Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(1): 260-265
Received: 01-10-2022
Accepted: 06-11-2022

Akakpo-Akue Joel
Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

KPLÉ Kangah Tatiana Mireille
Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

KRA Adou Koffi Mattieu
Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

KONAN Gbe Kouakou N'Dri Ange
Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

KOUASSI Konan Armand Marcelin
Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

OUATTARA Sitapha
Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

Golly Koffi Julien
Institut Pasteur of Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire

FOFIE Yvette
Pharmacognosy laboratory, UFR of Pharmaceutical and Biological Sciences, University of Félix Houphouët-Boigny, Côte d'Ivoire

SANOGO Ibrahime
Clinical Hematology Department, University Hospital of Yopougon, Abidjan, Côte d'Ivoire

YAPO-CREZOIT Claire Chiayé Antoinette
Institut Pasteur of Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire

DJAMAN Allico Joseph
1. Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire
2. Institut Pasteur of Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire

Corresponding Author:
KPLÉ Kangah Tatiana Mireille
Laboratory of Biology and Health, UFR Biosciences, University of Félix Houphouët-Boigny (UFHB), 22 BP 582 Abidjan 01, Côte d'Ivoire

Phytochemical investigation and antisickling properties of a Poly-herbal formula on the HBSS Red Blood Cells

Akakpo-Akue Joel, KPLÉ Kangah Tatiana Mireille, KRA Adou Koffi Mattieu, KONAN Gbe Kouakou N'Dri Ange, KOUASSI Konan Armand Marcelin, OUATTARA Sitapha, Golly Koffi Julien, FOFIE Yvette, SANOGO Ibrahime, YAPO-CREZOIT Claire Chiayé Antoinette and DJAMAN Allico Joseph

DOI: https://doi.org/10.22271/phyto.2023.v12.i1d.14583

Abstract
The aim of this study was to evaluate the sickle-formation inhibition of a recipe of three plants: *Zanthoxylum leprieurii, Xylopia aethiopica* and *Harungara madagascariensis.* The 70% hydroethanolic extract (EZHm) and the decoctate (DZHm) were used to determine the chemical groups by colorimetric and precipitation reactions. These extracts were also used to evaluate the antisickling properties by Emmel's method, following the protocol described by Image. The biological tests revealed that the total extracts have concentration-dependent and time-dependent anti-sickle cell activity. These extracts act by reducing the polymers of sickle cells. After 120 min the inhibition percentages were 87.35% DZHm, 87.28% EZHm and 85.07% phenylalanine in the presence of 10 mg/mL of the test substances. Plant extracts show a strong antisickling activity which would be due to its phytochemical constituents such as primary (aromatic amino acid) and secondary (alkaloids, phenolic compounds, saponosides, sterols, polyterpenes) metabolites found in these extracts. These extracts, even if they are crude, constitute a hope for the discovery of many antisickling active principles. This natural poly-herbal formula product could improve the quality of life of people with sickle cell disease.

Keywords: Phytochemical investigation, Antisickling properties, Poly-herbal formula, HBSS

Introduction
Sickle cell is the most common hemoglobinopathy in black Africa. Its prevalence ranges from 16 to 40% depending on the region of Africa (Delicat-loembet *et al.*, 2014) [1]. In Côte d'Ivoire, the birth of 6,000 to 8,000 cases of children with sickle cell disease are recorded each year. Forty (40) percent of these children die before the age of five (5) years (Sawadogo *et al.*, 2014) [2]. Sickle cell is the cause of 5% of child deaths on the African continent (WHO, 2006) [3]. Sickle cell disease or sickle cell anemia is an inherited genetic disorder that is characterized by the alteration of normal hemoglobin, a protein that transports oxygen in the blood (Lamine *et al.*, 2017) [4]. It results from a point mutation at the 6th codon of the β-globin gene located on chromosome 11. This Mutation is done by substitution of glutamic acid by valine. Thus inducing the synthesis of an abnormal hemoglobin called hemoglobin S (HbS) (Renaudier, 2014) [5]. In a situation of hypoxia, acidosis, a high level of 2-3 DPG, hemoglobin S sees its solubility decreased. It will therefore polymerize by gelling, thus follows a dehydration of erythrocyte HbS (Aubry and Bernard-Alex, 2019) [6]. Thus leading to the deformation into abnormal or sickle-shaped red blood cells. Sickle-shaped red blood cells not being able to pass block the small vessels (capillaries), causing thrombosis and ischemia in the organs (Marilyn *et al.*, 2019; Kugler 2020) [7, 8].
This falciformation induces an increase in hemolysis, which is also strongly involved in the production of reactive oxygen species, by release of heme in the vessels. An inflammatory response characterized by the activation of the vascular endothelium, which then produces various molecules, including vascular adhesion molecules, certain modulators such as endothelin-1, and proteins involved in coagulation (Strader *et al.* 2020) [9]. This inflammatory response leads to a decrease in the lumen of blood vessels and thus to the blockage of dense GR and irreversible GR, thus causing vascular occlusions, which are a major source of morbidity and mortality. Vaso-occlusive crises, anemia and susceptibility to infections are clinical expressions of sickle cell disease. The intensity and frequency of these clinical expressions over time cause hemolytic complications, complications of vaso-occlusive type

