The Journal of Phytopharmacology 2021; 10(1): 1-6
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2021; 10(1): 1-6
January- February
Received: 22-11-2020
Accepted: 15-01-2021

doi: 10.31254/phyto.2021.10101

M'béfèhê Soro
Agro-valuation Laboratory and Agroforestry, UFR Agroforestrie, Université Jean Lorougnon Guédé (UJLoG), BP Daloa 150, Côte d'Ivoire

Joël Akakpo-Akue
Laboratory of Pharmacodynamic and Biochemistry, UFR Biosciences, Université Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

Jacques Auguste Alfred Bognan Ackah
Agro-valuation Laboratory and Agroforestry, UFR Agroforestrie, Université Jean Lorougnon Guédé (UJLoG), BP Daloa 150, Côte d'Ivoire

Jean-François Sibri
Agro-valuation Laboratory and Agroforestry, UFR Agroforestrie, Université Jean Lorougnon Guédé (UJLoG), BP Daloa 150, Côte d'Ivoire

Yayé Guillaume Yapi
Agro-valuation Laboratory and Agroforestry, UFR Agroforestrie, Jean Lorougnon Guédé University (UJLoG), BP Daloa 150, Ivory Coast

Tatiana Kangah Mireille Kple
Laboratory of Pharmacodynamic and Biochemistry, UFR Biosciences, Université Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

Correspondence:
M'béfèhê Soro
Agro-valuation Laboratory and Agroforestry, UFR Agroforestrie, Université Jean Lorougnon Guédé (UJLoG), BP Daloa 150, Côte d'Ivoire
Email: sorombefehe@gmail.com

Effect of *Rhynchospora corymbosa* and *Olax subscorpioïdea* two plants used in the management of Korhogo sickle cell disease

M'béfèhê Soro*, Joël Akakpo-Akue, Jacques Auguste Alfred Bognan Ackah, Jean-François Sibri, Yayé Guillaume Yapi, Tatiana Kangah Mireille Kple

ABSTRACT

Sickle cell disease is the first genetic disease in the world. *Rhynchospora corymbosa* and *Olax subscorpioïdea* are two plant species used in traditional management of sickle cell disease in the department of Korhogo. After a chemical study of the decocted and the aqueous extract of these two plants, their effect on the *in vitro* reversal of sickling of HbSS genotype erythrocytes, has been evaluated according to Emmel method. An abundance of alkaloids, tannins, saponins, polyterpenes and sterols was found in the decocted and aqueous extract of *Rhynchospora corymbosa* (DRC and MRC). As for the decocted and aqueous extract of *Olax subscorpioïdea* (DOSA and MOSA), polyphenols, leuco-anthocyanins and flavonoids were found in addition to the chemical compounds identified in *Rhynchospora corymbosa*. At the concentration of 0.312 mg/mL DRC, MRC DOSA and MOSA have obtained the reversal rate of 75, 50, 80 and 80% respectively. All four extracts demonstrated activity on *in vitro* reversal of sickle cells. This activity could be caused by the presence of chemical groups that would be used in the treatment of several diseases because of their anti-oxidant and anti-inflammatory properties. *Rhynchospora corymbosa* and *Olax subscorpioïdea* are both an effect on the *in vitro* sickling reversal. Their use in the management of sickle cell disease in northern Côte d'Ivoire might be justified.

Keywords: *Rhynchospora corymbosa*, *Olax subscorpioïdea*, Sickling reversal.

INTRODUCTION

Sickle cell disease is a hereditary disease with recessive transmission. It is due to a genetic mutation in chromosome 11 that leads to the synthesis and establishment of valine instead of glutamic acid in position 6 of β globin chain [1, 2]. The resulting hemoglobin is called hemoglobin S (HbS). It is the most common genetic disease in the world, since the sickle cell gene is found in more than 50 million people, with the highest frequencies in Africa [3]. Clinically, heterozygous subjects express the disease very little or not at all [4]. On the other hand, the homozygous subject suffer from frequent vaso-occlusive crises, hemolytic anemia with sometimes complications such as acute chest syndrome, kidney disease, skin ulcers, retinopathy [5].

