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HEMOPOIETIC EFFECTS OF SOME HERBAL EXTRACTS USED IN TREATMENT OF INFANTILE ANEMIA IN CAMEROON

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ABSTRACT

The hemopoietic activity of *Manihot esculenta*, *Plastotoma africanum*, *Ricinodendron heudelotii* and *Sacoglottis gabonensis extracts* used for treatment of infantile anemia were studied on hemolytic anemic rats. Anemia was induced by repeated intraperitoneal injection of phenylhydrazine (40 mg/kg of body weight/day) for two days. A drop in red blood cell count, haemoglobin concentration, and pack cell volume characterized the anemia condition. In addition, the histopathological examination of the bone marrow for blood cells further characterized the anemic condition. Results obtained revealed that oral administration of plant extracts to rats significantly ($p<0.05$) increased the packed cell volume, haemoglobin, red blood cells and precursors of hematopoietic cell line in bone marrow. Phytochemical analysis of the plants studied revealed the presence of alkaloids, coumarins, flavonoids, phenolic compounds, saponosids, steroids, tannins, terpenoids and triterpenoids. The extracts also contain mineral elements, such as iron, calcium, zinc and copper. The results obtained suggested that the plant extracts studied possess hemopoietic properties, justifying their use in the management of infantile anemia.

KEYWORDS: Infantile anemia, herbal medicine, Hematological Parameters, phenylhydrazine.

1. INTRODUCTION

Anemia is characterized by low hemoglobin concentration. A child is considered anemic when the hemoglobin concentration is below normal (11 g/dl). The most serious consequence on health is an increase in mortality risk, which is 3 to 4 times higher among anemic children.[1] In addition, anemia also reduces physical and working capacity, growth and immune status.[2]

Childhood mortality is a core indicator for community health. Nearly half of all under-five deaths (5.64 million) occur in Africa [3] The Major risk factors for child deaths in Africa relates to infectious diseases such as diarrhea and malaria in addition to under nutrition that accounts for more than 50% of these cases.[4] A common factor of diarrhea, malaria and under-nutrition is the occurrence of anemia, either by hemolysis, loss of red blood cells or decreased production of red blood cells.[5] Globally 273 million (43%) of preschool children are affected by anemia.[6] In Cameroon, 6 out of 10 children under five 5 years of age suffer from anemia[7] and are often treated by the administration of decoction, herbs, or suc of fresh leaves, roots, bark of trees or a combination of these.[8] Examples of such plants used indigenously for the management of anemia include *Manihot esculenta*, *Plastotoma africanum*, *Ricinodendron heudelotii* and *Sacoglottis gabonensis*.[8] Many data portray the anti-anemic activities of medicinal plants used in traditional medicine.[9, 10] However, most often researchers the activity of plant based medicine simply by studying extracts or identify new bioactive molecules without taking into consideration the traditional methods of preparation and application of such. In the present study, we evaluated the hemopoietic property of some traditionally used plants in the management of anemia in children following the preparation and applications laid down by the herbalists.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant materials

Fresh leaves of *Manihot esculenta* (42576/HNC), *Plastotoma africanum* (8456/SRF/Cam) and barks of

Ricinodendron heudelotii (16610/SRF/Cam) and *Sacoglottis gabonensis* (31117/HNC) were used in this study. Plants were collected from their natural habitat in the town of Douala of Cameroon and were authenticated by botanists of the Faculty of Sciences of the University of Douala and confirmed by the National Herbarium of Yaoundé Cameroon.

2.1.2. Animals

Young male *Wistar albino* rats aged 45 ± 5 days (average body weight 121 ± 10 g) were used for this study. They were housed in wire-mesh cages, having free access to water and food and were acclimatized for one week to the Laboratory conditions.

2.2. Preparation of extracts

The plant materials (300 grams of fresh leaves or 1000 grams of bark) were added to 1000 ml of tap water and boiled for 15 min cooled and filtered (Filter papers Whatman No 1). On the other hand, fresh leaves of *M. esculenta* were crushed in a mortar and the latex filtered. All the extracts were concentrated, dried in an oven and stored in a refrigerator at 4°C until required meanwhile part of the extract was used for the analysis of mineral elements composition. For the animal study extracts were prepared every three days under the same conditions.

