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Anti-Anaemic Activity and Potential Toxicity of Extracts of Four Tinctorial Plants Used in the Treatment of Anemia in Benin: *Gossypium barbadense*, *Sorghum bicolor*, *Hibiscus sabdariffa* and *Justicia secunda*

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Abstract

Justicia secunda, *Sorghum bicolor*, *Gossypiun barbadense* and *Hibiscus sabdariffa* are dye plants traditionally used in Benin for the treatment of anemia. This work is part of the therapeutic valorization of dyes from these plants. Its objective is to characterize their composition in chemical groups and evaluate their harmlessness and their anti-anaemic property in laboratory rats. Anemia was induced in Wistar rats by phenylhydrazine hydrochloride followed by treatment by gavage with hydroethanolic extracts of the plants studied. Phytochemical screening of these extracts made it possible to characterize the major chemical groups, in particular alkaloids, polyphenols including tannins, flavonoids and leucoanthocyanins, as well as reducing compounds and saponosides in the plants studied. Cytotoxic analysis of these extracts on *Artemia salina* shrimp larvae revealed globally high LC_{50} values of between 3.14 and 4.64 mg/mL, which testify a priori to the harmlessness of these extracts. The administration of the hydroethanolic extract of each plant to anaemic rats at doses of 2000 mg/kg/d promoted, after 15 days, an increase in hemoglobin levels, the number of red blood cells and hematocrit, going to more than 90% recovery of the hematological parameters involved. The highest rate, 99.06% being that of the species *Justicia secunda* followed closely by *Sorghum*

bicolor (96.80%) compared to 93.93% obtained by treatment with the Ranferon-12 positive control used. Indeed, these results confirm the therapeutic indication of these plants in the resorption of anemia in traditional medicine.

Keywords

Anemia, Resorption, Tinctorial Plants, Phytochemistry, Benin

1. Introduction

Anemia is defined as the reduction in the number of circulating red blood cells [1] or as a condition in which the number of erythrocytes and therefore, their ability to carry oxygen is insufficient to meet physiological needs [2]. It is one of the major public health problems affecting one-third (1/3) of the world's population, and targeting people of all ages [3]. In Benin, anemia frequency is around 80% in peri-urban areas [4] and in the absence of rapid treatment, it has negative effects on perinatal and neonatal health, in particular, low birth weight, premature birth and children developmental delays [5] and can cause decreased cognitive abilities, a weakened immune system and increased mortality [6].

The most effective method used to treat anemia is blood transfusion [7]. Oral administration of iron supplements is an inexpensive method used to treat patients with iron deficiency anemia, but despite its cost, it is often not available to impoverished populations. Indeed, in peri-urban and rural areas, people find it difficult to mobilize money for their health, all the more so since the period during which health needs are most pressing is also that of "lean season" between harvests, when cash availability is lowest [8]. Moreover, this method is associated with a number of side effects, such as nausea, vomiting, constipation and stomach pain, which limit its long-term use [6]. The work of Coyne and Auerbach [9] mentions that oral iron supplements are poorly absorbed in the intestinal tract, due to the overexpression of hepcidin, a peptide hormone that plays a central role in iron homeostasis. Furthermore, they lead to an inappropriate increase in the production of free radicals which could in turn induce oxidative stress [10]. Indeed, with the formation of oxidation products, oxidative stress, can lead to cardiovascular, neurological or cancerous conditions [11]. It is also important to note that the presence of excess free iron initiates the Fenton reaction, which leads to oxidative damage to cell membranes, proteins, lipids and nucleic acids, as well as the stimulation of inflammatory mediators [12].

Faced with the risk of side effects due to the long-term ingestion of pharmaceutical iron and other synthetic products combining iron; medicinal plants still remain a source of medical care for the prevention and treatment of anemia in developing countries. They present considerable socio-economic advantages related to their knowledge, their availability and their accessibility. Indeed, the inventory of plants used in the treatment of anemia through an ethnobotanical survey carried out by Lokossougba in the Allada region in southern Benin, showed

