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In Vivo Antianaemic Activity of *Dalbergia Ecastaphyllum* and *Millettia barteri*, Two Plants Used for Controlling Sickle Cell Disease and Associated Disorders

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Abstract | **Original Research Article**

Background: Sickle cell disease is a disease that is of a real public health concern in many West African countries such Côte d'Ivoire. The associated diseases are anaemia, pain, which are mainly managed with medicinal plants. The aim of this study was to evaluate the potency of *Dalbergia ecastaphyllum* (DA) and *Millettia barteri* (MB), used in traditional medicine for the treatment of sickle cell disease. ***Methods:*** The anti-anaemic activity of the aqueous extracts of leaves was studied in male Swiss Wistar mice aged 6-8 weeks. After induction of anaemia with Phenylhydrazine (PHZ) through intraperitoneal injection, the mice was subsequently treated with an 50, 100 and 350 mg/kg bw of aqueous extract of MB and DE over a 21-day period. Acute toxicity and Phytochemicals were assessed using standard procedures. Parameters such as body weight, white blood cells (WBC), haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV) were measured. ***Results:*** These two plants were not toxic at doses of 2000 and 5000 mg/kg body weight. The PHZ induced anaemia was corrected after three weeks of oral administration of extracts to mice and the effect was higher at 50 mg/kg body weight. The hemoglobin increased from 12.98 g/dL before treatment, increases to 14.04 g/dL after treatment with MB extract and from 12.66 g/dL to 14.06 g/dL with DE. Phytochemical analysis revealed these plants contained polyphenols, flavonoids, anthocyanins, tannins, coumarins and aromatic amino acids. ***Conclusion:*** These results validate the traditional use of DE and MB against anaemia, one of the main associated disorders to sickle cell disease.

Keywords: Anaemia; Sickle cell disease; Phytochemicals; Acute toxicity; *Dalbergia ecastaphyllum*; *Millettia barteri*.

INTRODUCTION

Sickle cell disease (SCD) or sickle cell anaemia is a genetic disorder that affects approximately 3-3.6% of the world's population (WHO, 2010). About 120 million people are affected by the S gene mutation (Weatherall & Clegg, 2001, N'draman-donou *et al*., 2015). Each year, around SCD 500,000 children are born worldwide (Modell & Darlinson, 2008). About 5 million people died each year and the rate can reach up 20% of the population in some African areas (Yuma *et al.*, 2013). Africa has been associated with the highest prevalence of the sickle cell trait, with figures suggesting that between 10% and 40% of the entire population may be affected (Agasa *et al.*, 2010). The incidence of sickle cell trait ranges from 20% to 30% in Cameroon, the Democratic Republic of the Congo, Gabon, Ghana, and Nigeria (Adigwe *et al*., 2023). In these African countries, the mortality in children under 5 years could be higher and close to 90% due to various factors such as malnutrition, poverty and default of early diagnostic (Piel, 2017).

Sickle cell disease is a great public health problem in Côte d'Ivoire, and a prevalence ranges from 10 % to 14% in the overall population (N'draman-donou *et al*., 2015; Kakou Danho *et al*., 2021). Common signs and symptoms associated to SCD are painful swelling, aplastic crisis, splenic sequestration crisis, acute chest syndrome and haemolytic crisis (Serjent, 2001; Tsaras *et al*., 2009). These various severe and chronic complications affected hands and feet, blood, skin, eyes, chest and include infections. The haemolytic anaemia is

a complication of great concern. The control of this disease is curved by the ignorance of people on the disease and the high cost of modern treatment. Interestingly medicinal plants are available, and many people depend on phytotherapy for relieving patients. Scientific discovery of antisickling properties of some medicinal plants revealed the efficacy and interest of *Cajanus cajan*, *Zanthoxylum zanthoxyloides*, *Carica papaya* and *Parquetina nigrescens* by reducing crisis and reversing sickling (). Some studies reported anthocyanins as plant metabolites responsible for the anti-sickle cell disease (Kambale *et al*., 2013; Ngbolua *et al*., 2013).

This study concentrated on the anti-anaemic potential of *Dalbergia ecastaphyllum* (L.) Taub and *Millettia barteri* (Benth.) Dunn (Fabaceae) used for treating anaemia, pain and other health conditions associated to SCD. Many ethnobotanical surveys documented their utilization in traditional medicine in Côte d'Ivoire and elsewhere in West Africa in receipts against sickle cell disease and anaemia (Koné & Kamanzi, 2006; Aké-Assi, 2011; Eklu-Natey & Balet, 2011; N'draman-donou *et al*., 2015; Béné *et al*., 2016).

Dalbergia ecastaphyllum is a vining shrub with tangled and flexible branches, forming impenetrable bushes 3-4 m high. This plant is localized on the coast, either on the parts directly in contact with the tides in the sands of the coast, or on the edges of the lagoons and the silted estuaries behind the mangroves (Eklu-Natey & Balet, 2011).

