size reduction, a 1 kg root sample was boiled in 1.5 litres of distilled water and the resulting solution was allowed to cool then sieved to remove non-soluble plant matter and finally filtered through Whatman filter paper number 4. The mixture was boiled to dryness on a heating mantle, the resulting brittle powder was weighed and kept at 4°C until further use. Various concentrations (w/v) of the extract were made by dissolving the appropriate quantity of the solid extract in distilled water to make a solution.

2.3 Animals and induction of Anemia

Sample size was calculated by executing the function in G*Power version 3.1.9.4 26, 27. Briefly, under test family, “F test” was selected, and the statistical test selected was “ANOVA: Fixed effects, omnibus, one-way”. Sample size was then computed as a function of power level (1 - β), a pre-specified significance level (α) and the population effect size to be detected with probability. The power level (1 - β) was set at 0.8 default, significance level α at 0.05 default and pre-detected effect size at 0.7, and 6 groups. Calculation using G*Power gave a sample size of 36. So a total of 36 male albino experimental animals weighing between 150 and 180 grams were selected and put into 6 treatment groups with 6 animals each using similar studies by 28. All laboratory work was done according to the Guidelines for the Care and Use of Laboratory Animals 29. Anemia was induced by bleeding the rats under light anesthesia using diethyl ether as an anesthetic 30, 31. The formula described by Lee and Blaufox 32 was used to determine the
quantity of blood removed through bleeding. At the end of the study, rats were euthanized in diethyl ether.

2.4 Administration of the plant extract and Hematological tests

Treatment started 24 hours after inducing anemia. Ranferon, the extract and distilled water were administered by oral intubation as shown in Table 4 - 1. Rats were anesthetised in diethyl ether, when they became unconscious, blood was collected from their retro-orbital plexus for hematological studies at the baseline of the study, after inducing anemia and after treatment.

2.5 Phytochemical Screening

Qualitative phytochemical screening of the aqueous plant extract was carried out at the University of Zambia, Department of Chemistry using standard procedures.

2.6 Mineral quantification and Statistical analysis

The mineral content was determined by AAS using Perkin-Elmer atomic analyst 400 while one way ANOVA followed by Bonferroni post hoc test to determine pairwise comparisons was executed in STATA software version 13.0. In each case, the negative control was compared with other categories. All results were expressed as percent means at 95% confidence level with respective standard deviations.

2.7 Quality control

To ensure reliability of results, quality control was performed on the AAS and hematocrit before each test was done according to the respective manufactures manual.

3. Ethical Considerations

Ethical clearance was sought from the University of Zambia Biomedical Research Ethics committee (UNZABREC- REF. No: 005-09-16). The experimental protocol was followed according to Guidelines for Care and Use of Laboratory Animals in Biomedical Research.

4. Results

The color of solid extract was dark brown and the percentage yield was 1.6 %. The extractive weight of the plant extract was 16 g with a mineral composition of 230.5 mg Fe, 273.5 mg Mn, and 138 mg Zn.

4.1 Phytochemical Screening

The results of phytochemical screening of the extract are shown in Table 4 - 1.
Table 4-1. Phytochemical Screening

| Test       | Observation       | Result |
|------------|-------------------|--------|
| Alkaloids  | Turbidity         | +++    |
| Sterols    | Green-blue colour | ++     |
| Triterpenoids | Violet upper layer | ++    |
| Flavonoids | Red colour        | +++    |
| Saponins   | Stable foam       | +++    |
| Glycosides | Brown ring        | +++    |
| Tannins    | Green precipitate | +++    |

Key: - Negative, + Trace, ++ Moderate, and +++ High.

Table 4 - 1. The phytochemical screening showed presence of alkaloids, sterols, triterpenoids, flavonoids, glycosides, saponins and tannins in appreciable amounts. The alkaloids, flavonoids, saponins, glycosides and tannins were in high amounts.

