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Haematic Potentials of *Rhynchospora Corymbosa* and *Olax Subscorpioïdea* Extract in Phenylhydrazine – induced Anaemic Rats

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Abstract

The decoction of the whole plant of Rhynchospora corymbosa and Olax subscorpioïdea leaves are used by the Local traditional healers in the

department of Korhogo (Northern Côte d'Ivoire) to treat patients with sickle cell disease. This study was designed to assess, the antianemic potential of these plants extract. Anemia was induced with phenylhydrazine hydrochloride in rats. Animals were divided in normal (N), control (C), test (T), and reference (R) groups. T group included ill-induced Animals treated with R. corymbosa and O. subscorpioidea extracts, and R group ill-induced animals treated with vitamin B9 for anemic animals. Dosing was made as 1-day single dose repeated dose. In anemic animals, the production rate of RBC was significantly ($p < 0.001$) higher in T group as compared to C group. R. corymbosa whole plant and O. subscorpioidea leaves extracts have a haematic potential. The anti-anaemic effect may partly explain their use in patients with sickle cell disease who are affected by a deficiency of erythrocytes and its components.

Keywords: Antianemic activity, R. corymbose, O. subscorpioïdea, sickle cell, decoction

Introduction

Anemia is defined as a decrease in the concentration of circulating hemoglobin compared to the limit values set by the WHO (Levy *and al*., 2016). There are three major types of anemia. According to the size of the red blood cells, there is microcytic anemia in which the red blood cells are smaller than normal. This type of anemia would be due to a low-level of iron or an inherited disorder of hemoglobin. The normocytic anemia is a kind of anemia following a chronic disease. Finally, there is macrocytic anemia related to alcoholism (Vert, 2019). Anemia affects physical growth, cognitive development, reproduction and physical work capacity, resulting in decreased human performance (FAO, 2013). The most vulnerable groups are children, adolescents and pregnant women. Anemia is also a factor in fetal growth retardation, a cause of postpartum hemorrhage thereby increasing maternal morbidity and mortality (Beucher *and al*., 2011). About 50% of anemia cases are due to iron deficiency (Vert, 2019). Anemia is a public health problem that affects populations in both rich and poor countries (De Benoist *and al*., 2008). The global prevalence of anemia is 24.8%. In developed countries the prevalence is 8% compared to 38% in developing countries (Dupont, 2017). In Côte d'Ivoire, 78% of a sample of 128 children aged from 6 months to 2 years are anemic. Seven (7%) of these 78% suffer from severe anemia (Righetti *and al*., 2012). Forty-five percent (45%) of women are affected by the less severe form, 20% by the mod *erate form and 2% suffer from the severe form. The proportion of anemic children and women depends on the place of residence, 21% in rural areas

against 9% in urban areas for children and 73% in rural areas against 60% in urban areas for women (MSHP, 2016).

Most often, blood transfusion remains an effective and rapid means in the management of anemia (Dupouy-Manescau, 2020). However, there are risks of infection during blood transfusion and taking the drugs could create drug-induced immuno-allergic haemolysis which is linked to drug sensitivity (Bachir, 2020). In addition, the use of drugs would be expensive for low-income population (Inkoto *and al*., 2018).

Facing this situation, the use of medicinal plants could culturally serve as an alternative treatment against anemia for remote populations. Indeed, medicinal plants contain a multitude of secondary metabolites with several biological activity and easy access (Coulibaly *and al*., 2020).

Many studies have been conducted to scientifically assess the safety, efficacy and quality of certain plant species from traditional medicine. This is how *Rhynchospora corymbosa* and *Olax subscorpioidea* caught our attention after an ethnobotanical survey in the department of Korhogo. The bibliographic review revealed that *Rhynchospora corymbosa* could promote the reversion of sickle cells (Soro *and al*., 2021). As for *Olax subscorpioïdea*, indigenous healers would use it in the treatment of jaundice (Kerharo & Bouquet, 1950) and could promote the reversion of sickle cells (Soro *and al*., 2020). These plants have displayed, an antioxidant and an anticytotoxic activities (Cantrell *and al*., 2003; Okoro *and al*., 2021). They have also demonstrated analgesic (Odoma *and al*., 2016), anti-inflammatory (Popoola *and al*., 2016), antimicrobial (Dzoyem *and al*., 2014), anti-infectious diseases (Garandi *and al*., 2018) activities.