(Aufradet, 2012) [10]. Therapeutically, bone marrow transplantation currently provides a curative solution while gene therapy is in development with the new Crisper Cas9 technology (Grégoire, 2019; Park and Bao, 2021) [11, 12]. However, these highly specialized therapies require appropriate infrastructure, qualified personnel with high financial means and a compatible donor which generally explains their non-availability in Black Africa where the disease is more frequent (Kunh *et al.*, 2017; Hsu *et al.*, 2018) [13, 14].

Côte d'Ivoire, does not escape this situation and the management is essentially symptomatic based on blood transfusion and analgesic drugs. It must be noted that these treatments are not only inaccessible, but also very expensive and constitute a risk of contamination. These psycho-social and economic repercussions of sickle cell crises make sickle cell disease a public health problem for low-income countries. Because of the hereditary nature of the disease, several children in the same sibling can be affected. The availability and wealth in secondary metabolites contained in medicinal plants would constitute an alternative to oppose the physio pathological of Sickle cell disease. Being given that sickle cell anemia is a multifactorial disease (Ngbolua *et al.* 2020) [15]. These plants could have effects on: inflammation, oxidative stress, hemolysis, anemia and falciformation of red blood cells which are the phenomena related to sickle cell disease. Although many plants are used in traditional Ivorian medicine by traditional practitioners, very few scientific studies have been conducted on the anti-sickle cell activity of medicinal plants in Côte d'Ivoire. Thus, in order to contribute to the management of sickle cell disease, our team has set itself the objective of evaluating the anti-sickle cell potential of a traditional recipe based on three medicinal plants.

Material and Methods

Plant materials

The plant material was composed of leaves of *Harungara madagascariensis* (LAM), bark of the trunk of *Zanthoxylum leprieurii* (GUILL) and fruits of *Xylopia aethiopica*. These plants were listed and harvested from December 2017 to February 2018 in the region of Abengourou, (eastern Côte d'Ivoire). They were identified at the National Floristic Center of Félix Houphouët-Boigny University in February 2018 by the late Professeur Aké Assi Jean. On the basis of the information collected from the tradithérapeutes the recipe was constituted. The powders obtained after pulverization of these plants were assembled in the quantities of 33.33 ± 1 g per plant to form a total composition of 100 g. The new composition obtained named ZHm, was used for the various tests.

Preparation of 70% hydroethanolic extract

The hydroethanolic extract was prepared according to the method of Zirihi *et al.* (2003) [16]. One hundred grams (100 grams) of vegetable powder were soaked in one liter of hydroalcoholic 70% ethanol. The mixture was homogenized 10 times for 2 minutes per revolution using a Severin ® brand blender. The obtained homogenate have been filtered using a square of cotton cloth then successively three times on cotton wool and then once with whatman paper (3 mm). The filtrate was evaporated at 45 °C using a Venticell® type oven for 24 hours. The dry powder obtained was codified EZHm.

Preparation of aqueous extract by decoction

According to Konkon's *et al.* (2008) [17] method one hundred grams (100g) of vegetable powder were brought to the boil for 20 min in 2 L of distilled water. The obtained mixture was cooled at room temperature (25 °C) and was filtered three times on cotton wool and once on Whatman 3 mm. The obtained filtrate was dried at 50 °C in the oven of the Venticell® type. The powder obtained was the total aqueous extract codified DZHm.