that a total of 53 plants species belonging to 49 genera and 39 families are used in the treatment of anemia, especially in pregnant women [13]. This work revealed that the plants *Justicia secunda* Vahl. (Acanthaceae), *Gossypiun barbadense* L. (Malvaceae), *Hibiscus sabdariffa* L. (Malvaceae) and *Sorghum bicolor* (L.) Moench, (Poaceae) have been listed among the plant species used very frequently in the treatment of anemia and related symptoms both in children and in adults, including pregnant women in particular. These plants are also known for their production of natural dyes variously used in the artisanal food and cosmetics field [14] [15]. Indeed, plants with the primary use of dye and tannin are often versatile and generally have four secondary uses, the most important of which is medicinal use [16]. Their active principles would often be the chemical compounds also responsible for the dyeing properties. These coloring principles, in particular made up of polyphenols, have a wide spectrum of biological activities linked to their affinity for proteins, and their reducing and chelating properties for metal ions [17]. They have well-established antioxidant properties [18] and they promote the regeneration of blood tissue by numerous young resistant cells [19]. This work is undertaken within the framework of the valorization of the therapeutic use of these four tinctorial plants in local traditional medicine. Its objective is to study their phytochemical composition to evaluate their anti-anaemic property as well as to test their safety on *Artemia salina* shrimp larvae.

2. Materials and Methods

2.1. Plant Material

The samples studied are plants harvested in the South Benin region and brought back to the laboratory the same day. The samples were spread out in a cold drying room (22˚C) for about 14 days, after which time they are practically anhydrous and brittle. They are then ground, sieved with a sieve with 710 µm diameter pores and then stored in airtight containers. These are the leafy stems of *Gossypium barbadense* (Gb) and *Justicia secunda* (Js), the leafy panicums of *Sorghum bicolor* (Sb) and the calyxes of *Hibiscus sabdariffa* (Hs).

2.2. Animal Material

The rats used for the anti-anaemic activity are from the wistar line of weight varying between 180 and 220 g. They are acclimatized and fed with water and appropriate pellets, one week before the start and then during the experiment.

The larvae used for the toxicity test come from *Artemia salina* shrimp eggs purchased on the market.

2.3. Methods

2.3.1. Preparation of Crude Extracts

The extraction of the total chemical principles was made for each plant according to the method of decoction as well as by maceration in accordance with the traditional use of these plants. Indeed, 50 g of powder are dissolved in 500 mL of distilled water. The mixture is brought to a moderate boil for 30 min. After cool-

ing, the mixture obtained is filtered three times on absorbent cotton and the filtrate is transferred to a 1000 mL flask then subjected to evaporation until dryness at 40˚C using a rotavapor (Heidolph Laborota 4000 efficient) coupled with a water cooler (Julabo FL 300), until the decoction is obtained.

To obtain the macerated, the mixture of 50 g of drug with 500 mL of the mixed solvent water-ethanol 96˚ (4:6, v/v) is left under continuous stirring for 48 hours. The mixture is then filtered and then evaporated to dryness. Each operation is repeated three times on the same quantity of powder.

The dry extracts obtained are weighed and their yield is calculated according to the expression:

$$\text{Rdt (\%)} = (\text{Mass of dry extract})/(\text{Initial mass of powder}) \times 100$$

2.3.2. Phytochemical Screening

Phytochemical analyzes carried out on the species studied are based on differential coloring and/or precipitation reactions of the main groups of chemical compounds contained in plants according to the classic method of Houghton and Raman [20], widely used in the literature [14] [21].

2.3.3. Shrimp Larval Toxicity Test

This test is based on the survival of *Artemia salina* shrimp larvae in seawater in the presence of the extracts studied, according to the method of Michael *et al.* [22] and Sleet and Brendel [23]. *Artemia salina* eggs are incubated for 48 hours in seawater until young larvae hatch. A stock solution of the aqueous and hydroethanolic extracts of each plant is prepared by dissolving 200 mg of extract in 4 mL of distilled water. Successive half dilutions of the stock solution with sea water are then carried out. The concentrations expressed in mg/mL of the solutions contained in the test tubes numbered from 1 to 10 are respectively 50/2, 50/4, 50/8, 50/16, 50/32, 50/64, 50/128, 50/256, 50/512 and 50/1024. Then sixteen (16) live larvae are introduced into each solution of extract to be tested and into that of the negative control without extract and the positive control containing Ranferon-12. All the solutions are left under stirring for 24 hours. The tests were repeated three times.

Counting the number of surviving larvae in each solution under a binocular magnifying glass makes it possible to assess the toxicity of the solution. If deaths are observed in the control medium, the data are corrected by Abbott's formula [24]:

$$\text{\% death} = [(\text{test} - \text{control})/\text{control})] \times 100$$

The LC_{50} values corresponding to the lethal concentration causing 50% death in each solution tested are determined by a linear regression from the equations of the graphs of sensitivity of the larvae to the various extracts and positive control applied.