The aim of the present study was to investigate the antianemic activity of these plants in wistar rats of the species *Rattus norvegicus*. As anemia is an aspect of the pathophysiology of sickle cell syndrome.

Material and methods

Plant material

The plant material consisted of the whole plant of *Rhynchospora corymbosa* and the aerial part of *Olax subscorpioidea*. These plants were collected in the departments of Korhogo (northern Côte d'Ivoire). They were harvested, washed, cut and then dried away from the sun rays at room temperature 25 to 30°C for three weeks, in a well-ventilated room. They were then reduced to powder using an electric grinder (Retsch sk 100). A decoction was made according to the method (Konkon *and al*., 2006). One hundred grams (100g) of powder from each species of plant were separately put in one liter of distilled water and boiled (100^0C) for 10 minutes. The obtained mixture was wrung out in a square of white cotton fabric, then filtered three times on absorbent cotton and once on Whatman paper (3mm).

The filtrate was evaporated at 50°C using an oven. The powders obtained after evaporation were called DRC corresponding to *Rhynchospora corymbosa* and DOSA for *Olax subscorpioidea*. Distilled water was used for the decoction and physiological water (NaCl 0.9% buffer solution at pH 7. was used for the dilutions of the extracts during the experiment.

Animal material

The animal material consisted of male and female rats of *Rattus norvegicus* (Muridae) of the Wistar strain. These animals came from the vivarium of the École Normale Supérieure (ENS) in Abidjan. They were fed daily with Ivorian Compound Food Manufacturing Compony (FACI) pellets and water. There were 45 rats with a body weight between 150-230 g and aged 4 to 5 months. These rats were used to the antianeic test and to determinate some zootechnical parameters.

Antianemic test

Anemia was induced by intraperitoneal administration of 40 mg/kg/bw of phenyl hydrazine (PHZ) for two days (D0 and D1). Animals with hemoglobin concentration <12 g/dL were considered anemic (Berger, 2007). Nine (9) groups of 5 rats were formed. the rats of the N or normal group (1) were given physiological water (NaCl 0.9%). The C or control group (2) received phenyl hydrazine and physiological water (NaCl 0.9%). The rats of R group or 3 received phenyl hydrazine and vitamin B9 syrup. The rats of groups 4, 5 and 6 received 200, 400 and 800 mg/Kg/bw of DRC respectively and the rats of groups 7, 8 and 9 received 200, 400 and 800 mg/Kg body weight of DOSA respectively.

The blood samples were collected on DO, D2, D7, D14 and D21 from the rats by incision of the tail before and after, the anemia was induced by intraperitoneal administration of 40 mg/kg/d of phenyl hydrazine for two days (D0 and D1). The method of (Leonard, 1986) was used. Physiological water, vitamin B9, DRC and DOSA were administered by gavage from D2 to D21. The collected blood of 1 mL was used for haematological tests: Red Blood Count (RBC), Hemoglobin (Hb) and packed cell volume or hematocrit (PCV). The hematological parameters were assayed on days D0, D2, D7, D14 and D21 in the nine groups of rats using an automatic blood cell counter (Sysmex KX 21) in the laboratory of the Pasteur Institute of Cocody. The rats of the nine groups were weighed before any sampling.
Zootechnical parameters values such as average weight (AW) and average daily gain (ADG) were determinate during this experiment (21 days). The effect of DRC and DOSA on these zootechnical parameters were studied.

Average Weight (AW)

The Average Weight was determined by calculating the ratio of the sum of the weights of the individuals of the same batch by their number according to the following formula:

$$\mathbf{AW} = \frac{\text{Sum of the weights of the individuals of the same group}}{\text{Total number of the group}}$$

Average Daily Gain (ADG)

Using weights, we calculated the Average Daily Gain by calculating the ratio of the average gain during a period over the duration in days. The formula is as follows:

$$\mathbf{ADG} = \frac{\text{Weight gain during a given period } (\mathbf{g})}{\text{Duration of period (Days)}}$$

Statistical analysis

Statistical analyzes were performed using GraphPad Prism software, version 9.3.1.471 (2021) (San Diego CA USA). Two-ways analysis of variance (ANOVA) was performed with this software. The hematological values were expressed as mean ± standard error of the mean (M ± SEM). The Analysis of variance and the Dunnet's test applicable to multiple comparisons of data were used for statistical analyses. The change in hematological parameters were considered significant at the threshold of $P < 0.05$.