4.2 Effects of the plant extract on the Packed Cell Volume and Hemoglobin

The study defined anemia as Hb < 12 g/dL. At the baseline of the study, the mean values for PCV and Hb in the 5 comparison groups were not different (P > 0.05) from the control group. However, repeated bleeding decreased PCV and Hb to values less than 32.5 % and 11.5 g/dL respectively. Treatment with P. muellerianus reversed anemia in a dose-dependent manner (Fig. 4 - 1). The mean PCV for the negative control was 35.5±0.84 % significantly lower than the means of normal control 42±1.7%, Ranferon 42±0.0%, 100 mg/kg (40.8±0.8%), 200 mg/kg (43.3±1.2%) and 400 mg/kg (46.2±1%) all at p-value < 0.05 as shown in Fig. 4 - 1. The normal control was significantly lower than the 400 mg/kg, Ranferon was lower compared to 400mg/kg, 100mg/kg was lower compared to 200 mg/kg and 400 mg/kg. Also the 200mg/kg was lower when compared to the 400 mg/kg extract (Fig. 4 - 2).

4.3 Effects of the extract on Hb levels

The mean hemoglobin levels for the negative control, normal control, Ranferon, 100 mg/kg, 200 mg/kg and 400 mg/kg were 11.8±0.3, 13.9±0.6, 14.0±0.0, 13.6±0.3, 14.5±0.4 and 15.4±0.3 respectively. Analysis showed statistical differences across the groups. The negative control was significantly lower compared to all other groups. There were also differences when the normal control was compared to 400 mg/kg. Ranferon was lower compared to 400mg/kg, 100mg/kg was lower compared to 200 mg/kg and 400 mg/kg. Also the 200mg/kg was lower when compared to the 400 mg/kg extract (Fig. 4 - 2).

4.4 Effects of the plant extract on RBC Count
The mean values of RBC count ranged from, 5.3 to 5.9 x 10^6 /µl (P > 0.05) at the baseline of the study, 3.9 to 5.7 x 10^6 /µL after inducing anemia, and 4.9 to 6.3 x 10^6 /µL after treatment, P< 0.05. RBC mean values for the negative control, normal control, Ranferon, 100 mg/kg, 200 mg/kg and 400 mg/kg were 5.1±1.4, 5.1±0.2, 5.1±0.1, 4.9±0.6, 5.9±0.9 and 6.3±0.3 respectively. One way ANOVA showed statistical differences across the groups. However, Bonferroni post hoc test showed that the difference was only between 100 mg/kg and 400mg/kg (Fig. 4 - 3).

### 4.5 Effects of the plant extract on MCV, MCH and MCHC

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) all showed no statistical differences across the groups at 95 % confidence level.

#### 4.5.1 MCV

Means of the Negative control (74.7±20.2), Normal control (81.8±0.9), Ranferon (82.4±1.0),100 mg/kg (83.7±9.2), 200 mg/kg (75.2±9.8) and 400 mg/kg (73.8±3.4) for the corpuscular volume showed no statistical significance between and across the treatment groups.

#### 4.5.2 MCH

Means of the Negative control (24.9 ± 6.7), Normal control (27.2 ± 0.3), Ranferon (27.4±0.3), 100 mg/kg (27.8 ±3.1), 200 mg/kg (24.9 ±3.3) and the 400 mg/kg (24.6±1.2) treatment groups showed no statistical significance across the treatment groups.

#### 4.5.3 MCHC

Negative control (33.3±0.06), Normal control (33.3±0.04), Ranferon (33.3±0), 100 mg/kg (33.4±0.05), 200 mg/kg (33.3±0.05) and the 400 mg/kg (33.3±0)