Millettia barteri is a large vine with a height of over 30 m. This is distributed in tropical and subtropical regions of Africa. This species has been recorded from Senegal east to Sudan and south to Angola (Nyunaï, 2011).

MATERIAL AND METHODS

Preparation of extracts

The leaves of *Dalbergia ecastaphyllum* and *Millettia barteri* were harvested at the University NANGUI ABROGOUA in October 2018. The plants were authenticated at the herbarium of Centre National de Floristique at the University Felix HOUPHOUËT-BOIGNY (Côte d'Ivoire) where voucher specimen is stored.

Figure 1: Leafy twig of *Dalbergia ecastaphyllum*

Figure 2: Leafy twig of *Millettia barteri*

The leaves were dried for 7 days under air conditioning (18±2 °C), then grinded with a blender to obtain fine powder. Ten (10) g of powder were added to 100 mL of distilled water and boiled (100°C) for 15 minutes. The concoction was filtered on Whatman paper. After drying at 30 °C, the aqueous extracts of *M. barteri* (AEMB) and *D. ecastaphyllum* (AEDE) obtained were weighted and kept until use for bioassay.

Qualitative phytochemical analysis

This screening was performed using methods described by Harbone (1998) and Fofana (2004). The presence of phytocompounds was accessed with the following tests.

Alkaloids characterization

A volume of 6 ml of each extract was dried and dissolved in 6 ml of ethanol 60%. Few drops of Dragendorff's and Bouchardat alkaloidal reagents were added to each tube and the presence or absence of any turbidity or precipitates was noted in each test tube.

Phenolic compounds characterization

A few drops of 2% $FeCl_3$ solution were added to 2 ml of each extract. The appearance of deep blue, black or green colour indicates the presence of phenolic compounds.

Tannins characterization

5 ml of each extract were evaporated and dissolved in 15 ml of Stiasny reagent (10 ml of formol 30%, 5 ml of concentrated HCl, v/v). The mixtures were boiled over a steam bath at 80°C during 30 min, and then allowed to cool. The positive reaction characterized by big brown flakes indicates the presence of non-hydrolysable tannins. The present extract was filtrated

and saturated with sodium acetate. When treated with few drops of 10% ferric chloride test solution, a deep green colour indicated hydrolysable tannins.

Flavonoids characterization

Total flavonoids were determined by adding 5 drops of sodium hydroxide (NaOH) 10% to 2 mL of each filtrate. The appearance of a yellow-orange coloration indicates the presence of total flavonoids.

Anthocyanins characterization

The anthocyanins were determined by adding few drops of sulfuric acid (H_2SO_4) 2N to 2 mL of each filtrate, followed by few drops of ammonia. The appearance of a purple coloration indicates the presence of anthocyanins.

Saponosids characterization

The filtrate was vigorously shaked and the height of foam observed. After 15 min, the persistence of a foam's height ≥ 1 cm indicates the presence of saponosids.

Aromatic amino acids characterization

Few drops of nitric acid were added to 2 mL of the filtrate. The presence of aromatic ring (aromatic amino acids) was detected by the appearance of a yellow coloration.

Study of toxicity and haematological parameters

Maintenance of animals

Swiss albino white mice, nulliparous and non-pregnant were maintained at a temperature of 25±2 °C and a photoperiod of 12 hours (light) and 12 hours (dark) at the unit. The mice were fed with rodent pellets and water *ad libitum*. These animals aged 6-8 weeks and weighted 20-30 g.

Animals were maintained and all experiments were conducted following the OECD guidelines 425 as recommended by the ethic group of our university.

Study of acute toxicity

The procedure is a sequential test that uses a maximum of 5 animals. The LD_{50} was determined in mice using the limit dose of 5000 mg/kg body weight (bw) (OECD, 2006). One mouse was starved for 3 hours but allowed free access to water. then weighed. The extract was orally administered at a single dose of 20 mL/Kg bw. After administration, the animal was still maintained fasting for 1 hour. Three (3) animals were treated successively for 48 hours and observed during 14 days for any signs of general illness, change of behavior and mortality.

Study of hematologic parameters

Induction of anaemic condition

Anaemic condition was induced experimentally by an intraperitoneal administration of 5 mg/kg body weight phenyl hydrazine (PHZ) daily from Sigma (Steinhein, Switzerland) to each mouse for seven days. On the eighth day, approximately 1 mL of venous blood was collected for hematological studies by nipping the tails of the mice. Collected blood was stored in EDTA treated plastic tubes at room temperature. 60 male white albino mice weighing on average 25 g were used. The hematological parameters determined using the Coulter Counter included white blood cells (WBC), haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), mean cell hemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV). Mice that were observed to have haemoglobin and RBC levels of below 11 g/dL and 3.0 × 1012/L, respectively were considered anaemic.