Phytochemical screening

The phytochemical screening was performed according to the method used by Nemlin and Brunel (1995) [18]. The phytochemicals sought and the reagents used are presented in Table 1.

Table 1: Reagents and tests for characterization of chemical groups

Secondary metabolites	Reagents	Reaction indicating that the test is positive
Alkaloïdes	Dragendorff Bouchardât	Precipitate or orange coloration Reddish-brown precipitate
Polyphenols	Ferrique Chloride	Blackish blue coloration
Flavonoides	Cyanidine	Pink-orange precipitation
Sterols et les polyterpenes	Liebermann	Green ring
Tannins	Stiasny	precipitate in large flakes
Quinones subtances	Bornstraegen	Red or violet coloration
Saponines	Agitation	Persistent moss with a height of 10 cm

Qualitative Research of Aromatic Amino Acids

Aromatic amino acids were sought through the xanthoprotein reaction. To 3 mL of extract, 1 mL of concentrated nitric acid was added. The tube was then boiled for 2 minutes. The appearance of a yellow coloration indicates a positive reaction which highlights the presence of aromatic nuclei (benzene ring); amino acids of the aromatic series: tyrosine, tryptophan, and phenylalanine. The test was performed according to Fofana (2004) [19].

Human Material

To be included in the study, the blood should come from homozygous sickle cell voluntary patients regardless of age and gender. The voluntary patients shouldn't have undergone blood transfusion for at least two months prior to the blood test and they must not be in crisis. In addition, the volunteers have given their consent on an ethical issue. Venous blood sampling of each patient was collected in tube (EDTA). The blood samples were placed in a cooler containing cold accumulators at 4°C and then conveyed to Pasteur Institute in Côte d'Ivoire.

Sickle cell inhibitory activity study

This test was conducted using Emmel [20] method, according to the protocol described by Imaga [21]**.** The assessment of the inhibition of the sickling-formation of red blood cells of SS genotype in a deoxygenate environment using a 2% (p/v) sodium Meta bisulfite solution as reductant.

Conditioning collected blood

After the sample was taken, the blood of Hb SS genotype was washed for five (5) minutes by centrifugation at 1500 rpm. This action was repeated three (3) times. After removing the supernatant using a Pasteur pipette, 1mL of washed red blood cells was resuspended in 1mL of physiological water (NaCl 0.9%).

Sickling cell inhibition test

Fifty microliters (50 µL) of the solution of red blood cells were mixed with 50 µL of a 2% (p/v) sodium meta bisulfite solution and 50 µL of plant extracts at the concentrations of 5 and 10 mg / mL were added to the mixture. A negative control was prepared by mixing 50 µL of washed blood with 50 µL of physiological water and 50 µL of sodium meta-bisulfite (2%, p/v). As for the positive control, it was prepared by mixing 50 µL of washed blood with 50 µL of a phenylalanine solution at 5 and 10mg/mL and 50 µL of sodium meta-bisulfite (2%, p/v). Totaling 7 test tubes. Each experiment was carried out in triplicate. Each of the 7 test tubes was sealed with paraffin. After 2 hours of time, a drop of each mixture was deposited between slide and coverslip and an observation was made under an optical microscope (X 40) for erythrocytes morphological analysis and counting of sickle cells. This was done every
30 minutes up to 120 minutes. The Sickling inhibitory activity of the plant is its ability to prevent the sickling formation of red blood cells in low oxygen pressure environment. It is expressed as a percentage of sickle cells formed in the presence of extracts compared to the number of sickle cells formed in the negative control. The average of sickle cell number at different times was given by SQA vision microscope and the inhibitory activity was determinate by the following formula:

$$AA = (P0-Px) / P0*100$$

AA refers to sickling inhibitory activity; P0 the average of the sickle cells in the control; Px the average of sickle cells on test slide in the presence of plant extracts at Tx = 0, 30, 60, 90 et 120 minutes.

Data analysis

Statistical analysis of the data and graphical representation was performed using Graph Pad Prism 9.0 software (San Diego, California, USA). Values were given as means followed by the standard error on the mean (M ± SEM). The difference between two values was given by student's t-test which was completed by Dunnet and Turkey tests as post tests. The significance level was set at $P < 0.05$ for the expression of the results.

Results

Phytochemical study

The qualitative phytochemical analysis performed on DZHm and EZHm revealed the presence of different groups of secondary metabolites. These results reveal the presence of polyphenols, tannins, flavonoids, alkaloids, steroids, triterpenes and quinone substances in DZHm and EZHm; in contrast, saponosides were absent in EZHm (Table 2).

