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Evaluation of antianemic potential compounds of the leaf of a plant in Togo Flora, *Lannea kerstingii* (Engl. and K. Krause)

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Abstract
Most medicinal plants are traditionally use all over the world to alleviate anaemia. Our study aimed at evaluating the antianaemic chemical composition of the leaf of *Lannea kerstingii* which is reported to be potent in the management of anaemia by traditional healers. The leaf was extracted using hot water. Phytochemicals, amino acid, vitamins and mineral composition was carried out using standard protocols. Phytochemical screening of the leaf extracts has revealed the existence of saponins, tannins, cardiac glycosides, flavonoids and alkaloids in the extracts. The elemental analysis of leaf extract has shown a good amount of some erythropoietic minerals such as iron, Selenium, zinc, Copper and Cobalt. The vitamins analysis has shown the presence of adequate amount of some haematopoietic compounds, such as folic acid, vitamin C, riboflavin. The amino acid analysis of the leaf has revealed the presence of essentials and non-essential amino acids. The amount of Cystine, tyrosine, methionine, phenylalanine, lysine and leucine are lower in *Lannea kerstingii* compare to the requirement for adults. The amount of Isoleucine, tryptophan, valine, histidine and threonine are higher in *Lannea kerstingii* compare to the requirement for adults. These results illustrate that the aqueous extracts of *Lannea kerstingii* leaf possess antianaemic properties The findings of our study suggest that the leaf of *Lannea kerstingii* contains haematopoietic properties thus, justifying the use of the plant to alleviate anaemia in Togo.

Keywords: *Lannea kerstingii*, anaemia, Togo, leaf extract, antianaemic properties

1. Introduction
Medicinal plants have been reported as having useful properties for the treatment of various diseases because they contain some bioactive compounds such as phytochemicals (Fasuyi, 2006) [11], secondary metabolites and components that can protect humans against ailments (Kumar *et al*., 2009) [16]. It is known that most of the rural population depends largely on herbal remedies, and there are so many beneficial advantages related and some plants have been reported all over the world as being rich source of therapeutic agents for the healing of diseases such as anaemia. Thus, medicinal plants are traditionally used to alleviate anaemia and are reported to be effective in the treatment of anaemia (Liu, 2003) [17].

Anaemia can be defined as the reduction of the total number of red blood cells in blood or the reduction of their quantity according to blood volume or the reduction of the blood haemoglobin content. Anaemia can be caused by low, insufficient or anomalous production of red blood cells, inappropriate red blood cells loss or destruction (Dacia and Lewis, 2004) [8].

According to WHO (2004) [24] reports, a third of the world populations are anaemic due to non-balanced food intake and anaemia can affect people of all ethnicities, races, countries or ages. Despite the incessant global efforts to provide food and the attempts to eliminate nutritional deficiency diseases, nutritional anaemia affects both emerging and developed countries with major bad outcome for human health and the development of their countries (WHO, 2005) [27]. Anaemia may also occur due to the abnormal intake of compounds which are important for erythropoiesis such as iron, folic acid and vitamin B12 (Howland and Mycek, 2009) [13] or because of persistent blood loss, bone marrow disorders, increased haemolysis, infection, malignancy, endocrine disorders and some diseases (Alper *et al*., 2000) [2].

The good news is that anaemia often can be successfully treated and even prevented. In nutritional deficiency anaemia, the deficient iron, folic acid, and vitamin b12 can be replaced by oral and injection forms. Depending on the type of anaemia, various treatments such as iron, vitamin B12, B9 or folate replacement, treatment with immune-suppressors or corticosteroids, erythropoietin injection, blood transfusion, or osseous marrow transplantation can be used (Tchogou *et al*., 2016) [24].

The reasons of the extensive use of medicinal plants in developing countries are their low price, their easy obtainability, their accessibility, their effectiveness and their impact on diseases (Ijioma *et al*., 2014) [14]. *Lannea kerstingii* Engl. & K. Krause (Anacardiaceae) is one of these medicinal plant which is locally used in West African countries such as Togo to treat several pathologies including anaemia (Diallo *et al*., 2009) [9]. In Côte d'Ivoire, it's stem bark and root are traditionally used for the management of diarrhea, gastritis, rheumatic, sterility, intestinal helminthiasis and also in some West African countries, *Lannea* plant species is used in management of oedema, rickets, wounds, scurvy, scorbut, epilepsy (Arbonnier, 2002) [3]. The raw fruit of *Lannea kerstingii* is also consumed in the Guinean pre-forest savannah of Côte d'Ivoire (Alain, 2001) [1].