2.3. Phytochemical Screening

The presences of bioactive components were assessed using methods earlier described by Harborne.[11]

Alkaloids: To 1 mL of extract, a few drops of 2% sulfuric acid were added and then a few drops of Mayer's reagent. The presence of alkaloids was indicated by obtaining a white precipitate or turbidity.

Coumarins: To 5 ml of extract, a few drops of potash 10% were added. The presence of coumarins was indicated by the appearance of a coloration varying from blue to purple yellow.

Flavonoids: To 2 ml of extract, a few drops of sodium hydroxide solution (1/10) were added. The presence of flavonoids was indicated by the yellow-orange coloring.

Phenolic compounds: To 5 mL of various solvent extracts of sample a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenol.

Saponins: Five milliliters of various extract were boiled in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponin contents in the sample.

Tannins: Four drops of 1% aqueous Iron chloride ($FeCl_3$) solution were added in 2 mL of extract. The appearance of intense green, purple, blue or black colour indicated the presence of tannins.

Triterpenoids and Steroids: In two milliliters of extracts a few drops of Libermann-Buchard reagents (1 ml of concentrated H_2SO_4, 20 ml of acetic anhydride, 50 ml of CHCl3) were added. A purplish color indicated the presence of triterpenoids and a bluish-green coloring the presence of steroids.

2.4. Quantitative analysis of phytochemicals

Total phenolic content were determined following the method described by Marigo. [12] In to a test tube containing 0.2 mL of diluted extract was added Folin-Ciocalteu's reagent (0.2 mL) and after 10 min of incubation, 20 % sodium carbonate solution (0.4 mL) was added. This was thoroughly mixed and incubated for 20 min. The optical density was read at 725 nm using a spectrophotometer and gallic acid was used as standard. The total phenolic content of extracts was expressed as µg/mL gallic acid equivalent.

Tannin content was determined using acidified vanillin and (+) cathechin as standard.[13] In to a test tube containing 1 mL of diluted extract was added 4 mL of freshly prepared 4% vanillin in ethanol. The mixture was stirred, treated with concentrated HCl and quantified by spectrophotometry at 500 nm. Tannin content of extracts was expressed as µg/mL cathechin equivalent.

2.5. Mineral analysis

The mineral content of the extracts were analyzed by applying the method of Pinta.[14] After drying the plant extract concentrates for 16 h at 70 ° C and allowed to cool for 30 minutes in the desiccator, 2 g of sample was put in a platinum capsule and oven-dried at 450 ° C for 2 h then cooled. The ashes were moistened with 3 ml of distilled water and 1 ml of concentrated hydrochloric acid. The mixture was heated on a hot plate until the first vapors appear, then filtered (Whatman No 1) the ashes and rinsed 4 times with warm water in a volumetric flask. The filter paper and its contents were incinerated for 30 min at 550 ° C. The ashes were moistened with 5 ml of hydrofluoric acid and then heated on a gentle hotplate at 100 ° C. One milliliter of concentrated HCl was added and washed with warm water and filtered. The volume was reduced to 100 ml and then cooled. This solution has been used for atomic absorption spectrometric assays of elements such as Fe, Ca, Cu and Zn.

2.6. Anti-anemic activity

Anti-anemic effects were tested by assessing the impact of decoctions and latex on hematological parameters and red blood cells synthesis in anemic Wistar rats. Hemolytic anemia was induced by intraperitoneal injection of phenylhydrazine at 40 mg/ kg of body weight / day[15] for two days. Only rats which developed anemia characterized by a hemoglobin concentration of less than 11 g/dl were included in the study.

The experimental animals were then divided into seven groups of five: six groups of anemic rats (named 1, 2, 3, 4, 5, 6) and a control non anemic group (7). The groups 1-4 were fed the different plants extract, group 5 the reference drug (Vitafer forte- Strides Shasun), group 6 anemic control (distilled water) and group 7 normal control (distilled water). The plant extracts and reference drug in their respective dose level as shown below were mixed with the animal feed (4g per animal) and fed to experimental animals daily for a period of 14 days.