### 5. Discussion

#### 5.1 Effect of *P. muellerianus* on the hematological parameters

Dosages of 100, 200, and 400 mg/kg of the root extract and ranferon were administered orally to anemic albino rats to monitor their effect compared to that of the control group that did not receive any drug but distilled water. The results revealed that the root extract and ranferon were able to restore the hematological indices of experimental animals to normal levels and the significant (p < 0.05) effect was found to be dose-dependent. In addition, the eta squared values for PCV and Hb of 0.66 suggested that the aqueous root extract of the plant was both statistically significant and efficacious against anemia. The Phytochemical and mineral compositions of the root extract seem likely to be responsible for the
hematinc effect of *P. muellerianus* and their presence in the plant extract agrees with previous studies. The blood parameters, Hb, PCV, and RBCs together with the level of iron are indices of anemia that could be used to indicate nutritional values of ingested diets as well. MCV, MCH and MCHC are constants for typing anemia hence they were not statistically different (*p* > 0.05) when experimental groups were compared to the control group after treatment. They, however, decreased after inducing anemia, indicating microcytosis as their decrease reflects a release of RBCs, which are less saturated in Hb (hypochromia). Therefore, occurrence of anemia observed in this study was attributable to lowered values of these indices and related to the report by Osman et al., Moreover, the increase in these hematological parameters was dose-dependent and could be due to high nutritional values of *P. muellerianus* particularly in minerals such as Fe (230.5 mg). Fe plays a significant role in erythropoiesis. It is required for the synthesis of Hb and myoglobin while its deficiency causes anemia. However, the therapeutic potential of *P. muellerianus* could not be established based on available Fe content alone as other factors play a role in its absorption in the body. In this context, Fe is a necessity for the formation of the heme part of Hb as reported by others. The high Fe content of the plant under investigation justifies and partly supports the traditional use of its roots in treating anemia. The results also place it under a group of plants with antianemic potential, which are richest in Fe content according to the related findings of Koné et al., However, Schmelzer et al., reported an insignificant Fe content of 15 mg per gram of dry fruits of *P. muellerianus*. The plant extract also had high content of Zinc (138 mg) and Manganese (273.5 mg). Zinc is required for the function of over 200 enzymes and is important in growth and sexual development in man. Mn is both nutritionally essential and potentially toxic. It is important for brain and nerve function (can bind with neurotransmitters and stimulate faster or more efficient transmission of electrical impulses throughout the body, in effect, speeding up cognitive function. The methanolic and ethylacetate leaf extracts of *P. muellerianus* were shown by Assob et al., to have a lethal dose (LD$_{50}$) of more than 4 g/kg body weight of male and female rats. Based on their study, they concluded that *P. muellerianus* is not toxic, taking into consideration the 5 g/kg threshold of toxic substances. On the other hand, Adedapo et al., showed that the leaves of *P. muellerianus* significantly (*p* < 0.05) reduced the hematological parameters, had toxic potential and were therefore, poisonous to the animals.

### 5.2 Observed Phytochemicals and their action

Plants used in the treatment of disease contain a wide range of active principles with biological activity, some of which are responsible for the characteristic odours, pungencies and colours of plants while others give a particular plant its culinary, medicinal or poisonous virtues, which could be used as the base for discovering modern drugs for curing various diseases. The phytochemical tests performed on the root extract of *P. muellerianus* showed presence of steroids, triterpenoids, alkaloids, flavonoids, saponins, cardiac glycosides and tannins as reported by in previous similar studies.

#### 5.2.1 Sterols
Phytosterols (PS) such as β-sitosterol, campesterol and stigmasterol have been shown to have anti-eryptotic effects on cells. Presence of a considerable amount of sterols may have had the anti-hemolytic property hence improving availability of intact cells in blood, ultimately alleviating anemia.

5.2.2 Alkaloids

As inferred from other reports, alkaloids, the most revered of all phytochemicals are said to be pharmacologically active and their action is felt in blood vessels. They inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase leading to accumulation of cAMP. This effect stimulates phosphorylation of proteins and synthesis of proteins, which improves erythropoiesis.

5.2.3 Saponins

Saponins are known to vitalize blood circulation and promote hemolytic activities. Since saponins are active agents that lyse the membrane of RBCs, it is likely that the plant extract used in this study first lysed RBCs. Then the test animals overcame this inhibition by producing a glycosidic enzyme that cleaves some of the terminal sugars from the saponins, which detoxified it. This detoxification of saponins reinforced the proper use of iron contained in the plant extract allowing it to synthesize hemoglobin for new RBCs, thus leading to an observed improvement of Hb, RBCs and PCV in the plant extract treated groups. Glycosides enhance the natural resistance and have the recovery powers for the body.

