Original Research Article

Effects of aqueous extracts of *Rhynchosporacorymbosa* and *Olaxsubscorpioïdea* on the inhibition of falciformation of SS genotype erythrocytes

ABSTRACT

Aims: Sickle cell disease is one of the world's leading genetic disorders. It is characterized by the presence of an abnormal hemoglobin HbS in the blood. The anomaly affects the primary structure of hemoglobin, through the substitution of valine for glutamic acid. In traditional medicine, two plant species, *Rhynchosporacorymbosa* and *Olaxsubscorpioïdea*, are used to treat this condition.

Place and Duration of Study: Agro-valuation Laboratory,Hematology Clinic laboratory of Cocody Hospital University(CHU), between June-Aout 2021 and June-July 2022

Methodology: Following a triphytochemical test, the effect of each species on the *in vitro* inhibition of SS erythrocyte falciformation was evaluated.

Results: Thus an abundance of alkaloids, tannins, saponins, polyterpenes and sterols were found in the families of molecules identified in *Rhynchosporacorymbosa*. As for the decocted *Olaxsubscorpioïdea*, in addition to the families of molecules identified in *Rhynchosporacorymbosa* the polyphenols, leuco-anthocyanins and flavonoids were found. In addition, at an equal concentration of 0.312 mg/mL, decoctions of *Rhynchosporacorymbosa* and *Olaxsubscorpioïdea* gave an inhibition rates of 80 and 84 % respectively. Both extracts showed activity on *in vitro* falciformation inhibition. This activity is thought to be due to the presence of chemical groups with anti-oxidant and anti-inflammatory properties.

Conclusion: The use of *Rhynchosporacorymbosa*and *Olaxsubscorpioïdea* in the treatment of sickle cell disease in Côte d'Ivoire would be justified.

Keywords: [Rhynchosporacorymbosa, *Olaxsubscorpioïdea*, inhibition, sickle cell disease.*}*

1. INTRODUCTION

Sickle cell anemia is an autosomal recessive inherited hemoglobinopathy. It is caused by the presence of an abnormal haemoglobin (Hb) whose expression product is haemoglobin S (HbS), a haemoprotein found predominantly in the red blood cells of sickle cell patients. HbS has the property of polymerizing into long fibers that stiffen and deform the red blood cell, giving it a sickle shape in hypoxic conditions. Sickle-shaped red blood cells are less efficient at diffusing oxygen, leading to hypoxia-induced cell damage. Sickle cell disease is reversible but after a while, lesions of the erythrocyte membrane lead to the formation of sickle cell “irreversibly sickle cell”.

Clinically, the subject with the sickle cell disease gene double (homozygous SS) or associated with another hemoglobinopathy (double heterozygous S/C, S-β-thalassemia, S/D-Punjab, S/0 Arabic) suffers from complications of the disease. Although the symptoms of sickle cell anemia are polymorphic depending on the individual carrier, both acute and chronic complications can occur [1]. Thus worldwide, an estimated that around 5 million people have sickle cell disease and several hundred million carry the sickle cell trait [2]. Every year, more than 500,000 sickle cell children are born worldwide, including 300,000 in Africa. Without appropriate treatment, half of sickle cell children in Africa die before the age of 5 years [3]. According to the LYA Foundation, in Côte d'Ivoire the prevalence of sickle cell disease is 14 % of the AS forme, with 4 % of major forms and there are 6,000 cases detected each year at birth, of which 60-70 % die before the age of 5 years [4].

To remedy this public health issue, several therapeutic options have been proposed. In Africa, as in other sickle-cell endemic regions, the difficulty of accessing primary health care, the inadequacy and poor distribution of medical staff and socio-cultural behaviours mean that over 80 % of the population rely on traditional medicine for treatment [5].

Thus, numerous studies have been carried out to scientifically assess the safety and efficacy of certain plant species used in traditional medicine [6]. It is in this same context that the antifalcemic activity of certain plant species was evaluated. However, very few studies exist in Côte d'Ivoire in this area [7].