Even if several studies have been carried out on the pharmacological effects of *Lannea kerstingii* Engl. & K. Krause (Anacardiaceae), much scientific research has not been conducted on the antianaemic aspect of the plant. Based on local claims on the antianaemic activity of *Lannea kerstingii* in Togo, our study is conducted to investigate the amino acid composition, the phytochemical screening, haematopoietic minerals and vitamin composition of the plat leaf. Consequently, our present research seeks to scientifically look at the antianaemic potential of *Lannea kerstingii*.

2. Materials and methods

2.1. Plant material

Young leaf of *Lannea kerstingii* was harvested in January 2019, washed and air-dried to a constant weight at room temperature for three weeks and then grounded at CERFOPLAM (Centre de Recherche et de formation sur les Plantes Médicinales), of the University of Lomé, TOGO using a mortar and a pestle. The powdered sample was sieved and then stored in polythene bags for further use.

2.2. Phytochemical analysis

5 grams of *Lannea kerstingii* leaf powdered samples was mixed into 50 ml of hot distilled water. After 15 minutes, the mixture was filtered for qualitative phytochemical analysis of alkaloids, flavonoids, tannins, saponins, terpenes/steroids, cardiac glycosides, balsams, carbohydrates, phenols and resins. The analysis was carried out on the filtrate using standard procedures for identification of phytochemical constituents as described by Sofowora, (1993) [22].

2.3. Vitamin Analysis

Vitamin C; Riboflavin and Folate analysis was carried out using the method described by Zhang *et al*., 2018 [28].

2.4. Elemental Analysis

The method was used to determine Iron, Copper, Selenium, Zinc and Cobalt.

2.5. Amino Acid Profile

The Amino acid analysis of the sample was carried out using methods described by Benitez (1989) [6]. The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

2.6. Extraction

Powdered samples were used for both phytochemical, amino acid and hematopoietic nutrient analysis. 350 g of grounded sample was mixed with 1000 ml of distilled water and the mixture was boiled in a 2500 ml flask for 15 minutes in order to obtain aqueous extract. The plant sample residue was filtered and then purified by filtration using Whatman No 1 filter paper. The filtrate was stored in an oven at 60 °C to dry completely in the Laboratory of the Department of Biochemistry, University of Jos. The dried extract was then grounded to have powdered extract which was stored in fresh sterile universal bottles in the refrigerator at 4 °C until required for use.

3. Results

3.1 Phytochemical screening

The qualitative phytochemical screening of the leaf of *Lannea kerstingii*, hot aqueous extract presented in Table 1 have shown the presence of saponins, terpenes/steroids, tannins, flavonoids, alkaloids, Cardiac glycosides, Balsams, and phenols.

Table 1: Phytochemical Screening of the Leaf

Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Terpenes/ Steroids	+
Cardiac Glycosides	+
Balsam	+
Carbohydrates	-
Phenol	+
Resins	-

(-) Negative result or absent, **(+)** = present or positive result. H. A. E= Hot Aqueous extract

Table 2: Anaemia-related mineral and vitamins

Component	*VALUE*
Vitamins	
Riboflavin (IU/l)	114.44±69.880
Folate (mg/ml)	0.608±0.304
Vit. C (mg/ml)	4.011±2.557
Elemental composition	
Fe (%)	0.065
Cu (mg/l)	7.50
Se (mg/l)	0.20
Zn (mg/l)	21.60
Co (mg/l)	0.320

Tabulated values expressed as Mean± SD, n=3.

3.2. Vitamin and mineral composition

The elemental analysis of *Lannea kerstingii* leaf has revealed the presence of a reasonable quantity of Copper and Zinc, iron, Selenium, and Cobalt. The vitamin analysis of *Lannea kerstingii* leaf has shown the presence of riboflavin, folic acid and vitamin C (Table 2)

3.3. Amino acid composition

Table 3 represents the amino acid concentration of *Lannea kerstingii* leaf. From these values, it can be seen that Cystine, tyrosine, methionine, phenylalanine, lysine and leucine are lower in *Lannea kerstingii* compared to the requirement for adults. Isoleucine, tryptophan, valine, histidine and threonine are higher in *Lannea kerstingii* compared to the requirement for adults.