For the significant difference the following notation is used:

- No significant ( ) : $p > 0,05$;
- Low significant (*) : $p < 0,05$;
- Significant (**) : $p < 0,01$;
- Very significant (***) : $p < 0,001$;
- highly significant (****) : $p < 0,0001$.

Results and discussion

Effect of DRC on some zootechnical parameters:

Table I. Effect of DRC and DOSA on average weights of the rats (g)

Lots	J0	J2	J7	J14	J21
G1 (Normal group)	162,67±6,74	170,07±4,19	177,77±4,74	177,77±7,65	179,33±7,62
G2 (Control group)	149,73±8,14	143,67±13,86	152,20±6,12	162,87±11,77	165,67±11,26
G3 (Refence group)	159,07±10,91	154,53±11,69	193,47±4,65*	206,93±4,19**	211,33±2,96**
G4 (DRC 200)	154,67±3,18	150,40±5,18	160,50±9,12	163,33±8,66	169,67±5,61
G5 (DRC 400)	156,67±13,67	149,10±15,21	160,13±11,94	181,9±4,22	185,67±4,81
G6 (DRC 800)	153,00±15,70	151,76±14,68	164,60±15,50	179,60±8,33	189,67±6,74
G7 (DOSA 200)	151,33±7,80	145,46±6,63	156,00±3,05	161,00±3,00	168,00±7,00
G8 (DOSA 400)	150,25±2,25	148,13±1,88	162±0,25	170,5±5,75	182,5±15,75
G9 (DOSA 800)	151,00±3,00	141±2,00	162±2,00	175±4,00	194,5±0,50

Table II. Effect of DRC and DOSA on average daily gain (g)

Lots	J0	J2	J7	J14	J21
G1 (Normal group)	0,00±5,86	4,55±5,04	9,28±5,38	13,38±4,98	18,44±4,96
G2 (Control group)	0,00±7,69	-4,05±10,63	1,65±6,87	8,77±9,84	10,64±6,63
G3 (Reference group)	0,00±9,70	-2,85±9,92	21,63±8,84	30,09±9,30	32,86±9,30
G4 (DRC 200)	0,00±2,91	-2,76±3,90	3,77±6,27	5,60±6,01	9,70±4,27
G5 (DRC 400)	0,00±12,34	-1,74±11,87	2,21±11,73	16,11±10,48	18,51±10,78
G6 (DRC 800)	0,00±14,51	-2,55±14,10	7,58±14,98	17,39±13,21	23,97±13,46
G7 (DOSA 200)	0,00±7,29	-3,88±6,62	3,08±5,68	6,39±5,83	11,01±7,36
G8 (DOSA 400)	0,00±2,12	-1,41±1,83	7.82±1,62	13,48±4,19	21,46±10,64
G9 (DOSA 800)	0,00±2,81	-6,62±2,28	7,28±2,51	15,89±3,51	28,81±2,58

Effect of DRC and DOSA on hematological parameters

Table III. Effect of the DRC and DOSA on the red blood cell count (**10^6/*u*L**)

Lots	J0	J2	J7	J14	J21
G1 (Normal group)	7.25±0.25	7.35±0.09	7.27±0.26	7.31±0.14	7.30±0.28
G2 (Control group)	7,75±0.20	3.12±0.7	4.70±0.09	5.20±0.39	5.95±0.27
G3 (Reference)	7,25±0.25	3.37±0.2	5.70±0.40	6.83±0.24	7.19±0.40
G4 (DRC 200)	7,75±0.25	3.4±0.46	4.85±0.69	6.71±0.46	7.15±0.02
G5 (DRC 400)	7,05±.015	3.11±0.06	4.30±0.32	6.02±0.20	7.03±0.18
G6 (DRC 800)	7.30±0.80	3.57 ± 0.32	5.04 ±0.45	6.61±0.12	7.12±0.06
G7 (DOSA 200)	7,85±0,35	3,44±0,22	4,42±0,55	6,23±0,50	7,20±0,10
G8 (DOSA 400)	7,17±0,17	3,21±1,32	3,97±0,29	6,110,82	7,25±0,25
G9 (DOSA 800)	7.00±0,50	3,46±1,05	4,22±0,20	6,08±0,11	7,23±0,48