Initially confined to malaria-endemic territories in Africa, South-East Asia and the Mediterranean basin, sickle cell disease has been spread to other regions due to population's displacement, migratory flows to North America and Western Europe. Its distribution coincides with areas where there is high malaria prevalence or malaria's history [6]. Each year, about 500,000 sickle cell children were born worldwide of which 200,000 in Africa. Half of all these children die in Africa before the age of 5 in the absence of adequate care [7]. Sickle cell disease is a public health problem for most sub-Saharan Africa countries. In Central and West Africa 20-40% of subjects carry the sickle cell trait [8]. In Côte d'Ivoire, 12% of the population carry hemoglobin "S" and sickle cell disease remains very pronounced in the infant-juvenile population affecting 16.25% of children under the age of four, 65.55% of children between the ages of 5 and 14 and 18.19% of those over the age of 15 [9].

Several treatment options have been proposed to fight against sickle cell disease. All of these therapeutic approaches have side effects and are often inaccessible to low-income populations. These populations most often turn to medicinal plants to treat the ailments they suffer from. Numerous studies have been conducted to assess the safety and efficacy of plant species used in traditional medicine [8]. Some investigations have been carried out in Africa, to assess the antisickling activity of certain plant species [10, 11, 12]. But very few studies have been done in Côte d'Ivoire [13]. This study aimed to assess the *in vitro* antisickling actvity of *Rhynchospora corymbosa* and *Olax subscorpioïdea* two medicinal plants used by traditional healers in the Department of Korhogo for the management of sickle cell disease. Because of that, the decocted and the aqueous extract of each plant were prepared, a chemical study to highlight the

secondary metabolites present in these extracts was carried out and to the effect of these extracts on *in vitro* reversal of sickle cells was evaluated.

MATERIALS AND METHODS

Conditioning and preparation of plant extracts

The entire plant of *Rhynchospora corymbosa*, the leaves and stems of *Olax subscorpioïdea* were used. The plants were harvested in the department of Korhogo in northern Côte d'Ivoire from September 2 to 5, 2019. The two plant species were identified with the planet application and then confirmed by the Agroforestry Agro-valuation Laboratory of the Jean Lorougnon Guédé University of Daloa (UJLoG).

The plants were washed, cut and dried away from the sun at room temperature 25^{0}C for three weeks at the UJLoG Biochemistry Laboratory. They were then powdered using a Retsch sk 100 electric grinder.

The maceration or aqueous extract was done according to the method of Zirihi [14]. One hundred grams (100g) of powder from each plant species were put separately in a liter of distilled water. The mixture was homogenized 10 times due to 2 minutes per turn using a Binatone brand blender. The resulting homogenate was wrung out in a square of white cotton fabric and filtered three times on hydrophilic cotton and once on Whatman (3mm) paper. The filter was evaporated at 50^{0}C using a venticle® type. The resulting powder was called MRC corresponding to *Rhynchospora corymbosa* and MOSA for *Olax subscorpioïdea*. The decoction was made using konkon method [15]. One hundred grams (100g) of powder from each plant species were put separately in a liter of distilled water and brought to a boil (100^{0}C) for 10 minutes. The resulting mixture was wrung out in a square of white cotton fabric and filtered three times on hydrophilic cotton and once on whatman (3mm) paper. The filter was evaporated at 60^{0}C using a steamer. The resulting powder was called DRC corresponding to *Rhynchospora corymbosa* and DOSA for *Olax subscorpioïdea*. The extraction yield was calculated using the following formula:

$$\mathbf{R(\%) = \frac{Mf}{Mi} \times 100}$$

R: yield of the extraction; Mi: Initial mass; Mf: Final Mass

CHEMICAL STUDY

The chemical study of plant extracts was carried out using the technique by precipitation or coloring according to the methods described by Wagner and Bladt [16] and Békro [17].

Alkaloids: It was carried out by precipitation reactions with Dragendorff and Bouchardat reagents. Alkaloids complex with heavy metals such as bismuth, iodine, mercury, tungsten and precipitate in the form of salt. Thus, they form an orange precipitate with Dragendorff's reagent and a reddish brown precipitate with Bouchardat's. Six (6) mL of MRC, MOSA, DRC and DOSA were evaporated dry in a porcelain capsule in the sand bath. The residue was diluted in 6 mL of alcohol at 60 degrees. The resulting alcoholic solution was distributed in two test tubes. In the first tube two drops of Dragendorff reagent were added. The appearance of a precipitate or orange coloration indicated the presence of alkaloids. In the second tube, two drops of Bouchardat reagent were added. The appearance of a reddish brown coloration indicated the presence of alkaloids [16].