Group 1: received oral single dose of *Platostoma africanum* extract (79.47 mg/kg)

Group 2: received oral single dose of *Sacoglottis gabonensis* extract (207.57 mg/kg).

Group 3: received oral single dose of *Ricinodendron heudelotti* extract (146.64 mg/kg)

Group 4: received oral single dose of *Manihot esculenta* extract (848.21 mg/kg).

Group 5 (Positive control): received oral single dose of reference drug 'Vitafer forte' syrup 5ml containing 200 mg ferric ammonium citrate (41 mg iron element). Dosage for children under 5 years: 5 ml twice a day, 400 mg of ferric ammonium citrate (28.57 mg/kg)

Group 6 (Anemic control): received distilled water.

Group 7 (Normal control): received distilled water.

The dose level of extracts fed to experimental animals was extrapolated from traditional usage of the respective plants.[8]

On days D1, D7 and D14, rats were anesthetized and approximately 1 ml of blood was collected in EDTA tube by retro orbital venous plexus puncture. The samples were used for red blood cell count, determination of hemoglobin, hematocrit and pack cell volume using an automatic blood cell counter (Sysmex XN-1000).

2.7. Bone marrow analysis examination

Histological examination of bone marrow was carried out as described by Smith and Bruton.[16] On day 14, rat's right femur was collected and fixed in Zenker reagent. The bone was allowed to last in the reagent for 3 days, rinsed with tap water and went through decalcification phase. It was then embedded in paraffin, cut into 5-µm section, and stained with hematoxylin and eosin (H&E). The slides were later examined using light microscopy (100 ×). Effect of plant extracts on the bone marrow was evaluated in selected areas analysis based on structural organization organ and number of hematopoietic cell lineages.

2.8. Statistical analysis

All data were represented as means ± SD. One way analysis of variance (One-way ANOVA) followed by Least Significant Difference (LSD) test were used to determine differences between groups at $P \leq 0.05$ using SPSS version 16.0 for windows.

3. RESULTS

3.1. Qualitative phytochemical composition

Phytochemical analysis of extracts revealed the presence of different classes of secondary metabolites: alkaloids, coumarins, flavonoids, phenolic compounds, saponosids, steroids, tannins, terpenoids and triterpenoids (Table 1). The phytochemical content varied between plants with exception of phenolic content and alkaloids that were present in all plants extracts. *P. africanum* extracts did not contain tannins. *S. gabonensis* extract was rich in secondary metabolites than the other plants.

Table 1: Qualitative Phytochemical Constituents of plant extracts.

Compounds / Extracts	*S. gabonensis*	*R. heudelotii*	*P. africanum*	*M. esculenta*
Alkaloids	++	++	++	++
Coumarins	++	-	-	-
Flavonoids	++	-	++	-
Phenolic compounds	++	++	++	++
Saponins	++	++	++	+
Steroids	-	-	-	-
Tannins	++	+	-	+
Terpenoids	-	+	++	++
Triterpenoids	++	-	-	-

-: Absent, +: Present, ++: Indicates strong presence

3.2. Mineral analysis

M. esculenta extract showed the highest levels of minerals (Fe (780.50 mg/kg), Ca (7520.00 mg/kg), Zn (475.20 mg/kg), Cu (190.20 mg/kg)) opposite to *R.heudelotii* which showed the lowest mineral levels for Fe (76.50 mg/kg), Zn (90.58 mg/kg) and Cu (55.40 mg/kg) (Table 2). *S. gabonensis* presented the lowest Calcium content (3520.00 mg/kg).

Table 2: Mineral content of plant extracts (mg/kg) DM.