Experiment design

The anti-anaemic assay was performed according to the method described by Musyoka *et al*. (2016). Forty (40) female albino white mice individually identified were involved in the present study and randomly distributed in 8 groups of 5 animals. These animal groups included the normal mice (reference group), anaemic control mice (positive control group), anaemic experimental mice orally administered with plant extracts as following:

- Group 1: normal mice (negative control or reference group)
- Group 2: anaemic control mice (positive control)
- Group 3 and 4: anaemic experimental mice orally treated with 50 mg/kg body weight of plant extract
- Group 5 and 6: anaemic experimental mice orally administered with 100 mg/kg bodyweight of plant extract
- Group 7 and 8: anaemic experimental mice orally treated with 350 mg/kg body weight of plant extract.

Blood sampling for determination of hematological parameters

21 days post-administration of extracts to anaemic experimental mice, 1 mL of blood were collected from fasting animal. Blood samples were drawn from cleaned tails (ethanol 90 °C) (Liu *et al*., 1996) in tubes treated with EDTA for the analysis. Hematological parameters such as red blood cell count, packed cell volume, hemoglobin, mean cell hemoglobin concentration, mean cell hemoglobin, mean cell volume and white blood cell count were determined using a Coulter Counter machine.

Statistical Analysis

Data was expressed as Mean ± standard deviation (SD). The differences between the means of the various groups of animals in the efficacy study (normal, untreated anaemic, anaemic treated with 50, 100, and 350 mg/kg body weight of the aqueous extracts) was done using ANOVA and post ANOVA statistical test while the difference between the means of the two

groups used in the toxicity study was done using student's T-test (in case of normality). The Kruskal-Wallis test (no normality) was performed when there was no stabilization of the variances of transformed variables (Logarithm and Root). The level of significance for all the analyses including Fisher F-test was set at a value of $p < 0.05$. All these tests were carried out using the software R. 3.5.1 version 2018.

RESULTS

Phytochemical content

Phytochemical screening carried out on the aqueous extracts from leaves of *D. ecastaphyllum* and *M. barteri* revealed the presence of phytocompounds such as polyphenols, flavonoids, anthocyanins, tannins, coumarins and aromatic aminoacids.

Acute toxicity of aqueous extracts in mice

The results of the acute toxicity showed no signs of general illness, change of behavior and mortality at 5000 and 2000 mg/kg body weight (bw) of aqueous extracts of *Dalbergia ecastaphyllum* and *Millettia barteri* (LD_{50} > 5000 mg/kg bw). AEMB and AEDE were classified in category 5 and non-toxic by oral route.

Hematologic parameters after induction of anaemia

Sub-chronic intoxication of mice with 5 mg PHZ/ /kg bw for eight days resulted in anaemia characterized by decreased red blood cell count, hemoglobin and packed cell volume and normal mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume and white blood cell count. The decrease was significant ($p < 0.05$) for erythrocyte count in the seven experimental groups but not significant ($p = 0.47$) in the negative control group ($7.4 \times 10^6/\mu L$). A significant decrease ($p < 0, 05$) of hemoglobin levels also was noted in experimental batches (9.48-10.50 g/dL) in comparison to the negative control group (12.48 g/dL).

Effect of aqueous extract of *Millettia barteri* (AEMB) on hematological parameters

The oral administration of aqueous extracts of *Millettia barteri* leaves in mice at doses of 50, 100 and 350 mg/kg body weight daily for three weeks had significant effect on all the measured hematological parameters as shown in Table III. There was a very high significant difference ($\alpha = 5\%$, $P < 0.0001$) between the different test batches and control for blood cell levels at all doses. Three weeks post-treatment with AEMB, the RBC were $7.55 \times 10^6/\mu L$, $8.40 \times 10^6/\mu L$ and $8.57 \times 10^6/\mu L$ at 50, 100 and 350 mg/kg bw respectively and were not statistically different. However, the dose 100 mg/kg bw significantly increased the reduced level of RBC (Figure 3A). In addition, an increase in hemoglobin levels was observed in animals treated with AEMB (Figure 3B), the highest increase was recorded at 50 mg/kg bw (14.06 g/dL), followed by 100 mg/kg bw (13.74 g/dL). This hemoglobin gain was significant in treated groups ($p < 0.05$) compared to anaemic experimental group (11 g/dL).

For WBC, there was no significant difference between untreated and treated mice ($p > 0.05$). However, at the end of treatment, there was a non-significant drop ($p > 0.05$) in all treated batches compared to the anaemic control group (Ta: Anaemic). The Leukocyte levels at 50, 100, and 350 mg/kg body weight remained lower than the negative control group (tr: untreated mice). Also, there was a difference in erythrocyte index in the treated groups compared to the negative control group (tr: untreated mice). However, this difference was not significant ($p > 0.05$).