Table IV. Effect of the DRC and DOSA on the hemoglobin rate (g/dL)

Lots	J0	J2	J7	J14	J21
G1 (Normal group)	12.65±0.15	12.46±0.77	12.83±0.22	12.29±0.26	12.93±0.5
G2 (Control group)	13.10±0.90	6.73±0.62	9.6±0.06	10.4±0.33	11.96±0.31
G3 (Reference group)	12.90±0.10	6.9±0.13	10.50±0.46	14.06±0.28	14.96±0.57
G4 (DRC 200)	12.70±0.30	6.43±0.48	10.40±0.86	12.93±0.64	12.86±0.51
G5 (DRC 400)	12.70±0.30	6.03±0.31	10.23±0.44	13.13±0.45	13.41±0.24
G6 (DRC 800)	12.98±0.05	6.66±0.51	10.20±0.46	13.16±0.35	14.66±0.16
G7 (DOSA 200)	12,67±0,44	6,70±1,21	10.00±0,10	12,50±0,10	12,25±0,05
G8 (DOSA 400)	12,33±0,67	7,83±1,39	11,47±0,68	11,25±0,05	13,95±0,15
G9 (DOSA 800)	12,75±0,25	6,90±0,70	10,43±0,94	12,65±0,15	14,10±0,40

Table V. Effect of the DRC and DOSA on the hematocrit rate (%)

Lots	J0	J2	J7	J14	J21
G1 (Normal group)	43.00±0.00	42.93±1.08	43.66±1.02	43.03±1.31	43.96±1.35
G2 (Control group)	43.00±20.00	22.03±1.64	32.36±1.44	37.53±1.55	38.76±1.57
G3 (Reference group)	43.00±2.08	22.10±1.40	36.60±1.73	46.23±0.95	46.46±0.95
G4 (DRC 200)	41.00±40.00	22.23±1.28	36.10±1.60	42.63±0.95	43.73±1.15
G5 (DRC 400)	41,50±3.50	21.96±0.35	33.26±1.02	42.63±0.95	43.3±0.66
G6 (DRC 800)	41,50±6.50	22.03±0.95	34.86±0.95	42.76±1.08	43.23±0.84
G7 DOSA 200	44,50±0,50	21,75±2,54	40,07±2,40	48,71±1,15	46,96±3,70
G8 DOSA 400	40,50±0,50	32,66±4,75	33,04±8,01	42,17±0,12	49,87±5,93
G9 DOSA 800	44.00±1,00	26,25±8,62	33,97±13,45	50,35±0,24	45,54±12,44

The aim of this study was the assessment of the antianemic activity of two medicinal plants used in traditional medicine in northen Côte d'Ivore. *Rhynchospora corymbosa* known under the vernacular names of "Lôwonne or Tchang and Kômourouni" in Sénoufo and Malinké respectively and *Olax subscorpioïdea* known as "Nimbôchi or Korogbé" in Malinké. The decoction was chosen as the extraction mode because of its strong use in the preparation of herbal medicines. (Bla *and al*., 2015) indicated that the decoction is the most requested mode of preparation (65.38%). Moreover, the results (Gnagne *and al*., 2017) showed that the decoction is used at 88.2%. The phytochemical screening revealed the presence of alkaloids, catechin tannins, polyterpenoid, sterols and saponins in DRC. Also, (Soro *and al*., 2021) highlighted the presence of polyphenols, flavonoids, leuco-anthocyanins, alkaloids, saponins and steroids in DOSA. These chemical groups have been proved to exhibit biological activities. Indeed, phenolic compounds such as catechin tannins are known for their antioxidant