Extracts/ Minerals	Iron	Calcium	Zinc	Copper
M. esculenta	780.50±0.50[a]	7520.00±2.44[a]	475.20±0.20[a]	190.20±0.20[a]
S. gabonensis	305.30±1.3[b]	3520.00±8.16[b]	278.93±0.95[b]	184.45±0.45[a]
P. africanum	252.50±0.50[c]	4800.00±4.08[c]	176.40±1.40[c]	126.13±0.13[b]
R. heudelotti	76.50±0.50[d]	4320.00±1.63[d]	90.58±0.60[d]	55.40±0.4[c]

Values on the same column with the same subscript letters are not significantly ($p<0.05$) different, DM: Dry matter.

3.3. Quantitative Analysis of Phytochemicals

3.3.1. Total phenolic content

The phenolic contents varied between the extracts. *M. esculenta* showed the highest phenolic content (1586.06 µg/ml (15.86 mg/g DM)) as opposed to *R. heudelotii*, which presented a lower phenolic contents (14.15 µg/ml (0.84 mg/g DM)) (Table 3).

3.3.2. Total tannin content

Tannin content varied between extracts (Table 3). *S. gabonensis* showed the highest tannin content (75.85 µg/ml (3.26 mg/g DM)) opposite to *P. africanum* that showed a lower tannins content (1.31 µg/ml (0.14 mg/g DM)).

Table 3: Total phenolic and tannin content of plant extracts.

Extracts	Concentration mg/ml	Total phenolic content		Total tannin content	
		µg/ml*	mg/g DM**	µg/ml*	mg/g DM**
P. africanum	8.90	339.68 ±11.04[a]	38.04±1.2[a]	1.31±0,34[a]	0.14 ± 0.03[a]
S. gabonensis	23.25	350.05 ±50.30[a]	15.05±2.1[b]	75.85±0.34[b]	3.26 ± 0,01[b]
R. heudeloti	16.42	14.15 ±1,06[b]	0.84±0.06[c]	2.36±1.36[a]	0.14 ± 0.08[a]
M. esculenta	95.00	1586.06 ±20,21[c]	15.86±0.2[b]	30.55±0.35[c]	0.30 ± 0,00[a]

For each compound, the assigned values of the same superscript on the same column are not significantly different ($p<0.05$); *: potion; **: dry matter; DM: dry matter

3.4. Effect of extracts in hematological parameters

Phenylhydrazine administration had a damaging effect on the hematological parameters of rats. This was seen in the significant ($P<0.05$) decreases of red blood cell, hemoglobin and hematocrit levels (Tables 4, 5 and 6) as the rats became anemic.

3.4.1. Red blood cell count

Results of red blood cell count are shown in Table 4. After induction of anemia, the RBC values dropped to 4.40 ± 0.25 - 5.96 ± 1.79 10^6 /µL while the control group remains at 7.72 ± 0.05 106 / µL. On day 7th of treatment, a significant increase ($P<0.05$) was observed in the groups treated by *P. africanum*, *S. gabonensis*, *R. heudelotii* and positive control compared to the anemic group. *P. africanum* had the largest percentage (57.43%) recovery than the other plants studied. At day 14, values of the anemic and control group decreased, while those of treated groups still increased or remain unchanged. The most effective extract was *R. heudelotii* group (66.09%) better than *P. africanum* which was best in day 7. The RBC values for all treated groups ranged between 6.32 ± 0.03 and 7.62 ± 1.05 10^6/µL against 4.66 ± 1.03 10^6/µL for the negative control at the end of the experiment. Meaning that all treated animals recovered by the end of the study.

Table 4: Changes in red blood cells count of experimental animals during treatment.

Groups	RBC (10^6/µL)		
	Day 1	Day 7	Day 14
Group 1 (*P. africanum*)	4.41 ± 0.47[s]	6.95 ± 0.90 +57.43%[*]	6.61 ± 1.92 +49.77%[*#]
Group 2 (*S. gabonensis*)	4.57 ± 0.59[s]	6.81 ± 0.68 +49.14%[*#]	7.45 ± 0.58 +63.11%[*#]
Group 3 (*R. heudelotii*)	4.40 ± 0.25[s]	6.10 ± 0.23 +38.72%[*#]	7.31 ± 0.18[s] +66.09%[*#]
Group 4 (*M. esculenta*)	5.96 ± 1.79[s]	6.40 ± 0.27 +7.45%[#]	7.54 ± 0.79[s] +26.55%[#]
Group 5 (Positive)	4.62 ± 0.38[s]	6.64 ± 0.87 +43.72%[*#]	7.62 ± 1.05[s] +65.02%[*#]
Group 6 (Anemic (Negative))	4.50 ± 0.87[s]	5.20 ± 0.52[s] +15.70%[#]	4.66 ± 1.03[s] +3.64%
Group 7 (Control)	7.72±0.05	6.62±0,02 -14.24%[*]	6.32±0.03 -18.12%