2.3.4. Anti-Anaemic Activity

The anti-anaemic activity was evaluated according to the protocol of Kambou *et al.* [25], with some modifications. It is based on the red blood cell count and

blood hemoglobin (Hb) concentration or hematocrit of Wistar rats before and after induction of anemia. A preliminary preparation of the hydroethanolic extracts of each of the four species was carried out at 100 mg/mL.

Induction of anemia:

Each day, 20 mg/kg of phenylhydrazine hydrochloride was injected intraperitoneally for 3 consecutive days as described by Naughton *et al.* [26].

Verification of the presence of anemia:

Blood samples from anaemic rats were taken and then analyzed to ensure that the anemia was actually induced. Rats were considered anaemic when the red blood cell count and blood hemoglobin content decreased by 30% [27].

Treatment and monitoring of rats:

Once the anemia was induced, 6 batches of 4 rats were formed at random, including 4 batches for the test with the different plant extracts and 2 batches for the two controls (negative and positive).

The batches of rats formed were treated as follows:

- Lo (solution control−) with normal saline;
- LR (control+) with 0.15 mg/kg of Ranferon-12;
- LGb with 2000 mg/kg of *Gossypium.barbadense* extract;
- LJs with 2000 mg/kg of *Justicia secunda* extract;
- LSb with 2000 mg/kg of *Sorghum bicolor* extract;
- LHs with 2000 mg/kg of *Hibiscus sabdariffa* extract.

The administration by gavage of the extracts and control solutions to the rats was carried out over a period of 15 days at intervals of three days between two administrations. That is a total of 7 administrations (d1, d3, d5, d7, d9, d12, d15). This operation was guided by the work of Koffuor *et al.* [27] and Onoja *et al.* [28].

After treatment, red blood cell count and blood hemoglobin (Hb) concentration were determined using a KX-21 hematology analyzer (Sysmex, Kobe, Japan).

2.3.5. Data Processing

For statistical data analysis, GraphPad Prism Verion 5.0 software for Windows (GraphPad Sftware, San Diego, California, USA) was used. The hematological data were subjected to one-way Analysis of Variance (ANOVA), followed by Bonferroni's multiple comparison test (post-test); the $P < 0.05$ was considered statistically significant for the analyses.

At the end of the treatment, the results were expressed in concentration of red blood cells, hematocrits and hemoglobins.

3. Results and Discussion

3.1. Performance Extraction

The extractions carried out by decoction and by maceration produced variable yields depending on the solvents and the plant species studied (**Table 1**).

Table 1. Percent yield of aqueous and hydroethanolic extracts.

Species	Yield (%)	
	Aqueous extracts	Hydroethanolic extracts
Gossypium barbadense	14.56 ± 1.15	13.93 ± 1.05
Justicia secunda	06.91 ± 0.28	14.08 ± 0.23
Sorghum bicolor	05.85 ± 0.03	13.58 ± 1.18
Hibiscus sabdariffa	39.20 ± 1.54	15.26 ± 1.46

The highest yield, 39.20% ± 1.54%, is obtained with the aqueous extract of the *Hibiscus sabbariffa* plant and the lowest 5.85% ± 0.03% with the aqueous extract of the *Sorghum bicolor* plant. This difference in yields could be explained by the presence of polar chemical compounds sometimes highly, moderately or weakly concentrated in one species than others. The yields of the hydroethanolic extracts of *Justicia secunda* (14.08% ± 0.23%) and *Sorghum bicolor* (13.58% ± 1.18%) are 2.5 times higher than those of their decoctions. We deduce that these plants could contain moderately polar compounds having more affinity with ethanol than water. This disparity in yields could also be explained by the thermosensitivity of certain compounds during the decoction.

3.2. Phytochemical Screening

Phytochemical screening of the species studied revealed that they are rich in secondary metabolites and all contain gallic tannins, anthocyanins, reducing compounds, saponosides, terpenes and steroids. On the other hand, the cyanogenic and cardiotonic derivatives which are plant phytotoxins have not been characterized in the species studied (**Table 2**).

Apart from *Justicia secunda*, all the species studied contain flavonoids, while only *S. bicolor* does not contain an alkaloid. It should therefore be noted that the most common chemical groups in coloring plants traditionally used for their anti-anaemic effects are polyphenols (tannins, anthocyanins, flavonoids), alkaloids, reducing compounds and saponosides. These results are comparable to those obtained by Kambou *et al.* [25] to those of Yamoah *et al.* [29], and Gbénou *et al.* [30] who also noted the presence of these chemical groups in *J. secunda* plant and spirulina algae extracts tested for their anti-anaemic activity.