Table 2: Results of the phytochemical study of the extracts of the ZHm recipe

Decoction (DZHm)							Ethanolic Extrat				(EZHm)		
S/T	**POL**	**FLA**	**TAN**	**SQ**	**ALC**	**SA**	**S/T**	**POL**	**FLA**	**TAN**	**SQ**	**ALC**	**SA**
+	+	+	+	+	+	+	+	+	+	+	+	+	-

(+): Presence (-): Absence

ALC: Alkaloids; TAN: Tannin, FLA: Flavonoid; POL: Polyphenols; S/T: Sterol / Triterpenes; SQ: Quinones; SAP: Saponosides.

Aromatic Amino Acids Qualitative Research

The test carried out on DZHm and EZHm allowed detecting the presence of aromatic amino acids in both extracts. The amino acids present were characterized by the yellow coloration of the reaction (Table 3).

Table 3: Aromatic amino-acids presence

Extracts	Aromatic amino acids
Decoction (DZHm)	+
Ethanolic Extract (EZHm)	+

Sickling Inhibitory Activity

Effect of DZHm and EZHm of inhibition of sodium meta bisulfite induced HbSS erythrocytes sickling- formation

Effect of DZHm

HbSS erythrocytes with sodium metabisulfite (2%, w / v) resulted in a significant increase in sickle cell average between 0 and 120 min. Which explained the increasing pace of the curve of sickle cells rate against time. The average number of sickle cell increased from 56.67 to 98.38 at 30 to 120 min respectively. The average of sickle cells number increased over the time. The sickling of erythrocytes was time-dependent. Between 0 and 120 min of observation, DZHm at 5gm/mL caused a gradual drop in the average of sickle cells. The curve was decreasing allure, unlike that of erythrocyte treated with 2% NaCl only. The average number was 47.67, 43.33, 40.33 and 32.00 at 30, 60, 90 and 120 min respectively. Which led to increasing inhibitory activity of DZHm over the time to 15.88, 38.09, 54.68 and 67.45% at 30, 60, 90 and 120 min. At 10 mg/mL of DZHm, the average number of sickle cell was 21.00, 18.54, 15.50 and 12.4 corresponded to the inhibitory activity of 62.94, 73.51, 82.58 and 87.35%. The results showed that the longer the contact time between the erythrocyte cells and DZHm, the greater the inhibitory activity. In another hand the inhibitory activity of DZHm at 10mg/mL was higher than that of DZHm at 5mg/mL at each 30 minutes during 120 minutes. More the concentration was higher more the inhibition was stronger (figure 1). The inhibitory activity of DZH was concentration dependent.

Effect of EZHm

The erythrocytes treated with 2% NaCl in the presence of EZHm 5mg/mL gave as results the average number of Sickle cell of 39.00, 34.67, 31.00 and 28.33 which equated to 31.17, 50.47, 65.16 and 71.18% at 30, 60, 90 and 120 minutes respectively. At 10 mg/mL EZHm gave 23.33, 20.00, 17.33 and 12.50 as average number of sickle cells corresponding to 58.82, 71.42, 80.52 and 87.28% as inhibitory activity at 30, 60, 90 and 120 minutes respectively. Unlike the 2% NaCl solution, the curves of erythrocytes treated with both EZHm concentrations had a downward trend. This equated to a drop of the average number of sickle cells. The longer both concentrations of EZHm solution were in contact with the HbSS, the higher the sickling formation inhibition activity over the time up to 120min. Also, the inhibition value of EZHm at 10 mg/mL (87.28%) was higher than that of EZHm at 5mg/mL (71.18%). Increasing concentration of the extract led to a better inhibitory effect.

In comparison, the sickling formation inhibition activity of DZHm, EZHm and phenylalanine at 5mg/mL were 67.45,

71.18 and 68.81% respectively. Also, the inhibitory activity of DZHm, EZHm and phenylalanine at 10mg/mL were 87.35, 87.28 and 83.65% respectively. There was no significant difference (P>0.05) between DZHm, EZHm and the phenylalanine activity.