Values are mean ± Standard Deviation for five rats in each group. [s]significantly compared to control values (Group 7), [*]significantly compared to anemic control percentage values (Group 6), [#]significantly compared to control percentage values ($P < 0.05$).

3.4.2. Hemoglobin level

The results of the hemoglobin level are showed in Table 5. After induction of anemia, hemoglobin level ranged between 9.34 ± 0.81 - 10.12 ± 0.33 g/L in anemic groups against 13.60 ± 0.50 g/L in control group. After 7 days, treated groups quickly recovered compared to the control group. The largest percentage was *P. africanum* group (52.67%). Hemoglobin level in treated rat ranged between 13.56 ± 0.48 and 14.26 ± 0.71g/L against 10.82 ± 0.94 g/L in negative control group. Fourteen days later, only the value of the anemic group remains significantly low.

Table 5: Changes in hemoglobin level of experimental animals during treatment.

Groups	Hemoglobin level (g/L)		
	Day 1	Day 7	Day 14
Group 1 (*P. africanum*)	9.34 ± 0.81[s]	14.26 ± 0.71 +52.67%[*#]	14.38 ± 0.57[s] +53.96%[*#]
Group 2 (*S. gabonensis*)	9.70 ± 0.74[s]	13.56 ± 0.48[s] +39.97%[*#]	14.00 ± 0.33 +44.49%[*#]
Group 3 (*R. heudelotii*)	9.64 ± 0.28[s]	13.74 ± 0.69[s] +42.53%[*#]	14.08 ± 0.60[s] +46.05%[*#]
Group 4 (*M. esculenta*)	10.12 ± 0.33[s]	13.72 ± 1.17[s] +35.57%[*#]	14.42 ± 1.15[s] +42.49%[*#]
Group 5 (Positive)	10.00 ± 0.83[s]	14.08 ± 0.80 +40.80%[*#]	14.32 ± 0.53[s] +43.20%[*#]
Group 6 (Anemic (Negative))	9.42 ± 1.57[s]	10.82 ± 0.94[s] +14.86%	9.88 ± 1.79[s] +4.88%
Group 7 (Control)	13.60±0.50	14.84±0.72 +9.11%	13.24±1.13 -2.64%

Values are mean ± Standard Deviation for five rats in each group. [s] significantly compared to control values (Group 7), [*] significantly compared to anemic control percentage values (Group 6), [#]significantly compared to control percentage values ($P < 0.05$).

3.4.3. Hematocrit

The results of the red blood cell count are showed in Table 6. After induction of anemia, hematocrit ranged from 25.24 ± 0.80 to 28.16 ± 4.46 % in anemic groups against 43.58 ± 1.21 % in control group. On the 7th day of treatment, a significant increase ($P < 0.05$) was observed in all treated groups compared to the anemic group. The highest percentage recovery was observed in *P. africanum* group (101.01 %). Compared to control, all groups of rats under treatment recovered. At day 14, values of the anemic and control group decreased while those of treated groups still increased or remain unchanged. The most active extract was *R. heudelotii* with 113.15 % of recovery. Hematocrit values ranged between 46.00 ± 13.15 and 57.98 ± 4.97 % against 25.94 ± 3.79 % for the anemic group.

Table 6: Changes in hematocrit values of experimental animals during treatment.

