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Assessment of sickling inhibitory activity of *Ficus carpensis, Newbouldia laevis, Carpolobia lutea* and *Carpolobia caudata* on human erythrocytes HbSS

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ABSTRACT

Sickle cell disease is a hemoglobinopathy. In hypoxia situation, homozygous individuals might suffer from vaso-occlusive seizures, hemolytic anemia and increased susceptibility to infections. A recipe of the leaves of *Ficus carpensis*, *Newbouldia laevis*, *Carpolobia lutea* and *Carpolobia caudate*, four plants used in the traditional treatment of sickle cell disease in the Daloa region, was the subject of this study. This investigation was initiated in order to assess sickle cell inhibitory activity of the recipe, by using Emmel's method. The decocted (DS) and the aqueous extract (EA) of the recipe brought the residual sickle cell rate at 10 and 11% respectively. Which correspond to sickle cell inhibitory activity of 90% for the decocted (DS) and 89% for the aqueous extract (EA). These activities were higher than that of phenylalanine (83%) which is used as an antisickling amino acid reference. The chemical study of the both extracts of the recipe revealed the presence of compounds known for their anti-oxidant and anti-sickle cell activities. Those flavonoids, polyphenols and alkaloids could be partly responsible for the sickle cell inhibitory activity of the recipe. These results showed that both the extracts of the recipe had antisickling activity. The use of this recipe of four plants in the traditional treatment of sickle cell disease in the Daloa region might be justified.

Keywords: *Ficus carpensis*, *Newbouldia laevis*, *Carpolobia lutea* and *Carpolobia caudata*, sickle cell disease, antisickling activity.

INTRODUCTION

Sickle cell disease or sickle cell anemia is a severe hereditary genetic condition with autosomal recessive transmission in which red blood cells take the form of sickle instead of their normal disk form. It is a hemoglobinopathy caused by the replacement of glutamic acid by valine in position six of the β hemoglobin chain. This substitution reduces the hemoglobin's affinity for oxygen and its solubility under low oxygen pressure conditions. The decrease solubility leads to the polymerization of the hemoglobin HbSS and to the sickling of blood cells [1]. Severe sickle cell forms are found in SS homozygous individuals or double composite heterozygous if individuals possess HbS allele and another hemoglobinopathy, such as hemoglobin C or 'thalassemias' [2].

The severe form is characterized by its high frequency; more than 120 million people are affected on the planet [3]. This hemoglobinopathy is mostly found throughout Sub-Saharan Africa and is a real public health problem in some countries such as Côte d'Ivoire where there is a prevalence of 14% [4]. Sickle cell disease involves two major changes: the polymerization of deoxygenated HbS and the sickle-formation of red blood cells that cause the painful crisis. It is considered a serious disease with clinical manifestations dominated by painful vaso-occlusive crises, hemolytic anemia, ischemic and infectious complications. This makes sickle cell disease an important cause of morbidity and mortality [5].

Currently, many genetic diseases such as sickle cell disease do not have a proven cure [6]. However, several therapeutic solutions are proposed to fight this disease: bone marrow transplantation, chemotherapy and the prospects for gene therapy. Symptomatic treatments such as blood transfusion and analgesic intake are also used [7]. These methods are expensive and highly specialized for some or requiring a minimum of health infrastructures and financial resources for others. Also, they are not accessible to low-income populations. The difficulty of accessing primary health care directs three-fourths of the population to medicinal plants [8]. In Côte d'Ivoire several studies have been conducted to assess the antisickling activity of some medicinal plants used in traditional environments [9, 10, 11].

With this in mind, an ethno botanical survey was carried out on medicinal plants used in the management of sickle cell disease in the High Sassandra department from 03/09/2019 to 12/10/2019.

Among the treatments recommended by traditional practitioners was a recipe that consists of 4 plant species: *Ficus carpensis, Newbouldia laevis, Carpolobia lutea* and *Carpolobia alba*.

This study was initiated to assess the antisickling potential granted to this recipe of four plant species by some traditional practitioners. To achieve this goal, two points have been targeted. Achieving the chemical study and assessing sickling inhibitory activity of the recipe.

MATERIALS AND METHODS

Collecting and preparing plant extracts.

The plant material consisted of the leaves of *Carpolobia alba, Newbouldia laevis, Ficus capensis* and *Carpolobia lutea* was harvested from Daloa department on 28 and 29 September 2019. The leaves were washed, cut and dried, away from the sun, at room temperature 25^{0}C for three weeks at the University of J. Lorougnon Guede's Biochemistry Laboratory. They were then powdered with an electric grinder. Two hundred grams (200g) of the mixture of 50g leaf powder from each of the four species were composed into a recipe.

The Preparation of the recipe's decoction: One hundred grams (100g) of powder from the recipe were brought to a boil for 20 minutes in 2 L of distilled water. The decoction was filtered once on a square of white cotton fabric. Then, three times on cotton and finally once on Whatman filter paper (3mm). This extract was then dried in the oven at 50^{0}C, to give the dry decocted of the recipe called DS [12].