properties (Ebrahimzadeh *and al*., 2010). These antioxidant compounds could, activate the immune defense and protect erythrocytes against the oxidation of membrane proteins and lipid peroxidation (Mpondo *and al*., 2012). According to (Létapin, 2016), the consumption of tannin-rich plants in small ruminants seems to represent an alternative in order, to anthelmintics to control gastrointestinal nematodes and make the skin resistant to internal parasites. Parasites such as ticks cause up to 37% anemia in cattle (Azokou *and al*., 2016). As for saponins, they would have antifungal, antibacterial and antiviral properties. They would also present protective activities of veins and capillaries (Macheix *and al*., 2005). In addition to these compounds, alkaloids have antibiotic, antiparasitic and analgesic activities (Koua *and al*., 2018) and would also have an effect on the central nervous system (Bruneton *and al*., 2009). Finally, terpenoids and steroids derived from terpenoids constitute the largest known set of secondary plant metabolites (Yamunadevi *and al*., 2011). Steroids are secondary metabolites known for their analgesic and cardiotonic properties, regulate protein and carbohydrate metbolism, increase muscle and bone synthesis (Bruneton *and al*., 2009). Note that polyphenols, flavonoids, leuco-anthocyanins and quinones are absent in DRC. Quinones are also absent in DOSA. With regard to the effect of DRC and DOSA on the zootechnical parameters (average weight and average daily gain) of the rats, the results revealed that all the T-group showed a gradual increase in the weight of the rats throughout the experiment. The results in Table I shows the evolution of the average weight of the rats. The body weight of all animals, at the initial time, ranged from 149.73±8.14 to 162.67±6.74 grams. There is low significant difference ($p < 0.05$) between the average weights of N-group compared to the other groups at D0 and D2. At week three (Day 21) the groups R and T recorded an increased average weight of 32.86±9.30% for R-group, 9.70±4.27% for DRC 200 ; 18.51±10.78% for DRC 400 ; 23.97±13.46% for DRC 800 ; 11.01±7.36% for DOSA 200 ; 21.46±10.64% for DOSA 400 ; 28.81±2.58% for the DOSA 800 compared to the C-group which was 10.64±9.63%. The increased average weight in T-groups was concentration dependant. This observation could attest that DRC and DOSA have a positive effect on the average weight growth of rats. This same trend was observed with the values of the average daily gain. The table II shows the evolution of the Average Daily Gain (ADG) of the weight of the rats in the T-groups compared to the C-groups during the 21^{th} days of the experiment. On the first day (J0), the average daily gain were 0.00±7.69, 0.00±9.70, 0.00±2.91, 0.00±12.34, 0.00±14.51, 0.00±7.29, 0.00±2.12 and 0.00±2.81% respectively for C-group ; R-group, DRC 200, DRC 400, DRC 800, DOSA 200 ; DOSA 400 and DOSA 800 compare to that of N-group which was 0.00±5.86%. These values were almost zero in all groups. On day

(J21), the average daily gain recorded were 1.56±0.44 ; 0.46±0.20 ; 0.88±0.51 ; 1.14±0.64 ; 0.52±0.35 ; 1.02±0.51 ; 1.37±0.12% for R-group, DRC 200, DRC 400, DRC800, DOSA 200, DOSA 400 and DOSA 800 compared to the C-group which was 0.51±0.32% (table II). DRC and DOSA acted in a dose-response manner because the more the dose increases the more average weight increases, the better growth was observed with the rats of R-group. DRC and DOSA are beneficial on the weight evolution. This could, also, confirm the safety of DRC and DOSA which could be a food supplement for rats. The chemical composition of *Rhynchospora corymbosa* plants could indicated these observed beneficial effects. Indeed, for Létapin (2016), plants rich in tannins have beneficial effects in terms of weight gain and contribute to better milk and wool production in animals.

The haematic potential effect of DRC and DOSA in comparison to control and reference groups is shown in Tables II, IV and V. Values are mean for n=5. The baseline values in control animals were 7.75±0. 20 T/L; 13.10±0.90 g/dL; 43.00±2.00% for RBC, Hb and hematocit percentage respectively. The administration of PHZ caused a decrease in red blood cells count, hemoglobin and hematocrit percentages. Indeed, on Day 2 we had 3.12±0.70 T/L (59.74 % reduction), 6.73± 0.62 g/dL (48.62 % reduction) and 22.10 ±1.49 (48.60 % reduction) for the red blood cells count, hemoglobin and hematocrit percentages respectively. Our results are in line with those of (Gbenou *and al*., 2006). These authors observed a decrease in the number of red blood cells, hemoglobin and hematocrit percentages after the administration of PHZ. According to (Sheth *and al*., 2021), PHZ causes hemolytic anemia in rats. Indeed, phenylhydrazine caused oxidative stress by production of free radicals. So lipid peroxidation is generated, which induces red blood cells lysis (Zangeneh *and al*., 2019). However, it should be noted that the anemia induced by PHZ is reversible. Indeed, after treatment with DRC and DOSA at concentrations of 200, 400 and 800 mg/kg/BW, a restoration of the hemoglobin level, the red blood cells count and the hematocrit percentage to normal was observed compared to rats that did not receive phenyl hydrazine. In other words, PHZ does not destroy the stem cell producing blood cells (Berger, 1986).