Polyphenols: Polyphenols have been demonstrated by reaction to iron chloride. Phenols form with iron chloride (FeCl3), a blue-blackish or green colored precipitate. The assessment of this coloration was made in relation to a control test. At two (2) mL of MRC, MOSA, DRC and DOSA, a drop of aqueous solution of iron chloride (2%, v/v) was added. The appearance of more or less dark blue-blackish or green coloration reflected the presence of phenolic compounds [17].

Tannins: Stiasny's reagent has helped to highlight catechin tannins and gallic tannins. The cathechic tannins, in condensed form, were precipitated into large flakes by heating followed by cooling. The gallic tannins, which come in the form of hydrolysible heterosides, were hydrolyzed after the addition of sodium acetate, and then form a blue-black precipitate in the presence of iron chloride [17].

Catechic tannins: Five (5) mL of MRC, MOSA, DRC and DOSA were added to 15 mL of Stiasny reagent. The mixture was kept in a double boiler at 80^{0}C for 30 minutes and then cooled under water t. The observation of large flake precipitates characterized the catechic tannins.

Gallic tannins: The solution containing the flakes was filtered on Whatman No. 4 filter paper and the collected filter was then saturated with sodium acetate. Three drops of iron chloride (2%, v/v) were added to the mixture. The appearance of an intense blue-black coloration indicated the presence of gallic tannins.

Flavonoids: Flavonoids were highlighted by the so-called cyanidine reaction. In alcoholic solution, flavonic derivatives were colored variously according to their chemical structure. Thus, flavones gave an orange coloration, flavonols turned red and flavonones red-purple [17]. Two (2) mL of MRC, MOSA, DRC and DOSA were evaporated on a sand bath and the residue was taken back into 5 mL of diluted hydrochloric alcohol 2 times. By adding 2 to 3 magnesium shavings, there was a heat release, then a pink-orange or purple coloration. The addition of 3 drops of alcohol isoamylic intensifies this coloration which confirms the presence of flavonoids. An alcoholic quercetin solution was used to serve as a control.

Saponosides: Saponosides were highlighted by the foam production test. In aqueous solution, saponosides have a very high foam index. They produce a large and persistent moss [17]. Ten (10) mL of MRC, MOSA, DRC and DOSA were put in a test tube and then sealed tightly using a capsule. After agitated energetically for 1min, the foam height was measured after 3 min of rest. The persistence of the moss at a height of 1 cm indicated the presence of saponosides.

Polyterpenes and sterols: The search for sterols and polyterpenes was carried out by Libermann's reaction. Sterols and terpenes react with sulfuric acid in the presence of acetic anhydride to form a purple or purple colored complex, turning blue and then green. This analysis was done in comparison to the cholesterol that serves as a control [17]. Five (5) mL of MRC, MOSA, DRC and DOSA were dried under Rotavapor R-215 BUCHI rotary evaporator. The residue was hotly dissolved in 1 mL of acetic anhydride and collected in a test tube. Along the tube, a volume of 0.5 mL of concentrated sulfuric acid was poured. The appearance of a purple or purple ring on the interphase, turning blue and then green, indicated the presence of polyterpenes and sterols.

Leuco Anthocyanin: Two (2) mL of MRC, MOSA, DRC and DOSA were evaporated on sand bath. After cooling on mats, 5 mL of concentrated hydrochloric acid, 37% and 1 mL of isoamylic alcohol were added to the residue. The solution was heated in a double boiler at 80^0C for 30 minutes. The appearance of a cherry-red or purple coloration characterized the presence of anthocyanin leuco [16].

Free or combined quinones: Borntraeger's reagent helps to highlight free quinonic substances. For combined quinonic substances, prior hydrolysis (HCl at 1/5) [17]. The trial involved immediate hydrolysis of extract solutions by adding hydrochloric acid to 1/5 to characterize total quinonic substances. Two (2) mL of MRC, MOSA, DRC and DOSA were evaporated dry on sand bath. The residue was taken up in 5 ml of 37% diluted hydrochloric acid at 1/5. The solution was then warned in a boiling water bath for 30 minutes. After cooling under a cold water, it was extracted by 20 mL of chloroform in a test tube. Half diluted ammonia (0.5 mL) was added to the chloroformic solution. A red or purple coloration indicated the presence of quinones.