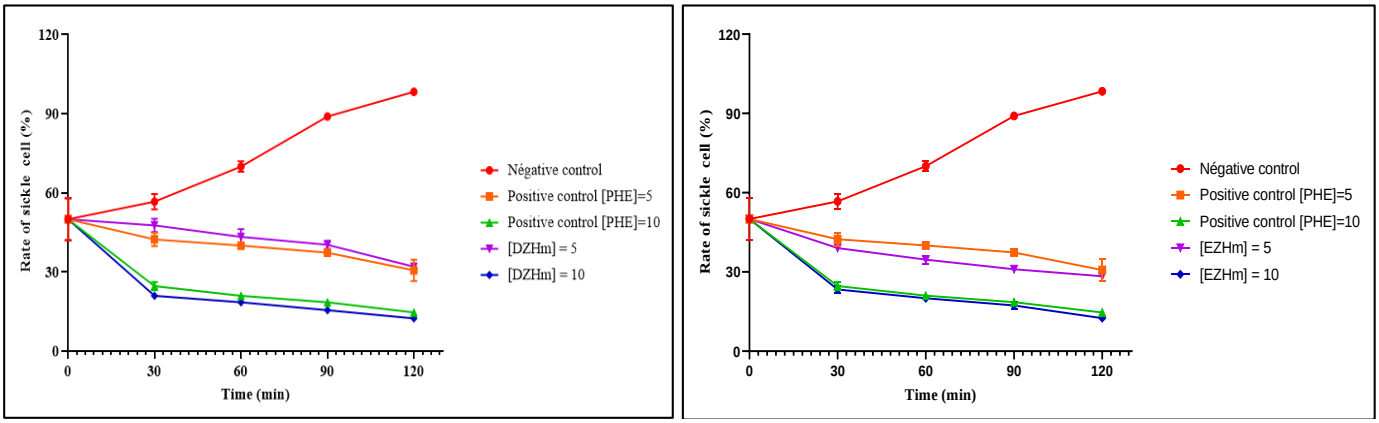

Fig 1: Effect of DZHm and phenylalanine on the evolution of sickle cell count in the presence of sodium metabisulfite as a function of contact time. Values are presented as mean ± standard deviation

Discussion

The traditional use of medicinal plants for the treatment of sickle cell disease has led to productive results, proving useful in reducing the crisis and also in reversing sickle cell disease in vitro. Under hypoxic conditions, the aqueous and hydroethanol extracts of the plant inhibited the falciformation of SS red blood cells. Analysis of the results reveals that sodium metabisulfite generates a progressive increase in the rate of sickled cells. Within the first 30 minutes of observation, the cumulative sickle cell rate was 56.67%. At the end of the observation (120 min), the sickle cell rate is 98.33%. Indeed, the polymerization which leads to falciformation is preceded by the nucleation stage which consists in the formation of polymerization nuclei. This nucleation phase corresponds to the "Delay time" which can range from a few milliseconds (Steinberg, 1999) [22] to several days (Galactéros, 2001) [23] depending on the percentage of HbS (Steinberg, 1999) [22] and the age of the red cells (Galactéros, 2001) [23]. This explains the increase in the rate of falciformation over time. The percentage of sickle cells was 98.33% in 120 min. These results are in agreement with those of (Joppa *et al*., 2008) [24] who showed that the action of metabisulfite on red blood cells is time-dependent and increases progressively to 96.5% in 3 h. As for the work of (Elekwa *et al*., 2005) [25], the percentage of sickling cells was 80% after (1) hour of observation. These results show that sickle cell formation is not an instantaneous phenomenon. But it is a phenomenon that takes place over time and its kinetics depend on the genotype of the sickle cell patient (Steinberg, 1999) [22].

After treatment of red blood cells with phenylalanine, DZHm and EZHm at concentrations of 5 and 10 mg/mL, these substances inhibited sickle cell transformation. Indeed, for phenylalanine at the concentration of 5 mg/ml the percentage of inhibition of falciformation generated by sodium metabisulfite is 18.82% and at the concentration 10 mg/ml the inhibition of falciformation is 85.07%. At the end of observation, this falciformation inhibition increases from 18.82% in 30 min to 68.81% in 120 min and from 56.47 to 85.07% for the 5 and 10 mg/mL concentrations respectively. This activity of phenylalanine is close to that reported by (Seck *et al.*, 2020) [26] who obtained a similar falciformation reduction rate with phenylalanine as the results of this study.