Groups	Hematocrit (%)		
	Day 1	Day 7	Day 14
Group 1 (*P. africanum*)	25.52 ± 1.96[s]	51.30 ± 3.72 +101.01%[*#]	47.54 ± 11.30 +86.28%[*#]
Group 2 (*S. gabonensis*)	27.28 ± 3.54[s]	48.34 ± 1.45 +77.19[*#]	51.52 ± 6.38 +88.85%[*#]
Group 3 (*R. heudelotii*)	25.24 ± 0.80[s]	48.82 ± 2.89 +93.42%[*#]	53.80 ± 8.29[s] +113.15%[*#]
Group 4 (*M. esculenta*)	28.16 ± 4.46[s]	47.90 ± 1.36 +70.09%[*#]	57.98 ± 4.97 +105.89%[*#]
Group 5 (Positive)	27.22 ± 0.58[s]	48.12 ± 2.96 +76.78%[*#]	46.00 ± 13.15 +68.99%[*#]
Group 6 (Anemic (Negative))	26.5 ± 4.63[s]	30.02 ± 5.22[s] +13.28%	25.94 ± 3.79[s] -2.11%
Group 7 (Control)	43.58±1.21	51.34±3.80 +17.80%	46.66±7.97 +7.06%

Values are mean ± Standard Deviation for five rats per group. ^{s} significantly compared with control values (Group 7), * significantly compared to with anemic control percentage values (Group 6), $^{\#}$significantly compared to control percentage values ($P < 0.05$).

3.4.4. Histological Findings

Effect of plant extracts on the bone marrow is presented in Figure 1. Histological finding indicates greater cellular regeneration (Red blood cell and white blood cell precursors) in the extract treated groups and positive control group. Structural organization was similar in control group except positive control group. Anemic group had a very low proportion of white blood cells precursors and a greater proportion of red blood cell precursors.