No significant range was observed between the mean values of MCV in treated groups (52.60 FL -58.90 fL) and negative control (55.68 fL). For the MCH level of animals treated with AEMB, the values remained homogeneous and ranged from 15.98 pg to 16.54 pg for treated anaemic experimental mice and 16.48 pg for the negative control three weeks post-treatment. The administration of AEMB did not show any significant effect on MCHC with mean value = 29.58 -31.80 g/dL in the treated animals, and 29.56 g/dL in the negative control.

Effect of aqueous extract of *Dalbergia ecastaphyllum* (AEDE) on haematological parameters

Table 1 shows the effects of three weeks oral administration of aqueous leaves of *Dalbergia ecastaphyllum* in mice on haematological parameters. The continued daily administration of the three doses of this extracts significantly increased the reduced levels of RBC and Hb (Figure 3C). Interestingly 21 days post-treatment, AEDE returned the measured haematological parameters to normal values and animals almost completely recovery the starting haematological status in comparison to the anaemic positive control group (11 g/dL). This result was highly significant at dose 100 mg/Kg of bw with a value of 14.05 g/dL (Figure 3D).

As depicted in table IV, at the end of the third week, the WBC levels ($8.22\text{-}9.78 \times 10^3/\mu L$) were slightly lower in treated groups compared to negative control group ($10.16 \times 10^3/\mu L$). However, for MCV, no significant range ($p > 0.05$) was observed between AEDE-treated groups (52.96-54.78 fL), and the negative control (55.68 fL). For MCH, rates about 16.46 pg and 16.98 pg in animals treated with AEDE were low in comparison to the negative control Batch (46.48 pg). The oral administration of AEDE in the present study did not cause significant changes in the MCHC values (30.42-31.38 g/dL) compared to the negative control group (29.56 g/dL).

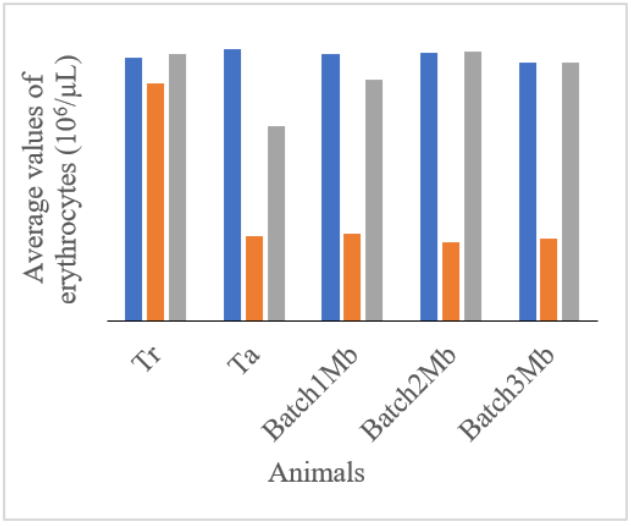

Figure 3A: **Effect of aqueous extract of *Millettia barteri* on erythrocytes**

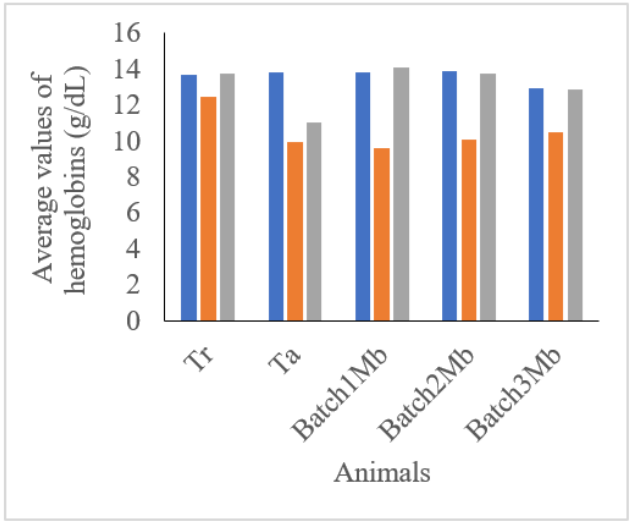

Figure 3B: **Effect of the aqueous extract of *Millettia barteri* on hemoglobin**

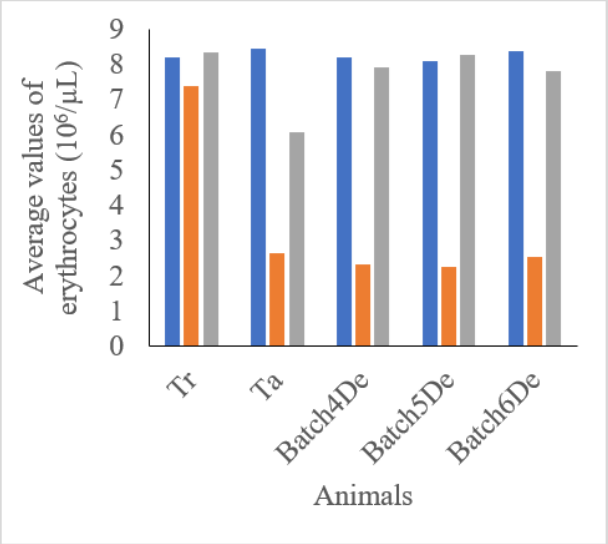

Figure 3C: Effect of aqueous extract of *Dalbergia ecastaphyllum* on mean values of erythrocytes