The absence of alkaloids was observed only in *Sorghm bicolor* as revealed by the work of Sènou *et al.* [31]. Similarly, the absence of flavonoids noted in *Justicia secunda* was confirmed by the work of Akibou *et al.* [32], however, the work of Abiodun *et al.* [33], revealed the presence of flavonoids in the extracts methanolic, ethanolic and aqueous of *J. secunda* studied for these antibacterial activities in Nigeria.

3.3. Brine Shrimp Cytotoxicity Test Results

The Artemia larvae lethality test, used in the interest of predicting the cytotoxicity

Table 2. Phytochemical characterization of species.

Phytochemical groups	Species				
	Gossypium barbadense	*Justicia secunda*	*Sorghum bicolor*	*Hibiscus sabdariffa*	Total*
Catechic tannins	+	+	+	−	3
Gallic tannins	+	+	+	+	4
Flavonoids	+	−	+	+	3
Leuco-Anthocyanins	+	−	+	+	3
Anthocyanins	+	+	+	+	4
Alkaloids	+	+	−	+	3
Reducing compounds	+	+	+	+	4
Mucilage	+	+	+	−	4
Saponoside	+	+	+	+	4
Cyanogenic derivatives	−	−	−	−	0
Triterpenes	+	+	+	+	4
Steroids	+	+	+	+	4
Coumarins	+	−	+	−	2
Quinone derivatives	−	+	+	−	2
Free antracenes	−	−	+	−	1
C-Heterosides	−	−	+	−	1
O-Heterosides	−	−	+	−	1
Cardiotonic derivatives	−	−	−	−	0
Total#	12	10	15	9	

Note: *: number of species containing a given chemical group, #: number of chemical groups characterized in a given species; +: presence of chemical group, −: absence of chemical group.

of the extracts studied, showed that the lethal doses causing the death of 50% of the larvae vary very little according to the plant and control extracts applied (Figure 1). They are between 3.74 ± 0.16 mg/mL and 4.64 ± 0.62 mg/mL provided respectively by the positive control Ranferon-12 and the aqueous extract of *S. bicolor* applied.

Analysis of these results reveals that it was at very high doses of substances applied that it was possible to achieve 50% mortality in the row of shrimp larvae. In comparison with the toxicity reference table of the brine shrimp test established by Mousseux [34], the LC_{50} values from the extracts tested are on average thirty times higher than the limit set (0.1 mg/mL) to declare that a substance is toxic. Consequently, these results reflect a priori the safety of the extracts and that of the Ranferon-12 control tested against these organisms.

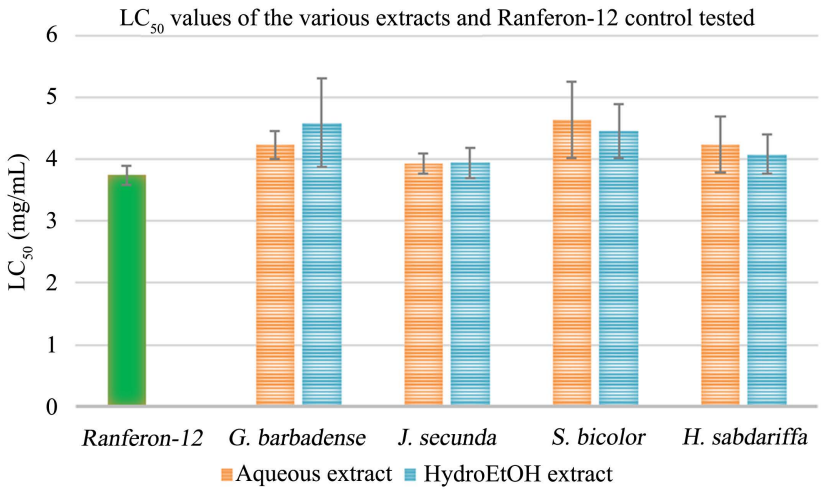

Figure 1. LC_{50} values of the various extracts and Ranferon-12 control tested.

It should be noted that the hydroethanolic extract provided a moderately acceptable extraction yield at the level of all the species and that there is no significant difference between their chemical composition and their toxicity, only the hydroethanolic extract is used for carrying out anti-anaemic activities.

3.4. Anti-Anaemic Activities of Plant Extracts Tested

Three hematological parameters were characterized during the induction of anemia and its treatment with hydroethanolic extracts of the plants studied and the reference anti-anaemic molecule used. These are the hemoglobin level, the hematocrit level and the red blood cell count.