Sickling reversal test

Collection and conditioning: Syringes and gloves were used to take blood samples from voluntary sickle cell patients selected at Cocody University Hospital center. SS genotype blood was confirmed by the electrophoresis method. In addition, the volunteers were not transfused for the two months prior to the blood test. The sample of the volunteers did not take into account age or gender. Also, consent was obtained from the volunteers. The blood was collected in EDTA tubes and stored at 4 degrees.

Blood sample washing and falciformation induction: Emmel's method was used [18] following the protocol described by Imaga *et al*. (2009) [19]. The blood sample was washed for five (5) minutes at 1,000 turns using a Jouan B4i-branded centrifuge. This action has been repeated three (3) times in a row. Once the supernatant was removed with a Pasteur pipette, 1mL of washed red blood cells was suspended in 9 mL of NaCl 0.9% physiological water. In a hemolysis tube, 50 µL of a washed red blood cell solution was added to 50 µL of a 2% sodium meta bisulfite solution, to induce and accelerate sickling-formation. The hemolysis tube containing the mixture was sealed with paraffin to prevent air entry and then placed in a darkroom. At T0, a drop of the mixture was removed and deposited between slide and coverslip for observation at the HumaScope Advanced Vision. The same operation was performed at the time T30, T60, T90, and T120. The observations were made to assessing the morphology of the erythrocytes and quantifying the sickle cells. This series of measurements has been served as a negative control to the sickling reversal test at T0, T30, T60, T90, and T120. The experiment was conducted in a triplicate.

Sickling reversal test: Six (6) solutions of MRC, MOSA, DRC and DOSA were prepared with physiological water at concentrations 0.3125; 0,625; 1, 25; 2, 5; 5 and 10mg/mL. A series of tubes containing 50 µL of a washed red blood cell solution and 50 µL of a 2% sodium meta bisulfitede solution were prepared and sealed with paraffin to prevent air entry, and then placed in a darkroom for 120 minutes. After this incubation time, 50 µL of each of the 6 different concentrations of MRC, MOSA, DRC and DOSA were added to one of the previously prepared tubes. Phenylalanine was used as a positive control during this experiment. Phenylalanine at concentrations of 0.3125; 0,625; 1, 25; 2, 5; 5 and 10mg/mL was treated as the extracts. A total of 30 tubes, 24 tests and 6 positive control tubes were prepared. One drop of each mixture was deposited between slide and coverslip for a morphological analysis of the erythrocytes and for a determination of the sickle cell rate, from the time of mixing and every 30 minutes up to 120 minutes. Corresponding to the times T0, T30, T60, T90, and T120. Pipettes, micropipettes, slide and coverslip were used for sampling and reading on a HumaScope Advanced Vision microscope linked to a tablet to determine the morphology and counting of cells in each drop analyzed.

The results were evaluated as a percentage of residual sickle cells. In the negative control tube, the number of sickle cells increased over time; it was considered that the 100% rate corresponded to the number of sickle cells obtained at 120 minutes. In the test tubes, this number decreased over time so the rate of 100% corresponded to the number of sickle cells at the initial time T0. Sodium meta bisulfite was used to induce sickling-formation and phenylalanine has been served as a positive control. The evolution of the percentage of residual sickle cells over time was given by the following formula:

$$RSR = \frac{\text{mean sickle cell disease at T0}}{\text{mean sickle cell disease at Tx}}$$

RSR= Residual sickle cells Rate; Tx = 0; 30; 60; 90 and 120 minutes; T0 = initial time

RESULTS AND DISCUSSION

Yields of extracts: aqueous extraction and decoction were chosen because of their high use in the preparation of plant-based medicines in traditional environments. DRC and DOSA had a yield of 14.13% and 13.40% respectively against a respective yield of MRC and MOSA of 11.50% and 10.76%. The decoction of each plant had a higher yield than that of aqueousr extraction. The results of the work of Gnagne coincide with ours [20, 21]. These authors indicated that decoction at 88.2% is the most requested method of preparation compared to aqueous extraction which is at 5.9%. Decoction could be the most active asset collection method compare to the aqueous extraction [22].

Chemical study of plant extracts: MRC and DRC extracts revealed an average presence of alkaloids and steroids. Catechic tannins and saponins had an abundant presence. Gallic tannins, polyphenols, flavonoids, anthocyanin leuco and quinones were absent in MRC and DRC. In contrast, MOSA and DOSA extracts revealed an average presence of all groups of chemical compounds studied except gallic tannins and quinones that were found to be absent (Table 1).