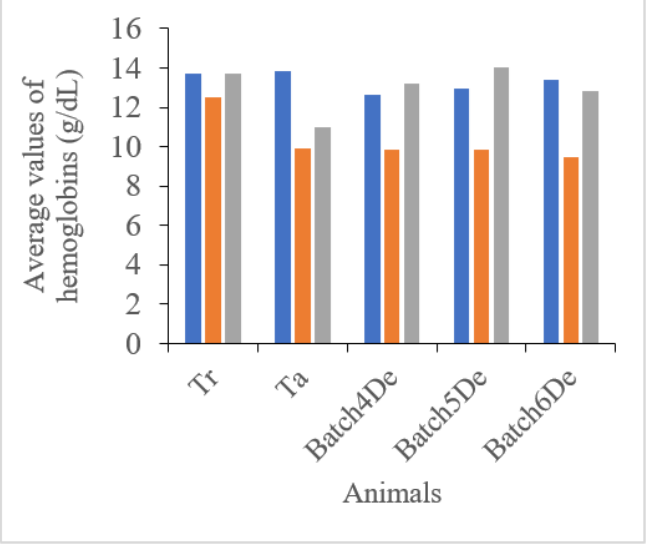

Figure 3D: **Effect of the aqueous extract of *Dalbergia ecastaphyllum* on mean values of hemoglobin**

Day 0 Day 8 Day 29

Tr: negative control group; Ta: anaemic control group; Batch1Mb: anaemic + 50 mg/kg bw; Batch2Mb: anaemic + 100 mg/kg bw; Batch3Mb: anaemic + 350 mg/kg bw; Batch4De: anaemic + 50 mg/kg bw; Batch5De: anaemic + 100 mg/kg bw; Batch6De: anaemic + 350 mg/kg bw; J0: day of collection before induction of anaemia; J8: day of collection after induction of anaemia; J29: day of collection after three weeks of treatment; n : 5 animals per group; Mb: *Millettia barteri*; De: *Dalbergia ecastaphyllum*

Table III: Rates of leukocytes (WBC) and erythrocyte index (MCV, MCH, MCHC) of mice after administration of the aqueous extract of *Millettia barteri*