Rats of T groups were given 200,400 and 800mg/kg bw of DRC and DOSA respectively after induction of anemia by phenylhydrazine. The administration of both extracts and vitamin B9 allowed a significant increase of the RBC count, hemoglobin (Hb) level and the hematocrit percentage from the 7th day of treatment up to D21 compare to the N and C groups. Indeed, from D2 to D21 the tables III, IV and V showed the following results : 3.4±0. 46 to 7.15±0.02 T/L, 6.43±0. 48 to 12.86±0.51g/dL , 22.23±1.28 to 43.73±1.15% for RBC count, hemoglobin (Hb) level and the hematocrit percentage respectively for DRC T-200. 3.44±0. 22 to 7.20±0.10

T/L, 6.70±1.21 to 12.25±0.05g/dL , 21.75±2.54 to 46.96±3.70% for RBC count, hemoglobin (Hb) level and the hematocrit percentage respectively for DOSA T-200. The concentations of 200, 400 and 800 mg/mL of both extracts have restored the anemia in rat by increasing RBC count, hemoglobin (Hb) level and the hematocrit percentage. (Soro *and al.*, 2021) about chimial analysis on DRC and DOSA revealed the presence of alkaloids and phenolic compounds which are known for their anti-anemic activity.II Indeed, alkaloids, flavonoids have antioxidant activity which prevent and repair the free radicals ations on the erythrocytes by enhancing the resistance of RBC to hemolysis (Itodo *and al*., 2011)**;** (Gui *and al*., 2019). So according to Turaskar *and al*., (2013) those compounds reverse anemic conditions. For example, alkaloids inhibit cyclic adenosine monophosphate (cAMP). However, the results of this treatment by DRC and DOSA was inversely proportional to the extracts concentartions. The more the concentations increased, the more the RBC count and the hematocrit percentage decreased and the more hemoglobin level decreased. Also, the phytochemical screening performed by Soro *and al.*, (2021) on DRC and DOSA revealed the presence of saponins. This compound is known to denature the membrane structure of erythrocytes, thus increasing the hemolysis of the RBC. The more DRC and DOSA concentrations increase, the higher saponins content and the slower the regeneration of RBC occures and the hématocrit percentage increases. On D21, the RBC count were 7.15±0.02, 7.12±0.06, 7.20±0.02 and 7.23±0.48 for DRC-200, DRC-800, DOSA-200 and DOSA-800 respectively (Gui *and al*., 2019) (table III).

Despite the presence of saponins, others phytochemicals such as polyphenols and alkaloids presents in DRC and DOSA may have contributed to PHZ-induced hemolysis inhibition. (R-Tiendrebeogo *and al*., 2019) showed in their study that the anti-hemolytic and anti-lipid peroxidation activity of *Ficus sycomorus* were due to the antioxidant activity of phenolic compounds. Also, Yenon *and al*., (2015) showed that the anti-anemic activity of *E.angolense* bark was linked to the presence of these compounds.

The Literature alleged that one of the clinical signs of sickle cell disease is anemia and one of the therapeutic means sought is transfusion therapy (Lemonne *and al*., 2013); (Stuart *and al*., 2019). DRC and DOSA by increasing hemoglobin, hematocrit and red blood cell count could help sickle cell patients prevent anemia associated with sickle cell disease.

Conclusion

The whole plant of *Rhynchospora corymbosa* and the aerial part of *Olax subscorpioidea* significantly increased the the RBC count, hemoglobin level and hematocrit percentage in phenyl hydrazine anemic-induced rats. This anti-anemic activity could be related to the antioxidant properties

characterized by the presence of phenolic compounds. The use of *Rhynchospora corymbosa* and *Olax subscorpioidea* in traditional area to fight anemia related to sickle cell disease could be justified.

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