3.4.1. Evolution of the Hemoglobin Level

Table 3 presents the evolution of hemoglobin level in rats before and after treatment with plant extracts.

These results show that the administration of phenylhydrazine hydrochloride caused on day D3 a significant reduction in the hemoglobin level of 42.29% in the rats compared to the values recorded before induction (day D0).

After induction, a gradual recovery is observed on the following days (D7, D12, D18) both at the level of the batch of LR control rats and at the level of the LGb, LSb LJs and LHs batches having received the hydroethanolic extracts of the plants (Figure 2).

Indeed, the average recovery of the hemoglobin level of the rats treated with the *G. barbadense* extract is 17.80 g/dL on day D18 compared to 9.80 g/dL on day D3, that is a significant increase of 81.63%. The improvement observed in the hemoglobin level due to treatment with the *S. bicolor* extract on day D18 is 90.62%, an increase of 18.30 g/dL compared to 9.60 g/dL obtained on day D3. For the extracts of *J secunda* and *H. sabdariffa*, it went from 9.35 to 18.40 g/dL and from 9.7 to 18.00 g/dL on day D18, corresponding respectively to an improvement evaluated at 96.79% and 85.56% due to these species.

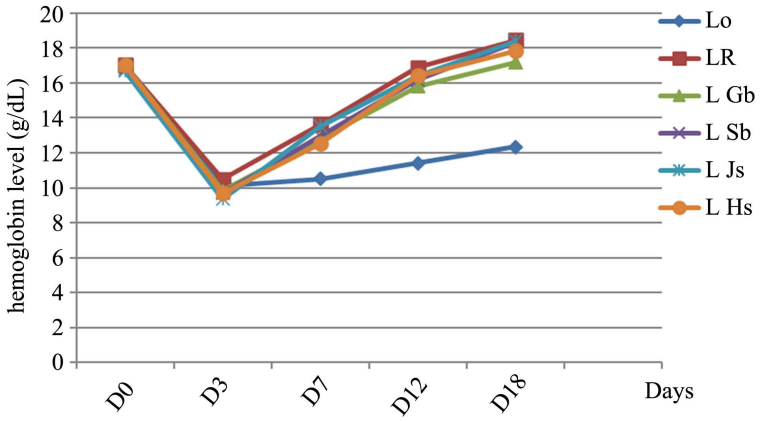

Figure 2. Evolution of the hemoglobin level during the treatments.

Table 3. Effect of 2000 mg/kg per day of hydroethanolic extracts of each plant on hemoglobin level.

Tests	Hemoglobin level (g/dL)				
	D0	D3	D7	D12	D18
Lo (**control**−)	17.10	10.1	10.5	11.4	12.03
	-	−40.93^{a*}	+3.96	+12.87	+19.10
L_R (**control**+)	17.05	10.00	13.60	16.70	18.47
	-	−41.34^{a}	+36^{b}	+67^{b*}	+84.70^{b*}
L_{Gb}	17.00	9.80	12.80	15.85	17.80
		−42.35^{a*}	+30.61^{b}	+61.73^{b*}	+81.63^{b*}
L_{Sb}	16.80	9.60	13.00	16.20	18.30
	-	−42.85^{a*}	+35.41^{b}	+68.75^{b*}	+90.62^{b*}
L_{Js}	16.70	9.35	13.50	16.40	18.40
	-	−44.01^{a*}	+44.38^{b}	+75.40^{b*}	+96.79^{b*}
L_{Hs}.	17	9.7	12.5	16.30	18
	-	−42.29^{a*}	+28.8^{b}	68.04^{b*}	85.56^{b*}

Note: a: percentage variation with respect to day Jo; b: percentage change compared to day D3; *: significant difference ($P < 0.05$).

In addition, on the 18th day, there is a remarkable improvement in the hemoglobin level of the rats treated with the extracts of *S bicolor* (90%) and *J. secunda* (96.79%) compared to that of the treated control (76.47%). Indeed, the comparison of the action of the extracts with that of the Ranferon-12 control shows that a normal progression of the hemoglobin level was induced in the rats treated with the extracts from the plants studied. The strongest recovery of the hemoglobin level being that induced by the extract of *J. secunda* followed closely by that of *S. bicolor*. However, a very weak increase in the hemoglobin level is recorded in the saline solution constituting the negative control used.

3.4.2. Evolution of the Hematocrit Level

The evolution of the hematocrit level in rats before and after treatment with the different extracts as well as with the control substances is summarized in Table 4.