Blood parameters	Batches	Day 0	Day 8	Day 29	P values
Hb g/dL	Tr : Negative control (–)	13.68 ± 0.60 [a]	12.48 ± 0.50 [a]	13.72 ± 0.40 [a]	0,38
	Ta : Anaemic (+)	13.84 ± 0.67 [b]	9.92 ± 0.95 [a]	11.00 ± 1.27 [a]	0,0001
	Batch1Mb: Anaemic + (50 mg/Kg bw)	13.80 ± 0.36 [a]	9.60 ± 1.23 [b]	14.06 ± 0.50 [a]	0,0001
	Batch2Mb: Anaemic + (100 mg/Kg bw)	13.86 ± 1.39 [a]	10.06 ± 0.73 [b]	13.74 ± 1.09 [a]	0,0001
	Batch3Mb: Anaemic + (350 mg/Kg bw)	12.94 ± 0.78 [a]	10.50 ± 0.55 [b]	12.86 ± 0.65 [a]	0,0001
RBC **10^3/µL**	Tr : Negative control (–)	8.22 ± 0.49 [a]	7.40 ± 0.81 [a]	8.35 ± 0.32 [a]	0,47
	Ta : Anaemic (+)	8.47 ± 0.41 [a]	2.66 ± 0.15 [b]	6.07 ± 1.01 [a]	0,0001
	Batch1Mb: Anaemic + (50 mg/Kg bw)	8.33 ± 0.69 [a]	2.73 ± 0.67 [b]	7.55 ± 1.78 [a]	0,0001
	Batch2Mb: Anaemic + (100 mg/Kg bw)	8.37 ± 1.07 [a]	2.46 ± 0.21 [b]	8.40 ± 0.65 [a]	0,0001
	Batch3Mb: Anaemic + (350 mg/Kg bw)	8.08 ± 0.47 [a]	2.57 ± 0.32 [b]	8.05 ± 0.60 [a]	0,0001
WBC **(10^3/µL)**	Tr : Negative control (–)	10.10 ± 1.61[a]	10.46 ± 0.78[a]	10.16 ± 1.19[a]	0.58
	Ta : Anaemic (+)	9.30 ± 1.95[a]	8.00 ± 2.03[a]	8.86 ± 1.87[a]	0.51
	Batch1Mb: Anaemic + (50 mg/Kg bw)	7.24 ± 0.74[a]	8.78 ± 1.96[a]	7.02 ± 1.05[a]	0.32
	Batch2Mb: Anaemic + (100 mg/Kg bw)	7.10 ± 1.40[a]	7.48 ± 2.26[a]	7.10 ± 2.60[a]	0.68
	Batch3Mb: Anaemic + (350 mg/Kg bw)	8.20 ± 3.54[a]	8.26 ± 1.96[a]	7.30 ± 1.61[a]	0.56
MCV (fL)	Tr : Negative control (–)	55.10 ± 1.47[a]	56.16 ± 5.47[a]	55.68 ± 2.60[a]	0.89
	Ta : Anaemic (+)	53.84 ± 1.49[a]	67.38 ± 2.28[b]	54.60 ± 1.81[a]	0.0001
	Batch1Mb: Anaemic + (50 mg/Kg bw)	53.46 ± 4.24[a]	62.86 ± 9.18[a]	58.90 ± 8.82[a]	0.19
	Batch2Mb: Anaemic + (100 mg/Kg bw)	52.96 ± 3.46[a]	69.08 ± 3.08[b]	55.46 ± 3.24[a]	0.0001
	Batch3Mb: Anaemic + (350 mg/Kg bw)	53.94 ± 2.51[a]	70.46 ± 2.16[b]	52.60 ± 1.19[a]	0.0001
MCH **(pg)**	Tr : Negative control (–)	16.64 ± 0.57[a]	16.98 ± 1.43[a]	16.48 ± 0.41[a]	0.75
	Ta : Anaemic (+)	16.36 ± 0.42[a]	27.30 ± 0.64[b]	16.54 ± 0.60[a]	0.0001
	Batch1Mb : Anaemic + (50 mg/Kg bw)	16.60 ± 1.01[a]	27.34 ± 0.77[b]	16.32 ± 0.42[a]	0.0001
	Batch2Mb : Anaemic + (100 mg/Kg bw)	16.62 ± 1.19[a]	26.98 ± 1.05[b]	16.38 ± 0.87[a]	0.0001
	Batch3Mb : Anaemic + (350 mg/Kg bw)	16.00 ± 0.49[a]	27.48 ± 2.14[b]	15.98 ± 0.61[a]	0.0001
MCHC **(g/dL)**	Tr : Normal (–)	30.22 ± 1.56[a]	30.32 ± 1.66[a]	29.56 ± 1.40[a]	0.70
	Ta : Anaemic (+)	30.36 ± 1.21[a]	40.56 ± 1.41[b]	30.38 ± 1.58[a]	0.0001
	Batch1Mb : Anaemic + (50 mg/Kg bw)	31.14 ± 1.77[a]	35.54 ± 5.23[a]	31.80 ± 3.29[a]	0.17
	Batch2Mb : Anaemic + (100 mg/Kg bw)	31.42 ± 2.31[a]	39.08 ± 0.46[b]	29.58 ± 2.50[a]	0.0001
	Batch3Mb : Anaemic + (350 mg/Kg bw)	29.74 ± 1.63[a]	39.00 ± 3.13[b]	30.38 ± 0.89[a]	0.0001

MCV: mean of cell volume; MCH: mean of cell haemoglobin; MCHC: mean of cell haemoglobin concentration ; WBC: white blood cell; Tr: negative control group; Ta: anaemic positive control group; batch1De: 50 mg/kg bw; Batch2De: 100 mg/kg bw; Batch3De: 350 mg/kg bw; P: probability; Day 0: collection before induction of anaemia; Day 8: collection after induction of anaemia; Day 29: sampling after three weeks of treatment with the aqueous extract of *M. barteri*.

The values with the same superscript letters on the same line are not statistically different.

DISCUSSION

This study was carried out for identifying medicinal plants for the management of sickle cell anaemia. Phytochemical screening of aqueous extracts from leaves of *D. ecastaphyllum* and *M. barteri* revealed the presence of phytocompounds such as polyphenols, flavonoids, anthocyanins, tannins, coumarins and aromatic aminoacids. The presence of these compounds may help predict the therapeutic interest of the two studied plants in sickle cell management. Many studies targeted anthocyanosids for the control of sickle cell disease (Ngbolua *et al.*, 2013 ; Kambale *et al.*, 2013 ; Yuma *et al.*, 2013). Longanga *et al.* (2000) reported the anti-inflammatory activity of tannins, saponins, flavonoids, sterols, ploy terpenes and reducing sugars. In addition, phenolic compounds such as flavonoids, tannins, anthocyanins, coumarins are endowed with antibacterial and antiviral properties (Thati *et al.*, 2007; Bajerova *et al.*, 2014; Zerargui, 2015) and antisickle cell anaemia. The patients with sickle cell disease are sensitive to bacterial infections (Mabiala-Babela *et al.*, 2005; Lamarre, 2013).