Regarding the activities of DZHm and EZHm extracts, the falciformation inhibition rates were 67.45% and 71.18% at the 5 mg/mL concentration, respectively. While at the 10 mg/mL concentration of DZHm and EZHm extracts the respective inhibition rates were 87.35% and 87.28%. Thus, these results reveal that phenylalanine and ZHm extracts are capable of attenuating the deleterious effects generated by sodium metabisulfite. The comparison of the inhibitory activities of the two substances shows that ZHm extracts have a capacity of inhibition of falciformation which is 2.25 times stronger than that of phenylalanine. This good performance would be related to the richness of phenolic compounds and amino acids in ZHm extracts.

These results are similar to those of several authors who studied individually the antisickling activities of the ZHm plants. Indeed in 2008, Uwakwe and Nwaoguikpe [27] showed that the aqueous extract of *X. aethiopica* inhibits 85.12% of the polymerization of HbS. According to the results, this extract is also able to reverse falciformation of erythrocytes and improve the Fe2+/Fe3+ ratio. These authors reported that the inhibition of falciformation generated by this extract was due to the presence of amino acids such as phenylalanine glutamic acid, arginine, Tyrosine and aspartic acid in varying concentrations. In addition, authors such as (Nur *et al.*, 2012; N'draman-Donou *et al.*, 2015; Nurain *et al.*, 2017) [28, 29, 30] claim that aromatic amino acids have antisickling activity. The presence of these amino acids in our plant extracts could participate in the fight against sickle cell disease by having a polymerization inhibition activity.

In addition to these works, the results of investigations by (Fatokun *et al.*, 2015; Mukeba *et al.*, 2020) [30,31] on the antisickling activity of some Congolese and Nigerian medicinal plants respectively showed that aqueous and ethanolic extracts of *H. madagascariensis* inhibited HbS polymerization by 42% and reversed sickle cells by 52%. In addition, results from previous work (Tatiana *et al.*, 2020) [32] showed that aqueous and ethanolic extracts of *Z. leprieurii* inhibited sickle cell formation by 89% and 81% respectively. This activity would be due to the presence of phenolic compounds in both extracts of *Z. leprieurii*.

Indeed, the reversion of sickling cells is generally attributed to the inhibition of hemoglobin S polymerization. It is well known that the formation of intra-erythrocyte tactoids is the

basis for the falciformation of SS red blood cells (Elion et Labie, 1996) [33]. Some phytoconstituents such as terpenoids, polyphenols, phenylalanine, and hydroxybenzoic acid components possess antisickling properties (Biapa *et al.*, 2019) [34]. The work of (Kitadi *et al.*, 2015) [35] showed that phenolic compounds have the ability to interact with proteins. This interaction of certain metabolites present in plant extracts with hemoglobin S would inhibit its polymerization, thus preventing the falciformation of erythrocytes, but they would also act by stabilizing the membrane of erythrocytes by bringing oxygen molecules into the red blood cells (Ngbolua *et al.*, 2019; Wembonyama, 2021) [15, 36].

The excellent antisickling activity of ZHm extracts is thought to be due to a combination of the effects of several factors including the presence of amino acids, polyphenols, and the antioxidant power of these extracts. According to (Nur *et al.*, 2012; Sawadogo *et al.*, 2017) [28, 37], the antioxidant properties of phenolic compounds present in plant extracts could prevent membrane lipid peroxidation and thus prevent erythrocyte lysis.

Conclusion

These extracts, although crude, are a hope for the discovery of many anti-sickle cell active ingredients. This natural product based on a poly-herbal formula could improve the quality of life of people suffering from sickle cell disease. It is therefore necessary to identify by LC-MS analysis the anti-sickle cell compounds in order to develop new therapeutic agents that could space or mitigate sickle cell crises. The results of the current study validate the traditional use of this recipe by some communities in Côte d'Ivoire.

Competing interests

Authors have declared that no competing interests exist.

Consent

An agreement was obtained from the ethic committee and an informed consent approved by each patient wishing to participate to the study.