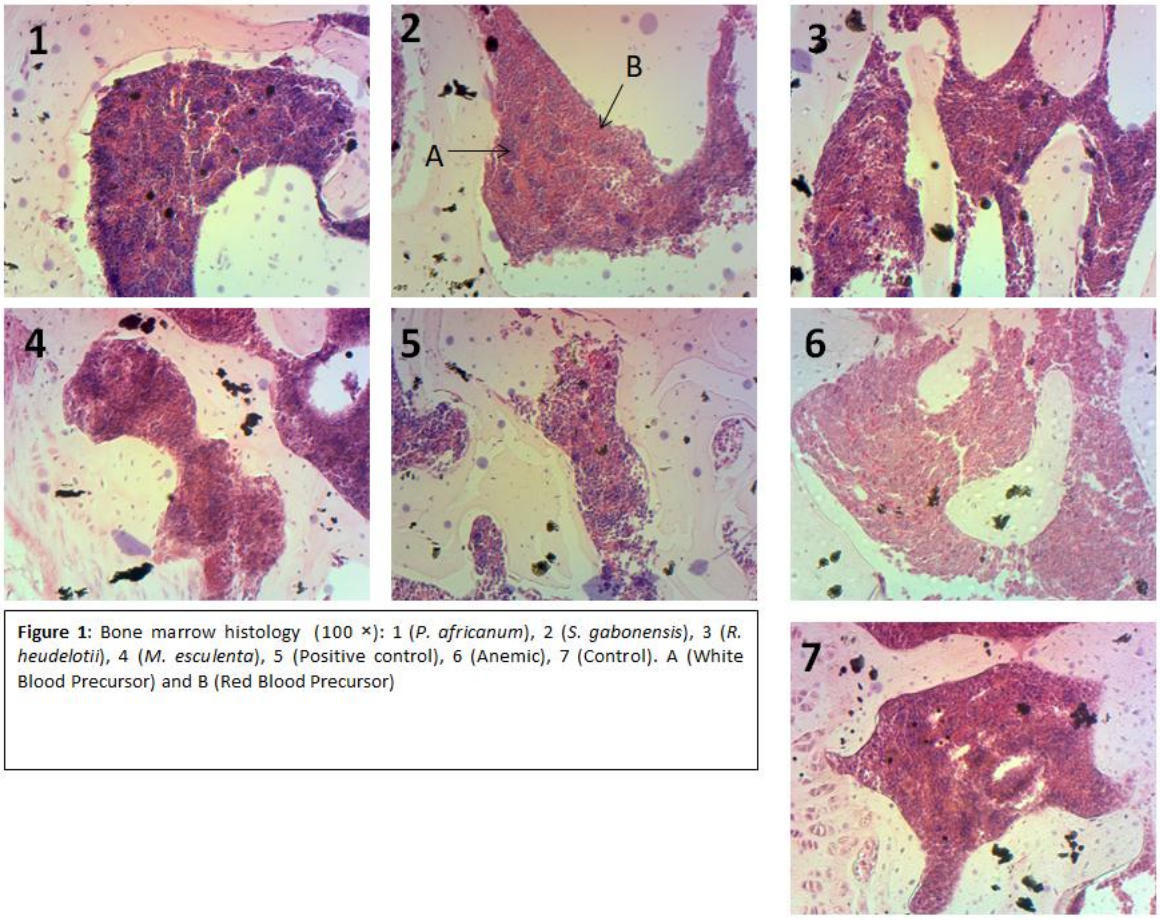

Figure 1: Bone marrow histology (100 ×): 1 (*P. africanum*), 2 (*S. gabonensis*), 3 (*R. heudelotii*), 4 (*M. esculenta*), 5 (Positive control), 6 (Anemic), 7 (Control). A (White Blood Precursor) and B (Red Blood Precursor)

4. DISCUSSION

Phenyl hydrazine administration in rats causes oxidative stress, generates reactive oxygen species (ROS) in red blood cells (RBC) and cell lysis.[17] In addition to hemolysis of the RBC, phenyl hydrazine also modifies iron metabolism by increasing ferrous transporter (DMT1) expression in the spleen, duodenum and liver.[18] These decrease hematological parameter levels and stimulate a self recovery (positive response of bone marrow) process through the iron metabolism pathway. However, this may not appear immediately.

Effect of restoring hematological blood parameters in treated groups could be due to the presence of some elements that made it possible. These elements could reduce the action of the ROS and increase request of iron. Activity of medicinal plants are based on their composition in bioactive components. Bioactive compounds identified in the plants used in this study belong mainly to the following group: Alkaloids, Coumarins, Flavonoids, Phenolic compounds, Saponins, Steroids, Tannins, Terpenoids and Triterpenoids. This profil is similar to that found in other plant extracts such as *Eremomastax speciosa*, *Hibiscus sabdariffa* and *Moringa oleifera* which have also shown antianemic activity.[10, 19, 20] Steroids have been agured to stimulate growth of early and old erythroid progenitor cells (BFU-E and CFU-E) in the presence of erythropoietin.[21] Other compounds such as saponins break the cell membrane of red blood cells by interaction with sterols. This hemolytic activity decreases as the osidic chain becomes longer. Saponins are not dangerous by oral ingestion because absorption is slow. Also, they go through hydrolysis in the digestive system.[22] Some time during anemia, inflammation could appear and saponins are also

known to inhibit platelet aggregation and thrombosis in the inflammatory process by promoting blood flow.[23] Alkaloids and terpenoids are also anti-inflammatory compounds.[24,25]