Oral administration of a single dose of aqueous extracts of the leaves of *D. ecastaphyllum* and *M. barteri* revealed that these plant species were not toxic (LD_{50} > 5000 mg/kg bw). Such a result is of great importance for the consumer security when using these plants or their derived products. After administration of phenylhydrazine®, the examination of blood parameters (RBC, Hb) showed that anaemia was induced in mice of all batches except negative control. Hemoglobin and red blood cell counts were below 11 g/dL and 3.106 g/µL, respectively. For example, the hemoglobin level moved from 12.66 ± 0.72 g/dL before induction to 9.82 ± 0.37 g/dL in batch4De, after induction. In addition, the red blood cell count (prior induction), decreased from 8.20 ± 0.32 × 106/µL to 2.31 ± 0.26 106/µL (Group Batch4De) at the end of induction. These values below the limits indicate that the animals were anaemic. Similar results have also been reported in mice given phenylhydrazine to induce anaemia. It has been reported that

administration of PHZ to mice results in decreased red blood cell counts, hemoglobin and hematocrit and normal mean cellular hemoglobin, mean cellular hemoglobin concentration, mean cell volume and white blood cell count (Agbor *et al*., 2005; Musyoka *et al*., 2016).

Table IV: Rates of white blood cells (WBC) and erythrocyte index (MCV, MCH, MCHC) of mice following administration of the aqueous extract of *Dalbergia ecastaphyllum*

Blood parameter	Batchs	Day 0	Day 8	Day 29	P
Hb g/dL	Tr : Negative control (–)	13.68 ± 0.60 [a]	12.48 ± 0.50 [a]	13.72 ± 0.40 [a]	0,64
	Ta : Anaemic (+)	13.84 ± 0.67	9.92 ± 0.95 [b]	11.00 ± 1.27 [a]	0,0001
	Batch4De : Anaemic + (50 mg/Kg bw)	12.66 ± 0.72 [a]	9.82 ± 0.37 [b]	13.18 ± 0.94 [a]	0,0001
	Batch5De : Anaemic + (100 mg/Kg bw)	12.98 ± 0.92 [a]	9.86 ± 0.68 [b]	14.04 ± 0.80 [a]	0,0001
	Batch6De : Anaemic + (350 mg/Kg bw)	13.40 ± 1.42 [a]	9.48 ± 1.20 [b]	12.80 ± 0.48 [a]	0,0001
RBC 10^3/µL	Tr : Negative control (–)	8.22 ± 0.49 [a]	7.40 ± 0.81 [a]	8.35 ± 0.32 [a]	0,61
	Ta : Anaemic (+)	8.47 ± 0.41 [a]	2.66 ± 0.15 [b]	6.07 ± 1.01 [a]	0,0001
	Batch4De : Anaemic + (50 mg/Kg bw)	8.20 ± 0.32 [a]	2.31 ± 0.26 [b]	7.93 ± 0.96 [a]	0,0001
	Batch5De : Anaemic + (100 mg/Kg bw)	8.09 ± 0.55 [a]	2.25 ± 0.17 [b]	8.28 ± 0.42 [a]	0,0001
	Batch6De : Anaemic + (350 mg/Kg bw)	8.38 ± 0.74 [a]	2.52 ± 0.37 [b]	7.81 ± 0.52 [a]	0,0001
WBC (10^3/µL)	Tr : Negative control (–)	10.10 ± 1.61[a]	10.46 ± 0.78[a]	10.16 ± 1.19[a]	0.58
	Ta : Anaemic (+)	9.30 ± 1.95[a]	8.00 ± 2.03[a]	8.86 ± 1.87[a]	0.51
	Batch4De : Anaemic + (50 mg/Kg bw)	8.36 ± 2.48[a]	9.30 ± 2.30[a]	8.22 ± 1.93[a]	0.71
	Batch5De : Anaemic + (100 mg/Kg bw)	8.16 ± 1.96[a]	8.08 ± 2.92[a]	9.78 ± 1.00[a]	0.38
	Batch6De : Anaemic + (350 mg/Kg bw)	8.40 ± 2.62[a]	9.78 ± 4.00[a]	8.62 ± 1.07[a]	0.37
MCV (fL)	Tr : Negative control (–)	55.10 ± 1.47[a]	56.16 ± 5,47[a]	55.68 ± 2.60[a]	0.89
	Ta : Anaemic (+)	53.84 ± 1.49[a]	67.38 ± 2.28[b]	54.60 ± 1.81[a]	0.0001
	Batch4De : Anaemic + (50 mg/Kg bw)	55.38 ± 1.69[a]	74.98 ± 7.49[b]	54.78 ± 1.92[a]	0.0001
	Batch5De : Anaemic + (100 mg/Kg bw)	52.76 ± 2.88[a]	78.26 ± 10.77[b]	54.20 ± 2.50[a]	0.0001
	Batch6De : Anaemic + (350 mg/Kg bw)	52.90 ± 3.06[a]	70.02 ± 3.17[b]	52.96 ± 3.03[a]	0.0001
MCH (pg)	Tr : Negative control (–)	16.64 ± 0.57[a]	16.98 ± 1.43[a]	16.48 ± 0.41[a]	0.75
	Ta : Anaemic (+)	16.36 ± 0.42[a]	27.30 ± 0.64[b]	16.54 ± 0.60[a]	0.0001
	Batch4De : Anaemic + (50 mg/Kg bw)	15.44 ± 0.96[a]	27.44 ± 1.23[b]	16.66 ± 0.93[a]	0.0001
	Batch5De : Anaemic + (100 mg/Kg bw)	16.04 ± 0.71[a]	27.82 ± 1.62[b]	16.98 ± 1.00[a]	0.0001
	Batch6De : Anaemic + (350 mg/Kg bw)	15.96 ± 0.55[a]	28.16 ± 1.95[b]	16.46 ± 0.67[a]	0.0001
MCHC (g/dL)	Tr : Negative control (–)	30.22 ± 1.56[a]	30.32 ± 1.66[a]	29.56 ± 1.40[a]	0.70
	Ta : Anaemic (+)	30.36 ± 1.21[a]	40.56 ± 1.41[b]	30.38 ± 1.58[a]	0.0001
	Batch4De : Anaemic + (50 mg/Kg bw)	27.86 ± 1.46[a]	36.92 ± 4.41[b]	30.42 ± 1.14[a]	0.0001
	Batch5De : Anaemic + (100 mg/Kg bw)	30.44 ± 2.01[a]	36.08 ± 5.25[a]	31.38 ± 1.58[a]	0.06
	Batch6De : Anaemic + (350 mg/Kg bw)	30.09 ± 1.70[a]	40.16 ± 1.14[b]	31.08 ± 0.78[a]	0.0001