Table 1: Chemical study results

		Rhynchospora corymbosa		*Olax subscorpioidea*	
Chemicals groups		**DRC**	**MRC**	**DOSA**	**MOSA**
Alcaloïds	Dragendorff	+	+	+	+
	Bouchardat	+	+	+	+
Polyphenols		-	-	+	+
Tannins	Catechids	+ +	+ +	+	+
	Gallics	-	-	-	-
Flavonoïds		-	-	+	+

Saponines	+ +	+ +	++	++
Polyterpenes and Stérols	+	+	+	+
Leuco anthocyanins	-	-	+	+
Quinones	-	-	-	-

a) - Absence; b) + : Average presence; c)++ : Abundant presence; d) +++ : Very abundant presence

Analysis of the results of the chemical study reveals the presence of alkaloids, catechins, poly terpenic sterols and saponins in DRC and MRC. In addition to these compounds, polyphenols, flavonoids and leuco-anthocyanins were found in DOSA and MOSA.

These families of chemical compounds have been shown to exhibit biological activities. Indeed, cathechic tannins are phenolic compounds known for their antioxidant properties [23]. They could fight oxidative stress from self-oxidation of hemoglobin, which produces free radicals such as O^{2-} oxide super anions [24]. These antioxidants could activate the immune defense and also protect the erythrocyte's membrane from damage caused by oxidative stress [25]. Alkaloids, tannins, flavonoids, anthocyanins and leucoanthocyanes have antioxidant power. They promote tissue regeneration, decrease the permeability of blood capillaries and strengthen their resistance to hemolysis [26]. Polyphenols and their derivatives have been cited as having antisickling activity in various studies [27, 8, 19, 28]. Also, alkaloids have several biological properties [29] According to Badiaga, alkaloids are highly sought after for their wide spectrum of biological activities including antibiotic, antiparasitic, anaesthetic, and analgesic properties [26]. Anesthetic, analgesic and analgesic activities would be essential in the management of sickle cell disease during the pain that would cause; vaso-occlusive crises. Saponosides have antifungal, antibacterial and antiviral properties. These antimicrobial activities might be beneficial to sickle cell disease patient that is susceptible to infection. This family of molecules would also exhibit protective activities of veins and capillaries that are tested because of the abnormal adhesion of sickle cell red blood cells to endothelial cells [30]; finally, terpenoids and steroids are the largest known of secondary metabolites [31]. Steroids are secondary metabolites known for their analgesic properties [32, 33]. It should be noted that polyphenols, flavonoids, leuco anthocyanins and quinones were absent in DRC and MRC but present in DOSA and MOSA. These different groups of compounds would partly explain the antisickling effect sought by the use of these plant species by traditional practitioners

In vitro effects of DRC, DOSA, MRC and MOSA on sickling reversal

Morphological analysis and determination of the percentage of residual sickle cells versus the negative control were performed. After 120 minutes all cells went from the normal rounded shape to the abnormal sickle shape. Hypoxia induced sickle shape to the erythrocytes HbSS.

The results of the effect of MRC, DRC, DOSA and MOSA at concentrations between 0.3125 and 10 mg/mL, on the sickling reversal of induced sickling of HbSS erythrocytes were used to draw curves of residual sickle cell over time (Figures 1 and 2). Analysis of the results in the form of an activity curve generally shows that the MRC curves (0.3125 to 10mg/mL), DRC, MOSA and DOSA at 0.3125 mg/mL have a decreasing slope. The first thirty minutes, from 0 to 30 minutes, the slope of all curves is steep. From 30 minutes to 120 minutes, the slope of the different curves is more or less low. All curves follow the same pace as phenylalanine, which is the positive control. Indeed, no blood cells could be observed, following the treatment of erythrocytes washed by DRC, DOSA and MOSA, at concentrations between 0.625 and 10mg/mL. Fields observed under a microscope showed a mass without a form. The red blood cells did not have a well-defined shape. Toxicological investigations would be required for concentrations between 0.625 and 10mg/mL of DRC, DOSA and MOSA to assess their safety. For the RCM with a concentration of 10 to 0.3125 mg/mL, the TDR was 15, 20, 30, 35, 45, 50% or a sickling reversal rate of 85, 80, 70, 65, 55 and 50%, respectively. The TDR decreased as the RMC concentration increased from 0.3125 to 10mg/mL. RMC therefore has a dose dependent activity. In this study, all extracts had the ability to reverse the abnormal sickle form into a normal rounded form. MRC at 10mg/mL had the best activity on the reversion of sickle cells, at 85%. But at equal concentration of 0.3125 mg/mL, the MOSA and DOSA extracts achieved a better sickling reversal rate compared to DRC with a sickling reversal rate of 75% and finally MRC that had a sickling reversal rate of 50%. Only MRC had a lower activity than phenylalanine the reference amino acid.