References

1. Delicat-loembet L, Elguero E, Arnathau C, Durand P, Ollomo B. Prevalence of the Sickle Cell Trait in Gabon: A nationwide study. Elsievier. 2014;5:18-24.
2. Sawadogo D, Tolo-Dilkébié A, Sangaré M, Aguéhoundé N, Kassi H, Latte T. Influence of the clinical status on stress reticulocytes, CD36 and CD49d of SSFA2 homozygous sickle cell patients followed in Abidjan. Advances in Hematology. 2014;27:38-60.
3. OMS. La drépanocytose dans la region africaine: situation actuelle et perspectives. Cinquante sixieme session, Rapport du Directeur regional, AFR/RC/56/17; c2006. p. 1-22.
4. Lamine T, Assane D, Isabelle ZC, François ND, Ndiogou S, Djibril B, *et al*. Profils épidemiologiques, cliniques et hématologiques de la drépanocytose homozygote SS en phase inter critique chez l'enfant à Ziguinchor, Sénégal. The Pan African Medical Journal. 2017;28(208):1-6.
5. Renaudier P. Physiopathologie de la drépanocytose. Transfusion clinique Biologique. 2014;21(4-5):178-81.
6. Aubry P, Bernard-Alex G. Hémoglobinoses Actualités. *Médecine Tropicale*, Université de Bordeaux, 33076 Bordeaux (France); c2019. (Consultée le 23/03/2022)
7. Marilyn J, Punam M, Gregory MV. Therapeutic strategies for sickle cell disease: towards a multi-agent approach. Nature Reviews Drug Discovery. 2019;18:139-158.
8. Kugler M. Sickl cell disease: symptoms, causes, diagnosis. Accès internet: (Page consultée le 23/03/2022), 2020.
9. Strader MB, Jana S, Meng F, Heaven MR, Shet AS, Thein SL, Alayash AI. Modification post-traductionnelle en réponse au stress cellulaire induit par l'oxydation de l'hémoglobine dans la drépanocytose. Rapports scientifiques. 2020;10(1):1-14.
10. Aufradet E. Drépanocytose et activité physique: conséquences sur les mécanismes impliqués dans l'adhérence vasculaire, l'inflammation et le stress-oxydatif. Université de Lyon, Science et Technique des Activité Physique et Sportive, Lyon, France, 2012, 264p.
11. Grégoire C. Génotoxicité des systèmes CRISPR-Cas9. Doctorat de l'Université de Bordeaux, 2019, 193p
12. Park SH, Bao G. Modification du gène CRISPR/Cas9 pour guérir la drépanocytose. Science de la transfusion et de l'aphérèse. 2021;60(1):1-8.
13. Kunh V, Diederich L, Suvorava T. Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. Forum Review Article. 2017;26(13):16-17.
14. Hsu L, Obiageli EN, Biobele JB, Furahini T, Shonda K, Livingstone GD, *et al*. White Paper: Pathways to Progress in Newborn Screening for Sickle Cell Disease in Sub-Saharan Africa. International Journal of Tropical diseases. 2018;6(2):260-269.
15. Ngbolua KN, Mpiana PT, Mudogo V. Pharmacopée traditionnelle et lutte contre la drépanocytose: Méthodes de sélection et d'évaluation de l'activité des plantes médicinales. Editions Universitaires Européennes, Riga: Latvia. International Standard Book Number. 2019;978-613-9-51486-1.
16. 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.
17. Konkon NG, Adjoungoua AL, Manda P, Simaga D, N'Guessan KE, Koné BD. Toxicological and phytochemical screening study of MitragynaInermis (willd.) O ktze (Rubiaceae), anti-diabetic plant. Journal of Medicinal Plants Research. 2008;2(10):279284.
18. Nemlin J, Brunel JF. Fascicule de Travaux Pratiques de Matière Médicale (3ème année). Université Nationale de Côte-d'Ivoire. Faculté de Pharmacie. Département de Pharmacognosie. Laboratoire de Phytologie, 1995, 47p.
19. Fofana S. Exploration biochimique sur le pouvoir immunogène de trois plantes en Côte d'Ivoire: *Alstonia boonei* (Apocynaceae), *Mitragyna ciliata* (Rubiaceae), *Terminalia catappa* (Combretaceae). Thèse de pharmacie, UFR de médecine, de pharmacie et d'odontostomatologie, Université de Bamako (Mali), 2004, 123 p.
20. Emmel VE. A red cell study of the érythrocytes in case of severe anemia with elongated and sickle and shape dred blood corpuscule. Archives of International Medicine. 1933;7:769-789.
21. 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.