The preparation of the water extract: One hundred grams (100g) of powder from the recipe were mixed with one 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 then filtered three times on hydrophilic cotton and once on Whatman paper (3 mm). The filtrate was dried at 50^{0}C using a venticell-type steamer® to produce a powder called dry aqueous extract (EA) [13].

Chemical study

The characterization of the main chemical groups present in DS and EA was performed using the coloring or precipitation technique described by Nemlin [14].

Sickle cell inhibitory activity Study

This test was conducted using the Emmel method [15], according to the protocol described by Imaga [16] for the evaluation of the inhibition of the sickling-formation of red blood cells of SS genotype.

Sampling and conditioning: The SS genotype blood sample, confirmed by the electrophoresis method, came from voluntary sickle cell patients selected at the Cocody University Hospital Centre. These volunteers should not have been transfused for the two months prior to the blood test. The sample of the volunteers did not take into account age or gender. Consent was obtained from the volunteers. The collected blood was put in EDTA tubes.

Washing the blood sample and sickling induction: Washing of the blood sample was done directly after the sample was taken. The Hb SS genotype blood sample was washed for five (5) minutes at 1000 laps/min. This action was 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 1mL of physiological water (NaCl 0.9%).

Sickling cell inhibition test: 10 mg/mL DS and EA solutions with physiological fluid (NaCl 0.9%) have been prepared. A volume of 50 µL of each of each solution was mixed successively with a volume of 50 µL of washed blood and a volume of 50 mL of sodium meta-bisulfite (2%, p/v) in a test tube. Each of the 2 test tubes was closed with paraffin to prevent air from entering the tube. Two controls were also prepared. The 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 10mg/mL and 50 µL of sodium meta-bisulfite (2%, p/v). The control tubes underwent the same treatment as the test tubes. 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. Abnormal red blood cells have taken the form of sickle or banana, sometimes with fringed edges in "holly leaf". The Sickling inhibition activity of the plant is its ability to prevent the sickling formation of red blood cells in low oxygen pressure environments. 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. This activity is determined by the following formula:

$$AA = (P0-P1) / P0*100$$

AA refers to sickling inhibitory activity; P0 the average of the sickle cells of the controls; P1 the average of sickle cells on test slide in the presence of plant extracts.

RESULTS AND DISCUSSION

Chemical study

The results of the secondary metabolites identification were recorded in Table 1. The results revealed, in both extracts DS and EA, the presence of secondary metabolites known for their biological properties: alkaloids, catechins, flavonoids, polyphenols, sterols, terpenes, saponins and leuco-anthocyanins. On the other hand, tannins gallic and quinones were not presents in the two extracts. Many studies have shown the benefits of compounds found in this recipe. For example, alkaloids, tannins, flavonoids, anthocyanins and leuco-anthocyanins have antioxidant properties. They promote tissue regeneration, decrease the permeability of blood capillaries and strengthen their resistance to hemolysis [17]. Among these compounds, polyphenols and their derivatives were cited as having anti-sickling activity [18, 19, 20, 21]. *Ficus carpensis*, *Carpolobia lutea* and *Carpolobia alba* have had individually the same phytochemical results of the recipe. Our results are comparable to those of Daffalla and Oyeleke [22, 23]. These authors showed that the aqueous extract of *Ficus capensis* contained: alkaloids, tannins, flavonoids, anthocyanins and leuco anthocyanins. Also, Mitaine-Offre and Nwidu confirmed the presence of polyphenols, alkaloids, flavonoids, tannins, saponins and terpenoids in aquoues extracts of *Carpolobia lutea* leaves [24, 25]. Thus, the anti-anemic activity of *Carpolobia alba* is thought to be due to the presence of sterols, polyphenols, cathechic tannins and terpenoids. Comparing DS and EA, polyphenols, catechins, flavonoids and saponins were more abundant in DS while sterols and terpenes were more abundant in EA. Alkaloids and Leuco-anthocyanins were in equal proportion.

The *in vitro* study of DS and EA Sickle cell inhibitory activity

Morphological analysis, counting of sickle cells and percentage of residual sickle cells compared to the negative control were carried out.

The red blood cells in the presence of sodium meta bisulfite were all sickle-shaped after 120 minutes (Figure 1). After the simultaneous addition of sodium meta bisulfite with DS and EA at 10 mg/mL separately on washed blood, morphological analysis was performed. It revealed that under hypoxia conditions in the presence of DS and EA and compared to the negative control (Fig1), few erythrocytes took the abnormal sickle shape; on the contrary they kept their rounded and biconcave shape (Figure 2). After counting sickle cells 2 hours of time after contact with plant extracts, the sickling inhibitory activity of DS and EA at 10 mg/mL each, was determined. The residual sickle cell rate was 11% for EA and 10% for DS versus 100% for the negative control. Which give a sickling inhibitory activity of 89% for EA and 90% for DS. These activities are higher than that of phenylalanine which was 83%.