MCV: mean of cell volume; MCH: mean of cell hemoglobin; MCHC: mean of cell hemoglobin concentration ; WBC: white blood cell; Tr: negative control group; Ta: anaemic positive control group; Batch4De: 50 mg/kg bw; Batch5De: 100 mg/kg bw; Batch6De: 350 mg/kg bw; P: probability; ; Day 0: blood collection before induction of anaemia; Day 8: blood collection after induction of anaemia; Day 29: sampling after three weeks of treatment with the aqueous extract of *D. ecastaphyllum*.

The values with the same superscript letters on the same line are not statistically different.

After treatment of mice with the aqueous extracts of *D. ecastaphyllum* and *M. barteri*, changes in hematological parameters were analyzed. The white blood cell count (WBC) after treatment with the aqueous extract of *Millettia barteri* and *Dalbergia ecastaphyllum* did not show a significant difference at all doses administered. This result is in full agreement with that of toxicity study. Leukocytes have the role of protecting and defending the body against bacteria, foreign substances, viruses, parasites, toxins and tumor cells (Marieb, 2009).

Three weeks post-treatment with different doses (50, 100 and 350 mg / Kg of bw) of the aqueous extract of *Millettia barteri* (AEMB), a return to normal status (relieve of anaemia) was observed in treated mice. Indeed, the hemoglobin level, which was initially 9.6 g / dL after induction increased to 13.84 g / dL at 50 mg / Kg bw, 14.06 g / dL at 100 mg / Kg bw. At 350 mg / Kg bw, this rate increased from 10.06 g / dL to 13.74 g / dL. In addition, the erythrocyte level moved from 2.46 106 / µL after PHZ treatment to 8.4 106 / µL after treatment with plant extract. The 50 mg / kg bw dose was the optimal dose that induced the greatest increase in hemoglobin levels.

Oral administration of the aqueous extract of *Dalbergia ecastaphyllum* leaves to anaemic mice restored the hematological parameters the third week. The Hb and RBC levels after administration of the extract at 100 mg / kg bw were higher than before the induction of anaemia. These results may justify the ability of these extracts to boost hemoglobin and RBC levels.

At 50 mg / kg bw, *Millettia barteri* repaired anaemia by increasing the hemoglobin level. Among the two species, extracts of *Dalbergia ecastaphyllum* exhibited the significant activity at 100 mg / Kg bw.

CONCLUSION

Dalbergia ecastaphyllum and *Millettia barteri* are used in the treatment of several ailments, including SCD. The phytochemicals related to SCD treatment were detected in the extracts of these plants and were phenolic compounds and aromatic aminoacids. The aqueous extracts of *D. ecastaphyllum* and *M. barteri* were not toxic. Their administration to anaemic mice resulted in an improvement of blood parameters, showing that these medicinal plants could play a role in the control of haemolytic anaemia.

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