Table 3: The Amino Acid Content of *Lannea kerstingii*

Amino Acid Concentration: G/100g Protein	Raw Leaves	Leaves Extract	Requirement For Adults
Essential Amino Acids			
Leucine	5.75	4.09	5.9
Lysine	3.45	1.83	4.5
Isoleucine	3.21	2.03	3.0
Phenylalanine	4.26	2.13	3.8
Tryptophan	1.39	0.74	0.6
Valine	3.22	1.93	3.9
Methionine	1.44	0.86	2.2
Tyrosine	2.75	2.93	3.8
Histidine	2.21	1.25	1.5
Cystine	1.33	1.09	2.2
Threonine	3.22	4.11	2.3
Non-Essential Amino Acids			
Proline	4.06	2.34	
Arginine	5.33	6.19	
Alanine	4.02	3.19	
Glutamic acid	9.01	6.36	
Glycine	3.37	2.02	
Serine	4.00	2.00	
Aspartic acid	7.20	4.22	

4. Discussion

A large set of rural population mostly depends on herbal remedies, and there are many beneficial advantages related to the usage of these medicines. Human beings are used to therapeutic agents for good health, longevity, remedies against sickness and discomfort. This push people to explore their natural surroundings and led to the use of various medicinal plants (Nair & Chanda., 2017) [20]. Table 3 presents the amino acid concentration of *Lannea kerstingii* leaf such as the essential and non-essentials amino acids. The qualitative phytochemical screening of the extracts has revealed that the leaf of *Lannea kerstingii* contains saponins, terpenes/steroids, tannins, flavonoids, alkaloids, Cardiac glycosides, Balsams and phenols. These results are the conclusion of the fact that the leaf of *Lannea kerstingii* is constituted by a diversity of phytochemicals and these components has various protective and therapeutic effects (Asaolu *et al*., 2009) [4] and this is probably the reason why *Lannea kerstingii* is used the most in management of anaemia by Traditional Healers in Togo.

Flavonoids are a big group of plant phenols that furnish a great deal of flavour and colour to fruits and vegetables. This may be the reason for the purplish red colour released despite its green leaf when boiled. Flavonoids, phenolic compounds and tannins are polyphenols are some bioactive compounds identified in *Lannea kerstingii* which have powerful antioxidant properties (Beack *et al*., 2020) [5]; this can enhance an adequate utilization of the haematopoietic nutrients present in the aqueous leaf extracts of *Lannea kerstingii* to synthesize haeme/haemoglobin for new red blood cells. This can especially lead to improve Hb, PCV and RBC (Luka, Abdulkarim, Adoga, Tijjani & Olatunde, 2014) [18] when it is consumed. Flavonoids and glutamic acid are important for the synthesis of haemoglobin and can boost blood production in the bone marrow. Phytochemicals and amino acids present in the extracts can demonstrate haematopoietic potentials when consumed. Antioxidants presence in the plant can trigger erythropoiesis *Lannea kerstingii* has powerful antioxidant properties (Diallo *et al*., 2010) [9]. Thus, we can conclude that these phytochemicals can protect RBCs as powerful antioxidants which will prevent or repair damage done to red cells by the action of free radicals or highly reactive oxygen species.

The vitamins and mineral composition of the plant (Table 2) has shown an adequate presence amount of some haematinic compounds, such as folic acid, vitamin C, riboflavin, Se, Cu, Fe, Co and Zn. Folic acid and some other vitamin components in erythropoiesis has been documented as haematopoietic factors and have been found to be effective in relieving the symptoms of anaemia. Vitamins C contribute to the proper utilization of iron and improve intestinal absorption of non-haem iron by reducing ferric ion to a ferrous form (Umaru *et al*.,2018) [23]. Fishman *et al*. (2000) [12] stated that optimal presence of folic acid, vitamin C and riboflavin can be used to control anaemia. Folate can cure megaloblastic anaemia when Riboflavin enhances haematological response to iron. Copper acts in Hb synthesis and is necessary for oxidation of ferrous iron to ferric form and is important for the incorporation of iron into Hb. Cobalt act also for Vitamin B12 synthesis which in turn is necessary for the normal production of erythrocytes when Zn increases red cell resistance to oxidative stress. Selenium is a key component of the active site of GSH-Px which is responsible for the protection of haemoglobin in erythrocytes from oxidative stress, the decrease of lipid peroxidation of membrane lipids, the denaturation of haemoglobin, and haemolysis and increase osmotic resistance of erythrocytes. This probably will increase haemoglobin level in the body. Thus, *Lannea kerstingii* can contribute in the faster reversal of the effects of nutritional anaemia

Conclusively, the present study tends to suggest that the oral consumption of the aqueous leaf extract of *Lannea kerstingii* have antianaemic potential fashion due to its nutritional composition. Therefore, our study scientifically supports the traditional use of leaves of *Lannea kerstingii* in enhancing haematological parameters and improving health in the treatment of anaemia.

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