Compared to the work of Ismaila [34] which obtained for *C. cajan* leaves, *Z. zanthoxyloides* leaves, *C. cajan* seeds, and *C. papaya*, a TDR between 32 and 47%, our results are better with a TDR between 20 and 50%. These findings could be explained by the presence of groups of secondary metabolite families identified in the extracts. The presence of alkaloids, cathechic tannins, saponins, sterols and triterpenes, polyphenols, flavonoides leuco anthocyanins and quinones in the 4 extracts could explain the ability of these two plant species to reverse the sickle cells, so that they return to their normal, rounded and biconcious form.

As for the best performance, at the concentration of 0.3125mg/mL, of extracts of *Olax subscorpioid* compared to extracts of *Rhynchospora corymbosa*, it could find an explanation in the presence of polyphenols, flavonoids, leuco anthocyanins and quinones in DOSA and MOSA, while these molecules would be absent in DRC and MRC.

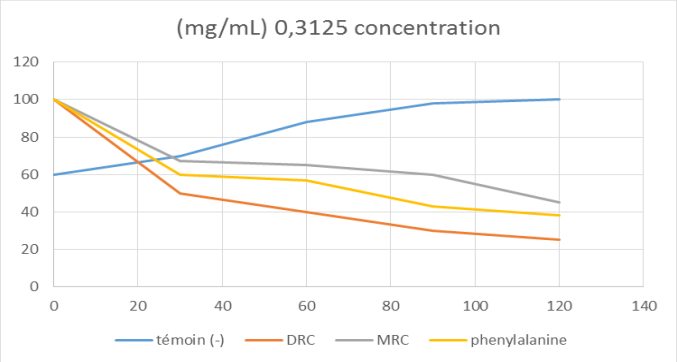

Figure 1: *In vitro* effet on sickling reversal of DRC and MRC at (mg/mL) 0,3125

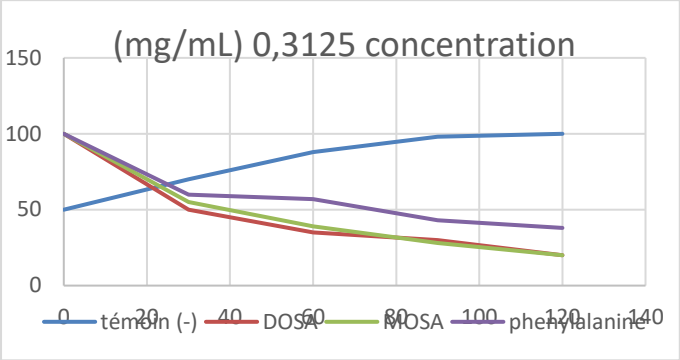

Figure 2: *In vitro* effet on sickling reversal of DOSA and MOSA at (mg/mL 0, 3125)

Table 2: Sickling reversal rate as function of MRC, DRC, MOSA and DOSA's concentrations

	RSR	SRR	RSR	SRR	RSR	SRR	RSR	SRR	RSR	SRR	RSR	SRR
Extracts	(mg/mL) 10		(mg/mL) 5		(mg/mL) 2,5		(mg/mL) 1,25		(mg/mL) 0,625		(mg/mL) 0,3125	
MRC	15%	85%	20%	80%	30%	70%	35%	65%	45%	55%	50%	50%
DRC	-	-	-	-	-	-	-	-	-	-	25%	75%
MOSA	-	-	-	-	-	-	-	-	-	-	20%	80%
DOSA	-	-	-	-	-	-	-	-	-	-	20%	80%

Légende: a) (-): absence of Cell. b) (%): percentage of sickling reversal; c) RSR: Residual sickle cells Rate; d) SRR: Sickling reversal rate

CONCLUSION AND PERSPECTIVES

The two extracts of *Rhynchospora corymbosa* and the two extracts of *Olax subscorpioïdea* were rich in various major chemical groups such as alkaloids, polyphenols, flavonoids, tannins, steroids and saponosides. These chemical groups would be used in the treatment of sickle cell disease due to their antioxidant, anti-inflammatory and analgesic properties. At equal concentration of 0.312 mg/mL, DRC, MRC, DOSA and MOSA achieved a sickling reversal activity of 75, 50, 80 and 80% respectively.