Analysis of the results from Table 4 shows on the third day of induction, a fall in the hematocrit level in the rats, evaluated on average at 40.75% under the effect of phenylhydrazine hydrochloride. Then, a gradual recovery of the hematocrit level was observed between the 7th and 18th day (Figure 3).

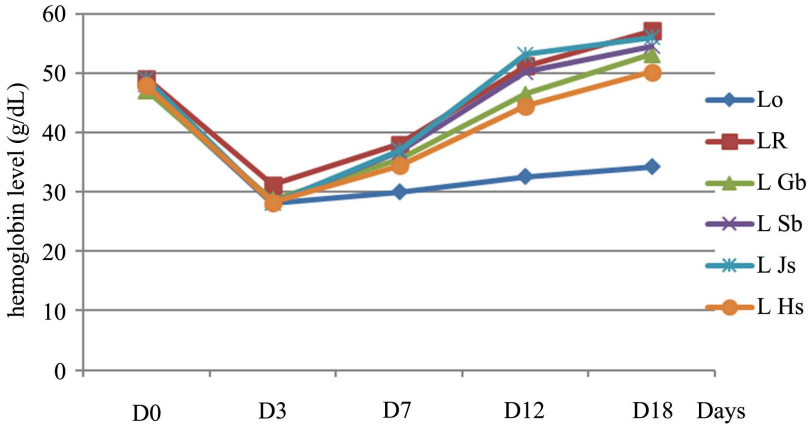

Figure 3. Effect of extract of each plant studied and of the reference anti-anaemic molecule on the evolution of the hematocrit level.

Table 4. Effect of 2000 mg/kg per day of hydroethanolic extract of each species studied on the hematocrit level.

Tests	Hematocrit level (%)				
	D0	D3	D7	D12	D18
Lo (**control**−)	47.5	27.9 −41.26^{a*}	30.01 +7.56	32.77 +17.45	34.15 +22.40
L_R (**control**+)	49	30.0 −38.77^{a}	38 +26.66^{b}	51 +70^{b*}	57 +90^{b*}
L_{Gb}	47	28.6 −39.14^{a}	35.6 +24.47^{b}	46.4 +62.23^{b*}	53.6 +87.41^{b*}
L_{Sb}	48	28.1 −41.45^{a*}	36.7 +30.60^{b}	50.2 +78.36^{b*}	54.4 +93.95^{b*}
L_{Js}	49	28.3 −42.24^{a*}	36.9 +30.38^{b}	53 87.27^{b*}	56 +97.87^{b*}
L_{Hs}.	48	28.0 −41.66^{a*}	34 21.42^{b}	45 60.71^{b*}	50.5 80.35^{b*}

Note: a: percentage variation with respect to day Jo; b: percentage change relative to day D3; *: significant difference ($P < 0.05$).

The recovery of the hematocrit level is favored by the administration of the plant extracts tested, the highest recovery rate being that induced by *J. secunda* (97.87%) followed by nearly *S. bicolor* (93.95%) then *G. barbadense* (87.41%) and *H. sabdariffa* (80.35%), on the 18th day of treatment. During this time, there is a fairly slow recovery estimated at 22.40% in the saline-fed rats, while those who received Ranferon-12 regenerated their hematocrit levels by 90%.

3.4.3. Evolution of the Red Blood Cell Count

The evolution of the red blood cell count in rats before and after treatment with the various extracts and control substances is summarized in Table 5.

Like the data from the evolution of hemoglobin and hematocrit levels, we also note a drop in the level of red blood cells of 43.81% in the rats on the third day of induction with phenylhydrazine.

During treatment, a gradual production of red blood cells is observed between the 7th and 18th day. These results show that on each day of blood sampling (D7, D12 and D18), the strongest regeneration of red blood cells is ensured by the extract of *J. secunda*, the highest rate of 99.06% being recorded on the 18th treatment day. It is followed by *S. bicolor* (96.80%) and the positive control Ranferon-12 (Figure 4). However, a low rate of red blood cell production (20.52%) is induced by the normal saline solution used as a negative control.

Overall, the analysis of the data from the anti-anaemic activity reveals rapid resorption of the incriminated hematological parameters, in particular by ingestion

Table 5. Effect of 2000 mg/kg per day of hydroethanolic extract of each species studied on the level of red blood cells.