5.2.4 Flavonoids

Flavonoids are known to possess a well-established protective effect against membrane lipoperoxidative damages. The antioxidant activity of phenols and flavonoids is mainly attributed to their redox properties because of which they act as reducing agents, electron / hydrogen donators, and singlet oxygen quenchers. It has been demonstrated that the antioxidants such as flavonoids can act: either by neutralizing reactive oxygen species (ROS) by directly reacting with superoxide anion, nitric oxide and peroxynitrite thereby preserving vascular function and protecting vascular injuries from ROS and perhaps from other oxidant species, or they could stimulate erythropoiesis.

5.2.5 Tannins

Tannins are well known for their anti-oxidant and skin regeneration. Tannins from many plants especially Euphorbiaceae are used to stop bleeding during circumcision. Steroids regulate carbohydrate and protein metabolism and possesses anti-inflammatory properties. This might correspond to their ethnomedicinal significance.

6. Conclusion

P. muellerianus was efficacious against anemia in a dose dependent manner, \( p < 0.05 \). The rich array of phytochemicals (especially alkaloids, saponins, and flavonoids) and high iron composition seem likely to
be responsible for its hematinic effect. Further studies are needed with this plant to evaluate the anti-
anemic active components and to elucidate their mode of action.

Declarations

Ethics approval and consent to participate

Ethical clearance to execute this research was sought under University of Zambia Biomedical Research
Ethics committee (UNZABREC- REF. No: 005-09-16).

Consent for publication

All the authors consented to publication of this manuscript.

Availability of data and material

Not applicable as all data is presented herein.

Competing interests

The authors declare no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-
for-profit sectors.

Author contribution

GML, collected samples and performed experiments and contributed to writing the manuscript, SG
contributed to writing the manuscript, DKC collected, accessioned the plant sample in the UZL and
contributed to writing the manuscript, JN and KMM designed experiments and critically analyzed data.
JN contributed to overall supervision. All authors read the manuscript and contributed to editing.

Acknowledgements

The authors would like to thank members of the Departments of Chemistry, Disease Control and
Biomedical Sciences of the University of Zambia for the equipment and services rendered.

References

1. W.H.O. Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity.
Vitamin and Mineral Nutrition Information System. Document Reference WHO.
NMH/NHD/MNM/11.1. http://www.who.int/entity/vmnis/indicators/haemoglobin..., 2011.
2. W.H.O. Nutritional anaemias: report of a WHO scientific group [meeting held in Geneva from 13 to 17 March 1967]: World Health Organization; 1968.

3. W.H.O. World Health Organization. Iron Deficiency Anaemia: Assessment, Prevention, and Control: A Guide for Programme Managers. Geneva, Switzerland 2001.

4. W.H.O UNICEF. UNU. Iron deficiency anaemia: assessment, prevention, and control. A guide for programme managers, Geneva, WHO 2001.

5. Guidelines: Intermittent Iron Supplementation in Preschool and School-age Children. PubMed abstract. Geneva 2011.

6. De Benoist B, McLean E, Egli I, Cogswell M. WHO global database on anaemia. Geneva: WHO 2008:1993-2005.

7. Murray RK, Granner DK, Mayes PA, Rodwell VW. Happer’s Illustrated Biochemistry. USA: Lange medical books / Mc Graw-Hill companies; 2003.

8. National Research Council (US) Committee. Update on the Guide for the Care and Use of Laboratory animals. 2011. In: Guide for the Care and Use of Laboratory animals. Washington DC: National Academies Press. 8th. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54052/.

9. Goddard AF, McIntyre AS, Scott BB. Guidelines for the management of iron deficiency anaemia. Gut 2000;46(suppl 4):iv1-iv5.

10. Osungbade KO, Oladunjoye AO. Preventive Treatments of Iron Deficiency Anaemia in Pregnancy: A Review of Their Effectiveness and Implications for Health System Strengthening. Journal of Pregnancy 2012;2012:7.