All four extracts demonstrated activity on the *in vitro* sickling reversal. The use of *Rhynchospora corymbosa* and *Olax subscorpioïdea* for the management of sickle cell disease in Korhogo department in northern Côte d'Ivoire would be justified. However, the extracts studied had excellent results at the 0.3125 mg/mL concentration, but appear to cause blood cell lysis at concentrations between 0.625 and 10 mg/mL. Following this study, it would be interesting to study the toxicity of these two plants, to identify and isolate the molecules responsible for the effect on the reversion of sickle cells.

REFERENCES

1. Ingram VM. A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. *Nature*. 1956; 178:792–794.
2. Marotta CA, Wilson JT, Forget BJ, and Weissman SM. -Human β-globin messenger RNA III. Nucleotide sequences derived from complementary DNA. *Journal of Biological Chemistry*. 1977; 252:040–5051.
3. Weatherall DJ. -Phenotype-genotype relationships in monogenic disease: lessons from the thalassaemias. *Nature Reviews Genetics*. 2001; 2:245-255.
4. Serjeant GR. Sickle Cell Disease. Oxford University Press, New York. 3rd edn, (in press), 2001.
5. Habibi A, Arlet J-B, Stankovic K, Gellen-Dautremer J, Ribeil J-A, Bartolucci P, *et al*. Recommandations françaises de prise en charge de la drépanocytose de l'adulte: actualisation. La Revue de Médecine Interne. 2015 ; 36(5):5S3–5S84.
6. free.fr/cours/hemoglobinoses.pdf Mise à jour le 07/01/2012
7. OMS. Bulletin de l'organisation mondiale de la santé. 2012 ; 86(6):417-496.
8. Mpiana PT, Ngbolua NK, Sha TDT. -Les alicaments et la drépanocytose. une mini revue C.R; Chimie, CRAS2C. 2016; 6:2.
9. La fondation LYA et Drépavie. Mission exploratoire d'évaluation des moyens nécessaires pour la mise en place d'un programme de formation dans le cadre du renforcement du système de santé dans la lutte contre la drépanocytose. Côte d'Ivoire, 2018.
10. Sibinga EMS *et al*. Paediatric patients with sickle cell disease: use of complementary and 49 alternative therapies. *Journal of Alternative and Complementary Medicine*. 2006; 12:291-98.
11. Sofowora EA. Medicine plants and traditional medicine in Africa. Spectrum books limited (Ibada) and John Wiley and Son. *Liyodia*. 1985; 4261:1-6.
12. Wambebe C, Khamofu H, Momoh JA, Ekpeyong M, Audu BS, Njoku SO, *et al*. Doubleblind, placebo-controlled, randomised cross- over clinical trial of NIPRISAN in patients with sickle cell disorder: *Phytomedicine*, 2001; 8(4):25261.
13. Kplé TKM, Akakpo-Akue J, Fofié Y, Ahon GM, Golly Koffi J, Kra AKM, *et al*. Sickling Cells Inhibition and Radical Scavenging Activities of Zanthoxylum leprieurii's (GUILL) Bark Extracts: Comparative Study. Journal of Pharmaceutical Research International. 2020; 32(11):42-50.
14. Zirihi G, Kra AKM, Guede-Guina F. - Évaluation de l'activité antifongique de Microglossa pyrifolia (Lamarck) O.Kantze (Astéracée) « PYMI » sur la croissance in vitro de Candida albicans, *Revue de Médecine et pharmacie Afrique*, 2003; 17:11-18.
15. Konkon NG, Simaga D, Adjoungova A. -Etude phytochimique de mitragyna inermis(willd.) o. ktze (rubiaceae), plante a feuille antidiabetique», *Pharm Méd Trad Afr*. 2006; 14:73-80.
16. Wagner H, Bladt S. Plant Drug Analysis: A thin Layer Chromatography Atlas (2nd ed.). Springer, Berlin. 2001; 349-364.
17. Békro YA, Janat A, Békro M, Boua BB, Fézan H, TRA BI, *et al*. Étude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana*