Tests	Red blood cell count (n.10^6 /µL)				
	D0	D3	D7	D12	D18
Lo (control−)	5.97	3.41 −42.88^{a*}	3.62 +6.15	3.86 +13.19	4.11 +20.52
L_R (control+)	6.0	3.3 −45^{a*}	4.75 +35.71^{b}	5.8 +75.75^{b*}	6.4 +93.93^{b*}
L_{Gb}	5.94	3.28 −44.78^{a*}	4.46 +35.97^{b}	5.49 +67.37^{b*}	6.15 +87.50^{b*}
L_{Sb}	5.7	3.13 −45.08^{a*}	4.35 +38.97^{b}	5.40 +72.52^{b*}	6.16 +96.80^{b*}
L_{Js}	5.75	3.20 −41.21^{a*}	4.59 +46.33^{b}	5.60 +81.25^{b*}	6.37 +99.06^{b*}
L_{Hs}.	5.80	3.25 −43.96	4.4 35.38b	5.4 66.15^{b*}	6.00 84.61^{b*}

Note: a: percentage variation with respect to day Jo; b: percentage change relative to day D3; *: significant difference ($P < 0.05$).

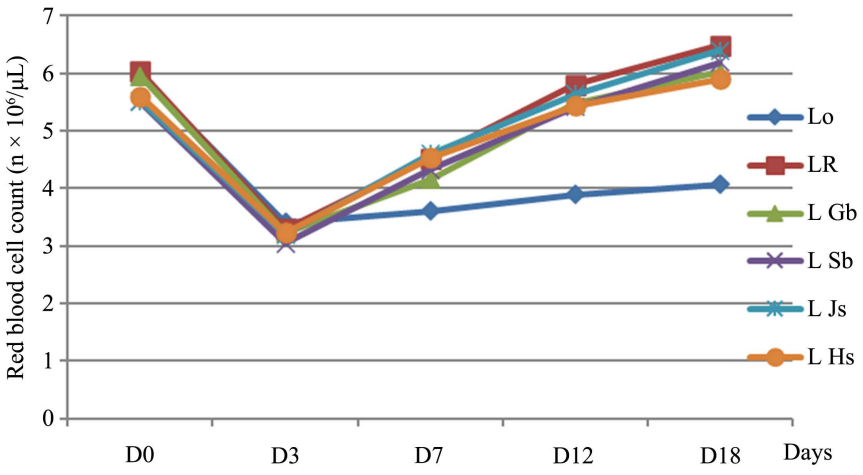

Figure 4. Evolution of the red blood cell count during the different treatments.

of extracts of *S. bicolor* and *J. secunda* with a high resorption power approaching 100%, noted at the level of *J secunda* after 15 days of treatment, as shown by the work of Onoja *et al.* [28]. These results are comparable to those of Gbénou *et al.* [30] who proved that extracts of *J. secunda* lead to resorption of up to 100.28% on day D15 of treatment. They are also close to those Yamoah *et al.* [29] on the quite remarkable resorption power of *Justicia secunda* against anemia.

3.5. Discussion

Administration of phenylhydrazine caused a moderate and significant drop in hemoglobin, red blood cell count and hematocrit. The results obtained are similar to those of Ryu and Yook [35] and Yamoah *et al.* [29] who observed a decrease in these hematological parameters of 50% and 55% respectively, with an administration of 40 mg/kg/d of phenylhydrazine in Sprague Drawley rats.

On the 18th day of treatment, the rats having received the control anti-anaemic molecule as well as those treated with the plant extracts studied had almost completely recovered, which was not the case in the negative control rats treated with the saline solution. Indeed, this reversibility of the anemia due to the discontinuation of the administration of phenylhydrazine was described by Ryu and Yook [35] and Criswell *et al.* [36].

Treatment of anaemic rats with Ranferon-12 resulted in increased red blood cell count and hemoglobin concentration compared to rats treated with normal saline. Ranferon-12 like Feroglobulin mainly contains vitamin B12, Zinc, folic acid and iron. Iron, a component of heme, forms the core of the iron-porphyrin heme ring and together with globin chains forms hemoglobin, so its availability may contribute to the regeneration and structural consolidation of hemoglobin. The B-complex vitamins, by acting as cofactors in hematopoiesis, would promote the synthesis of blood proteins [37]. This would explain the increase in the number of red blood cells and the concentration of hemoglobins in rats fed with Ranferon-12.

Similarly, the treatment of anaemic rats with hydroethanolic extracts of the plants studied showed a significant increase in hemoglobin concentration from 9.35 g/dL to 18.40 g/dL, with the number of red blood cells rising from 3.20 × 10^6/µL to 6.37 × 10^6/µL over the experimental period.