22. Steinberg MH. Management of sickle cell disease. New England Journal of Medicine. 1999;340:1021-30.
23. Galactéros F. Bases physiopathologiques de la drépanocytose, prise en charge et actualités thérapeutiques. Bulletin de la Société de Pathologie Exotique. 2001;94:77-9.
24. Joppa KM, Vovor A, Eklu-Gadegbeku K, Agbonon A, Aklikokou K, Gbeassor M. Effet de *Morinda lucida* benth. (Rubiaceae) et de *Newbouldia leavis* p. Beauv. (Bignoniaceae) sur la falciformation**.** Médecine Tropicale. 2008;68:251-256.
25. Elekwa I, Monanu OM, Anosike OE. Effects of aqueous extracts of *Zanthoxylum macrophylla* roots on membrane stability of human erythrocytes of different genotypes. Nigerian Society for experimental biology Biokemistri. 2005;17(1):7-12.
26. Seck M, Cheikh S, Gueye PM, Seck I, Mbaye DD, Zhour L, *et al*. Etude de l'activité antifalcémiante d'extraits de racines de *Leptadenia hastata* (Asclepiadacae). International Journal Biological and Chemical Sciences. 2015;9(3):1375-1383.
27. Nur E, Brandjes DP, Teerlink T, Otten HM, Oude ERPJ, Muskiet F, *et al*. N-acetylcysteine reduces oxidative stress in sickle cell patients. Annals of Hematology. 2012;91(7):1097–105.
28. N'draman-Donou E, Fofié Y, Adjambri E, Mélèdje M-F, Sawadogo D. Caractérisation et évaluation *in vitro* de l'effet antifalcémiant des graines de *Cajanus cajan* (Fabacées) sur les drépanocytes à Abidjan - Côte d'Ivoire. International Journal Biological and Chemical Sciences. 2015;9(5):23002308.
29. Nurain IO, Bewaji CO, Johnson JS, Davenport RD, Zhang Y. Potential of Three Ethnomedicinal Plants as Antisickling Agents. Molecular Pharmaceutics. 2016;14(1):172–182.
30. Mukeba FB, Mukoko JB, Mayangi MM, Ngondo MM, Ngelinkoto PM, Bamba JV, *et al*. Microscopic Features, Mineral Contents, Antisickling, Antioxidant and Antibacterial Activities of Stem Bark of *Harungana madagascariensis* Lam. ExPoiret (*Hypericaceae*). European Journal of Medicinal Plants. 2020;31(20):33-47.
31. Fatokun OT, Agbedahunsi JM, Elujoba AA. Antisickling Activities of Some Nigerian Medicinal Plants. Nigerian Journal of Natural Products and Medicine. 2015;19:92-1118-6267.
32. Tatiana KMK, Akakpo-Akue J, Golly JK, Fofie N'GBY, Ahon MG, Kra AKM, *et al*. Phytochemical Characterization of Three Plants and Their Antisickling Activity in the Management of Sickle Cell Disease. Journal of Biosciences and Medicines. 2020;32(11):42-50.
33. Elion J, Labie D. Bases physiopathologiques moléculaires et cellulaires du traitement de la drépanocytose. Hématology. 1996;2:499-510.
34. Biapa PCN, Yembeau NL, Chetcha B, Fotsing CK, Nkwikeu PJN, Telefo PB, *et al*. The antisickling mechanism of Amphimas pterocarpoides (Cesalpiniaceae) against sickle cell Anemia. Academia Journal of Medicinal Plants. 2019;7(4):117–124.
35. Kitadi JM, Mazasa PP, Tshibangu DST, Memvanga PB, Ngbolua KN, Taba NK, *et al*. Anti-sickling and antioxidant activities of anthocyanins extracts from Dissotis brazzae Cogn (Melastomataceae). Journal of Advancement in Medical and Life Sciences. 2015;3(4):6.
36. Wembonyama SO. Moringa oleifera, une aubaine dans la prise en charge du syndrome drépanocytaire majeur? Journal of Medicine, Public Health and Policy Research. 2021;1(1):31-35.
37. Sawadogo S, Sanou SD, Dabire P, Belemtougri GR, Sawadogo LJL, Tanguy S, *et al*. Activité antifalcémiante d'extraits de trois plantes médicinales du Bukina Faso: Jatropha curcas, Khaya senegalensis et Dichrostachys cinerea. International Journal of Biological and Chemical Sciences. 2017;11(5):2017-2027.