Some bioactive compounds identified in the plants studied such as coumarins, flavonoids, phenolic compounds and tannins are polyphenols which have antioxidant properties. Phenolic compounds and tannin contents were low compared to values found by other authors. Phenolic content were low compared to value of Quartey et *al*.[26] in the aqueous extract of dried leaves of *M. esculenta* (95.33 mg/g DM) and Tchuenguem et *al*.[27] in leaves of *R. heudelotii* (33.5 mg/g DM). With exception of *M. esculenta* for phenolic content and *S. gabonensis* for tannin content, the rest of the plant extracts had low tannin and phenolic content. However, this low concentration may not affect the antianemic properties of the plants since even lower concentrations in methanolic leaf extract of *Solanum nigrum* (70.60 mg/g ; 1.89 mg/g) were reported to show antianemic activity.[28] The low concentrations obtained in *in vitro* studies may be attributed to low solubility and slow diffusion in water.[29] Since tannins are more soluble in water which may be due to their chemical nature phenolic compounds containing tannnins may possess high antioxidant effects.[30,31] Equally phenolics containing tannins may have some unpleasant effects because of their chelating activity. Hence, serving as antinutritif since it chelates nutrients and decreasing their bioavailability. However, the balance between the beneficial and non-beneficial effects of plant bioactive compounds depends on concentration, chemical structure, exposure time and interaction with other dietary components.[32] Polyphenols are known for their antioxidant capacity because of their high reactivity as hydrogen or electron donors, the ability of their polyphenol-derived radical to stabilize and delocalize unpaired electron (chain-breaking function) and their ability to chelate transition metal ions (terminating the Fenton reaction that have prooxidant activity).[33] *M. esculenta*, *P. africanum* and *S. gabonensis* showed significant *in vitro* or *in vivo* antioxidant activity. The hexane and dichloromethane extracts of *P. africanum* showed significant antioxidant activity with the dichloromethane extract exhibiting an IC_{50} (90 %) comparable to that of butyl hydroxy toluene, a synthetic antioxidant.[34] The Aqueous ethanol bark extract of *S. gabonensis* reduced the SOD depressing effect of the experimental oxidant. Its also exerted a sparing effect on tissue antioxidant vitamins, ascorbic acid and vitamin E, effectively inhibiting their depletion by 2,4-DNPH on red blood cells.[35] Ethanolic extract of leaves of *M. esculenta* increased serum levels of the antioxidant enzymes exerting a potent antioxidant effect in a graded manner.[36] Antioxidants would reduce cell lysis. Any of these mechanisms may explain the role of the plant tested in reducing the oxidative effect of phenylhydrazine in cell lysis and further damage of the bone marrow cell lines.

Phenylhydrazine admistration decreased the production of RBC in the bone marrow as seen in Fig. 1. Administration of the plant extracts stimulated the stem cells in producing new blood cells through erythropoiesis as seen in the densed image in Fig 1A. This activity is to counter the effect of phynylhydrazine.[37] In the absence of treatment the hematopoiesis response was not as effective. This response may be slow and the state of anemia deteriorate. Similar results have also been report by other authors[9,38] as observed in the histological feature of the bone marrow (Fig 1). The greater proportion of red blood cell precursors may be a response to red blood cell losses and iron needs.[18]

Together with secondary metabolites, iron, zinc and copper were identified in significant quantities. *M. esculenta* extract, which presented the highest levels of minerals have got higher values for iron and calcium compared to *Hibiscus sabdariffa* juice (Fe (588.00 mg/kg), Ca (2800.00 mg/kg)) but had lower values for Zinc (814.00 mg/kg) and Copper (24.4 mg/kg).[39] *S. gabonensis* and *P. africanum* were found to have higher iron contents than *Eremomastax polysperma* (248 mg/kg) and *Solanum nigrum* (130 mg/kg).[28,40] Copper contents of our extracts are superior to those found in other antianemic plant (*Solanum nigrum* (21.2 mg/kg)). Zinc and copper are implicated in iron metabolism. These minerals could favor reversibility of anemic state of the test groups.

5. CONCLUSION

Decoction or latex of leaves of *Manihot esculenta*, *Plastotoma africanum* and barks of *Ricinodendron heudelotii* and *Sacoglottis gabonensis* possess anti-anemic activity. This may be attributed to their phytochemicals and minerals contents. *P. africanum* had the best hemopoietic activity. Though in very small concentration its effect on recovery of anemic rats was fastest as animals recovered within one week of treatment. This study therefore, supports the therapeutic use of these plants in the traditional medicine for the treatment of anemia.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Sandrine Suzanne Beack Bayengue carried out laboratory analysis prepared the first draf of the manuscript. Sabine Adeline Fanta Yadang, Dairou Hadjidatou participated in laboratory analysis. Theodora Kopa Kowa performed phytochemical tests. Loick Kojom, Aristide Kognou Mokale performed statistical analysis. Mathieu Ndomou, Gabriel Agbor Agbor, Rosalie Anne Ngono Ngane, Clerge Tchiegang designed the study, supervised the work, and participated in the manuscript writing.

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