(Baill.) Herend. et Zarucchi (Caesalpiniaceae). Sciences & Nature. 2007; 4(2):217-225.
18. Emmel VE. A red cell study of the erythrocytes in case of severe anemia with elongated and sickle and shape dred blood corpuscule. *Archives of Internal Medicine*. 1933; 769-789.
19. Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, *et al*. Antisickling property of Carica papaya leaf extract. *African Journal of Biochemistry Research*, 2009; 3(4):102-106.
20. Bla KB, Trebissou JND, Bidie A. Étude ethnopharmacologique des plantes antipaludiques utilisées chez les Baoulé- N'Gban de Toumodi dans le Centre de la Côte d'Ivoire. *J Appl Biosci.* 2015; 85:7775-7783.
21. Gnagne *et al*. Étude ethnobotanique des plantes médicinales utilisées dans le traitement du diabète dans le Département de Zouénoula (Côte d'Ivoire) *Journal of Applied Biosciences.* 2017; 113:11257-11266.
22. Salhi S, Fadli M, Zidane L, Douira A. Études floristique et ethnobotanique des plantes médicinales de la ville de Kénitra (Maroc). Lazaroa. 2010; 31:133-146.
23. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of H. Officinalis L. Var Angustifoluis, V. Odorata, B. hyrcana et C. Speciosum. Pak. J. Pharm. Sci. 2010; 23(1):29-34.
24. Mpondo ME, Dibong SD, Ladoh YCF, Priso RJ, Ngoye A. Les plantes à phénols utilisées par les populations de la ville de Douala. *Journal of Animal and Plant Sciences.* 2012; 15:2083-2098.
25. Hebbel RP, Morgan WT, Eaton JW, Hedlund BE. Accelerated autoxidation and heme loss due to instability of sickle hemoglobin. Proceedings of the National Academy of Sciences of the United States of America, 1988; 85(1):237–41.
26. Macheix JJ, Fleuriet A, Jay-Allemand C. Les composés phénoliques des végétaux : un exemple de métabolites secondaires d'importance écoomique. *Presses Polytechniques et Universitaires Romandes (PPUR) : Lausanne, Suisse*. 2005.
27. Okwu DE. NMAP. Science and Biotechnology, 2007; 1(1):9096.
28. Badiaga M. Etude ethnobotanique, phytochimique et activités biologiques de *Nauclea latifolia* Smith, une plante médicinale africaine récoltée au Mali. Université de Bamako. 2011; 136.
29. Thevenin BJ, Crandall I, Ballas SK., Sherman IW, Shohet SB. Band 3 peptides block the adherence of sickle cells to endothelial cells in vitro. Blood, 1997; 90(10):4172–9.
30. Bruneton J. Pharmacognosie, Phytochimie et plantes médicinales. *Tec & Doc et médicales internationales, 3eme édition. Paris (France),* 1120. Clinics in Mother and Child Health. 1999; 1(1):P37-42 20.
31. Yamunadevi M, Wesely EG, Johnson M. Phytochemical studies on the terpenoids of medicinally important plant Aerva lanata L. using HPTLC. A.Pacific *J. of Trop.Biomedicine.* 2011; S220-S225.
32. Hossain MA, Riyami QA, Raqmi AKASA, ZHA M, Weli AM. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grownT hymus vulgaris *Asian Pacific Journal of Tropical Biomedicine Pac J Trop Biomed*. 2013; 3(9):705-710.
33. Kade IJ, Kotila OO, Ayeleso AO, Olaleye AA, Olawoye TL. Antisickling properties of Parquetina nigrescens. Biomed. Res. (Alligarh). 2003; 14:185-188.
34. Ismaila O, Nurain CO, Bewaji JS, Johnson RD. Davenport, and Yang Zhang. Potential of Three Ethnomedicinal Plants as Antisickling Agents. Mol Pharm. 2017; 14(1):172–182.

HOW TO CITE THIS ARTICLE
Soro M, Akakpo-Akue J, Ackah JAAB, Sibri JF, Yapi YG, Kple TKM. Effect of *Rhynchospora corymbosa* and *Olax subscorpioïdea* two plants used in the management of Korhogo sickle cell disease. J Phytopharmacol 2021; 10(1):1-6.