11. D.F.I.D. Tackling Maternal and Child Undernutrition in Zambia. A Business Case. 2011.

12. N.H.S.P. National Health Strategic Plan 2011-2015. Zambia2011.

13. MOST. Report of the national survey to evaluate the impact of vitamin A interventions in Zambia, July and November, 2003. Zambia: Micronutrient Operational Strategies and Technologies, UNICEF, Centers for Disease Control and Prevention, Food and Nutrition Commission of Zambia, University of Zambia., 2003.

14. N.F.N.C. Zambia Food Consumption and Micronutrient Status Survey Report 2014.

15. USAID. Zambia : Nutrition Profile. 2014.

16. C.S.O. Zambia Demographic and Health Survey 2007. Lusaka2009.

17. Koné W, Koffi A, Bomoisso E, Bi FT. Ethnomedical Study and Iron Content of Some Medicinal Herbs Used in Traditional Medicine in Cote D’Ivoire for the Treatment of Anaemia. African Journal of Traditional, Complementary, and Alternative Medicines. 9(1):81-87. 2012.

18. Simute S, Phiri CL, Tengnäs B. Agroforestry Extension Manual for Eastern Zambia. Nairobi, Kenya: Majestic Printing Works Ltd.; 1998.

19. Burkill HM. The useful plants of West Tropical Africa.Families. E–I. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 1994;2nd Edition. Volume 2:636 pp.

20. Schmelzer, Harriët G, Gurib-Fakim, Ameenah. Medicinal plants: Prota; 2008.
21. Agyare C, Lechtenberg M, Deters A, Petereit F, Hensel A. Ellagitannins from Phyllanthus muellerianus (Kuntze) Exell.: Geraniin and furosin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. Phytomedicine 2011;18(7):617-24.

22. Boakye YD, Agyare C, Aboitsi WKM, Ayande PG, Ossei PPS. Anti-inflammatory activity of aqueous leaf extract of Phyllanthus muellerianus (Kuntze) Exell. and its major constituent, geraniin. Journal of ethnopharmacology 2016;187:17-27.

23. Calixto JB, Santos AR, Filho VC, Yunes RA. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential. Medicinal research reviews 1998;18(4):225-58.

24. Brusotti G, Cesari I, Frassà G, Grisoli P, Dacarro C, Caccialanza G. Antimicrobial properties of stem bark extracts from Phyllanthus muellerianus (Kuntze) Excell. Journal of ethnopharmacology 2011;135(3):797-800.

25. Doughari J, Sunday D. Antibacterial Activity of Phyllanthus muellerianus. Pharmaceutical biology 2008;46(6):400-05.

26. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods 2007;39(2):175-91.

27. Banda M. Assessing the anti-hyperglycemic and anti-hyperlipidemetic effects of an aqueous extract of Lannea edulis in alloxan-induced diabetic rats. Lusaka: University of Zambia; 2018.

28. Banda M, Nyirenda J, Muzandu K, Sijumbila G, Mudenda S. Anti-hyperglycemic and Anti-hyperlipidemic Effects of Aqueous Extracts of Lannea edulis in Alloxan-Induced Diabetic Rats. Frontiers in pharmacology 2018;9:1099.

29. National Research Council (United States) Committee. Update on the Guide for the Care and Use of Laboratory animals. 2011. In: Guide for the Care and Use of Laboratory animals. Washington DC: National Academies Press. 8th. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54052/.

30. Brain JD, Heilig E, Donaghey TC, Knutson MD, Wessling-Resnick M, Molina RM. Effects of Iron Status on Transpulmonary Transport and Tissue Distribution of Mn and Fe. American Journal of Respiratory Cell and Molecular Biology 2006;34(3):330-37.

31. Wallin DJ, Tkac I, Stucker S, Ennis KM, Sola-Visner M, Rao R, et al. Phlebotomy-induced anemia alters hippocampal neurochemistry in neonatal mice. Pediatr Res 2015;77(6):765-71.

32. Lee H, Blaufox M. Blood volume in the rat. Journal of Nuclear Medicine 1985;26(1):72-76.

33. Guidelines for the Euthanasia of Animals. 2013.

34. Stone SH. Method for Obtaining Venous Blood from the Orbital Sinus of the Rat or Mouse. Science (New York, NY) 1954;119(3081):100.