The presence of flavonoids and anthocyanins in the recipe might partly justify the observed sickling inhibitory activity. Indeed, researches have shown that the antisickling activity of many Congolese medicinal plants is attributed to this chemical group [28, 20]. Other authors claim that phenylalanine, phydroxybenzoic acid and its derivatives as well as maslinic, oleanolic and betulinic acids are also partly responsible for the antisickling activity activity of the studied plants [29, 30]. These compounds could be present in the recipe.

Table 1: Chemical compounds in the recipe.

Groupes chimiques	DS	EA
Alkaloids	+	+
Polyphenols	+ +	+
Sterols and terpenes	+	+ +
Catechic tannins	+ +	+
Gallic tannins	-	-
Flavonoids	+ +	+
Quinones	-	-
Saponins	+ + +	+
Leuco anthocyanins	+ +	+ +

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

Figure 1: Sickle cells after 120 min without DS nor EA

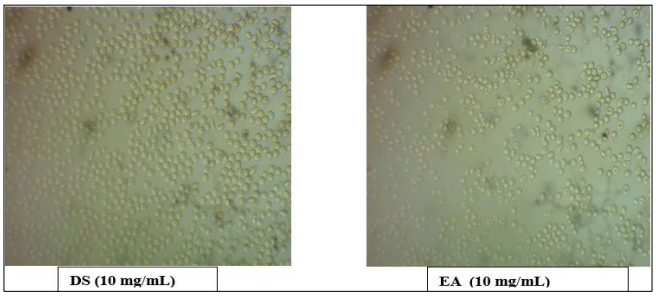

Figure 2: Red blood cell shape after 120 min contact with DS and EA

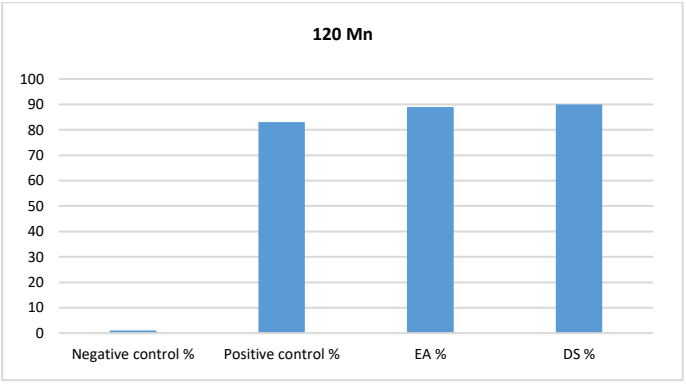

Figure 3: Sickling inhibitory activity

N'gbolua [27] reported that saponins, in addition to carboxylic acids and flavonoids, could be partly responsible for the sickling inhibitory activity of Hymenocardia acida leaves [31]. The normalization of sickle cells is generally attributed to the inhibition of hemoglobin S polymerization [32].

The interaction of some metabolites, extracted from plants, with hemoglobin S would inhibit its polymerization, thus preventing the sickling of erythrocytes. Indeed, Mpiana and N'gbolua [26, 27] have shown that anthocyanins would reduce the polymerization of HbS by engaging in a competitive reaction with polymerization [20, 21]. These compounds could also act by stabilizing the membrane of the erythrocytes. The compounds contained in the aqueous extract and the decocted could protect the membrane of the erythrocyte like the enzymatic defense system. They could also stabilize hemoglobin S by increasing its affinity for oxygen and promote better water intake in the red blood cell [33, 34]. The aqueous extract and the decocted of the recipe inhibiting sickle cell formation would prevent all complications associated with sickle cell such as pain, inflammation and anemia.

CONCLUSION

Natural substances are increasingly taking a prominent place in therapeutic means. Plants are made up of chemical compounds that could be useful for the well-being of people. It is with this in mind that our work is intended to be a modest contribution to the study of biological activities and phytochemistry of a recipe based on *Ficus carpensis*, *Newbouldia laevis*, *Carpolobia lutea* and *Carpolobia caudata*'s leaves. The *in vitro* study of the antisickling effect of the aqueous extract (EA) and the decocted (DS) at 10 mg/mL showed an inhibition of sickle cells. Both the aqueous extract and the decocted have a sickling inhibitory activity. The decocted had a maximum activity of 90% and the aqueous extract (EA) or macerated 89%. The phytochemical study of leaf extracts from *Ficus carpensis, Newbouldia laevis, Carpolobia lutea* and *Carpolobia caudata* revealed the presence of catechins, alkaloids, flavonoids, polyphenols, sterols, terpenens, saponins and leuco-anthocyanins. Thus, the anti-sickling activity of this recipe could come from the phytochemicals contained in the 4 plants. The use of this recipe in a traditional environment in Daloa region could be justified.

Considering the performance of this recipe for fighting sickle cell disease, it would be interesting to study its activity on the inhibition of hemoglobin polymerization which is the primary action in the physiopathology of sickle cell disease.

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