These results show that the anaemic rats treated with the extracts of *G barbadense*, *S. bicolor*, *J. secunda* and *H. sabdariffa* almost completely recovered with regard to the number of red blood cells and the concentration of hemoglobin at the 18th day of treatment. This increase in the number of red blood cells could be attributed to the groups of chemical compounds identified in the plant extracts, which were thus able to reverse the effects of anemia induced by phenylhydrazine hydrochloride, as described by the work of Koffuor *et al.* [27] and Diallo *et al.* [38] on extracts of *Solanum torvum* and *Tectona grandis* administered following the induction of anemia in rats by phenylhydrazine.

The phytochemical analysis carried out on the extracts of tinctorial plants studied, revealed that these species contain polyphenols in particular tannins, anthocyanins and flavonoids as well as reducing compounds. These compounds possess a wide range of biological activities related to their reducing character and their affinity for proteins and metal ions. They have well-established antioxidant properties linked to the inhibition of oxidative stress [18] as well as they promote the regeneration of blood tissue by numerous young resistant cells [39]. The presence of these major chemical groups, due to their properties, in particular those of iron chelators, justifies the high rate of regeneration of red blood cells recorded in rats treated with these extracts. The lowest regenerative power 84.61% was recorded at the level of treatment with *H. sabdariffa*. Although effective, this last position of *H. sabdariffa* compared to other species could be justified by the fact that it contains fewer secondary metabolites. On the other hand, the strong capacity for regeneration of red blood cells induced by extracts of *S. bicolor* (96.80%) compared to those of *G. barbadense* (87.50%) and *H. sabdariffa*, would be related to the many chemical groups identified in this species. In addition to the polyphenols, coumarins, terpenes, reducing compounds characterized in these plants, *S. bicolor* also contains quinone derivatives and glycosides.

Although less rich in chemical groups, compared to the extract of *S. bicolor*, *J secunda* displays a very high potential for cell regeneration (99.06%). This capacity for resorption of red blood cells by *J. secunda* compared to the other species tested, would be linked to its high iron content. Indeed, according to the work of Koné *et al.* [40] on the iron content of anti-anaemic plants used in traditional medicine, *J. secunda* would contain 26.6 mg of iron per 100 g of plant matrix, compared to 3.8 mg of iron for 100g of *H. sabdariffa* calyces and 4.2 mg of iron for 100 g of *S. bicolor* panicum revealed by work carried out on the chemical composition of these species respectively by Leung *et al.* [41] and Sawadogo [42].

This could justify a priori the order of regeneration of red blood cells observed in this study by the plant extracts used: (*H. sabdariffa* < *G. barbadense* < *S. bico-*

lor < *J. secunda*).

Iron plays an important role in hematopoiesis. However, the therapeutic potential of plants cannot be established on the basis of available iron content alone because other compounds play a role in iron absorption in the body [43]. These compounds are alkaloids, flavonoids, saponins, tannins, calcium, zinc, vitamins C and potassium [44]. For example, vitamin C contributes to the bioavailability of iron in the body [45]. Phytochemical compounds, in particular polyphenols, characterized in the extracts tested, due to their metal-chelating properties and their affinity for proteins, could also explain their anti-anaemic effects. Tannins form by complexation, iron tannates, and regulate by inhibition the intestinal absorption of iron by transforming soluble iron into ferric iron.

This study has shown that the dye plants studied can play a role in part in improving the health conditions of people with anemia, especially those suffering from iron deficiency anemia without the risk of probable side effects. Indeed, this work testifies to the safety of the extracts tested in view of the very high LC_{50} values compared to the reference concentration accepted by the applied test.

4. Conclusions

Phytochemical studies of the plant extracts studied revealed the presence of chemical groups, the most frequent of which in plants with anti-anaemic activity are alkaloids, polyphenols, reducing compounds and saponosides. These plant species are very rich in secondary metabolites and do not contain toxic compounds such as cardiotonic, heteroside and cyanogenic derivatives, which gives them a certain safety as to their use orally. These results are confirmed by toxicity tests which have proven the harmlessness of these extracts in the concentration ranges analyzed.

These studies, the aim of which is to know the chemical components of these plants, verify their harmlessness and evaluate the anti-anaemic properties of the hydroethanolic extracts of *Justicia secunda*, *Sorghum bicolor*, *Gossypium barbadense* and *Hibiscus sabdariffa* in order to confirm their use by the population, have shown that the pharmacological properties of the various secondary metabolites contained in these plants give them, in addition to job security, genuine anti-anaemic activity and an anemia-resorbing power comparable to that of the anti-anaemic molecule Ranferon-12 used.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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