35. Bull BS, Simson EV, Assendelft OW. Procedure for determining packed cell volume by the microhaematocrit method. 3rd edition; 20, 2.1958.

36. Thomas D, Hinchliffe R, Briggs C, Macdougall C, Littlewood T, Cavill L. Guideline for the laboratory diagnosis of functional iron deficiency. 161: 639-48. Br J Haematol 2013.

37. Trease GE, Evance WC. Pharmacognosy. London: Saunders Publishers; 2002.
38. Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan: Nigeria: Spectrum Books Ltd; 1993.

39. Katsayal UA, Lamai RS. Preliminary phytochemical and antibacterial screening of the ethanolic stem bark extract of Phyllanthus muellerianus. Nig Journal Pharm Sci 2011;Vol. 8 (No. 2): P. 121-25.

40. Yenon AA, Yapi H.F., Gnougue G., Yapo A.F., Nguessan J. D., Djaman AJ. Anti-anaemic activity of aqueous and ethanolic extracts of Entandrophragma angolense bark on phenylhydrazine- induced anemic rats. Asian Journal of Biochemical and Pharmaceutical Research 2015;Vol. 5(Issue 3).

41. Osman HM, Shayoub M. E., M. BE. The effect of Solenostemma argel on anemia related parameters in Albino Rats and Rabbits. Saudi J Health Sci 2013;2:81-6.

42. Assob JCN, Kamga HLF, Nsagha DS, Njunda AL, Nde PF, Asongalem EA, et al. Antimicrobial and toxicological activities of five medicinal plant species from Cameroon Traditional Medicine. 2011;11:70.

43. Adedapo AA, Abatan MO, Olorunsogo OO. Effects of some plants of the spurge family on haematological and biochemical parameters in rats. Vet arhiv 2007;77,29-38.

44. Okeniyi JO, Loto CA, Popoola AP. Electrochemical performance of Phyllanthus muellerianus on the corrosion of concrete steel-reinforcement in industrial/microbial simulating-environment. Portugaliae Electrochimica Acta 2014;32(3):199-211.

45. Alvarez-Sala A, López-García G, Attanzio A, Tesoriere L, Cilla A, Barberá R, et al. Effects of Plant Sterols or β-Cryptoxanthin at Physiological Serum Concentrations on Suicidal Erythrocyte Death. Journal of Agricultural and Food Chemistry 2018;66(5):1157-66.

46. Shami C, Aman M. Role of flavonoids in the treatment of hemolytic anaemia-a review. European Journal of Pharmaceutical and Medical Research 2016;3(5):212-16.

Figures
Figure 1

Mean Packed Cell Volume after treatment with the extract. The asterisks imply significance across the treatment groups. The results were expressed as mean ± SD at 95% confidence level, p<0.05 (One-way ANOVA followed by Bonferroni post hoc test executed in STATA version 13.1) The plot was generated using GraphPad Prism software version 6.01.
Figure 2

Shows mean Hb levels. The asterisks imply significance across the treatment groups. The results were expressed as mean ± SD at 95% confidence level, p<0.05 (One-way ANOVA followed by Bonferroni post hoc test executed in STATA version 13.1). The plot was generated using GraphPad Prism software version 6.01.
Figure 3 shows the results of treatment of the rats with extract. The Bonferroni post hoc test revealed that significance was only between the 100 and 400 mg/kg treatment groups. Double asterisks shows significance between these two groups. The results were expressed as mean ± SD at 95% confidence level, p<0.05 (Oneway ANOVA followed by Bonferroni post hoc test executed in STATA version 13.1). The plot was generated using GraphPad Prism software version 6.01.
Figure 4

showing the dose dependent decrease of Mean Corpuscular Volume (panel a) and Mean Corpuscular Hemoglobin Concentration (panel b) as shown by black arrows in the red box. The results were expressed as mean ± SD at 95% confidence level, p<0.05. One-way ANOVA followed by Bonferroni post hoc test executed in STATA version 13.1 showed that there was no significance across the treatment groups in each case. The plots were generated using GraphPad Prism software version 6.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Nyirendaetal1307GraphicalAbstract.tif