The aim of the present study was to evaluate the *in vitro* anti-sickle cell potential of two medicinal plant species, *Rhynchosporacorymbosa* and *Olaxsubscorpioïdea* used in traditional medicine in the Korhogo department (Côte d'Ivoire), for the treatment of sickle cell disease. More specifically, this is to assess the effect of these extracts on the in vitro inhibition of sickle formation and to highlight the major chemical groups that could be responsible this activity.

2. MATERIAL AND METHODS

2.1 Material

The biological material consisted of human blood from a sickle cell anemia population of SS types aged between 3 and 50 years. Plant material, consisting of two (2) plant species including the whole plant of *Rhynchosporacorymbosa* and the leaves and stems of *Olaxsubscorpioïdea.* As for the technical equipment includes EDTA tubes, syringes, gloves and laboratory equipment. Products and consumables include distilled water, phenylalanine, sodium metabisulfite, organic solvents (chloroform; 96 °C ethyl alcohol; sodium acetate; fuming hydrochloric acid; concentrated sulfuric acid (H_2SO_4); hydrochloric alcohol diluted 2-fold; isoamyl alcohol; ammonia diluted 2-fold; acetic anhydride), ferric chloride 2 %, magnesium chips, Bouchardat's reagent, Dragendorff's reagent, Stiasny's reagent and Borntraeger's reagent.

2.2 Methodology

2.2.1 Preparation of plant extracts

The whole plant of *Rhynchosporacorymbosa* and the leaves and stems of *Olaxsubscorpioïdea*, harvested in the department of Korhogo (Côte d'Ivoire) have been identified by the Agro-valorisation laboratory of the Jean Lorougnon Guédé University (Daloa).

After washing, cutting and drying at room temperature (25-30 °C) for three weeks at the Laboratory, the various parts of these plants were pulverized using a Retschsk 100 electric grinder. Subsequently a decoction was then prepared according to the method of Konkon*et al.* 2006 [8]. One hundred (100) grams of powder of each plant species were separately put into one liter (1 L) of distilled water and brought to a boil for 10 min. The resulting mixture was wrung out in a square of fabric and then filtered three (3) times on hydrophilic cotton and once (1) on whatman paper (3 mm). The filtrate was evaporated at 60 °C and the powder obtained was coded DRC for *Rhynchosporacorymbosa* and DOSA for *Olaxsubscorpioïdea*.

2.2.2 Chemical study

Extracts were analyzed using conventional precipitation and staining techniques. Alkaloids, polyphenols, tannins (catechic, gallic), flavonoids, saponosides, polyterpenes and sterols, leuco anthocyanins and quinones were sought.

2.2.3 Anti-sickling tests

For anti-sickle cell testing, blood samples were collected in EDTA tubes from sickle cell patients followed at the CHU de Cocody. These samples were transported in a cooler containing cold accumulators and kept in a refrigerator at 4 °C for further work.

2.2.3.1Falciformation inhibition test

The method used was that described by Emmel (1933) [9] and updated by Imaga*et al.*, 2008 [10]. It assesses the effect of the extracts studied on falciformation reversal.

2.2.3.2 Protocol

Thus, following confirmation of sickle cell disease by the hematology laboratory of Cocody Hospital University, the confirmed Hb SS genotype blood sample was washed for 5 min at 1,000 rpm. This operation was repeated 3 times to ensure complete removal of the supernatant. The supernatant was then removed using a Pasteur pipette and 1 mL of washed red blood cells was suspended in 9 mL of physiological water (NaCl 0.9 %). In addition, solutions of concentrations 0.3125; 0.625; 1.25; 2.5; 5 and 10 mg/mL of DRC and DOSA extract were prepared separately by the double dilution method using physiological fluid (NaCl 0.9 %).

A volume of 50 µL of each of the previously prepared solutions was homogenized, successively with 50 µL of washed blood and 50 µL of sodium meta-bisulfite (2 %, w/v) in a test tube. Each test tube was sealed with paraffin to prevent air ingress.

At the same time, 2 control solutions were prepared using the negative control (sodium metabisulfite) and the positive control (phenylalanine). Thus the negative solution was prepared by homogenizing 50 µL respectively of each solution (washed blood, physiological water and 2 % sodium metabisulfite). As for the positive control solution, a range of positive solution was realized with different concentrations of this control. Thus, this range was prepared by homogenizing respectively 50 µL of different solutions including washed blood, sodium metabisulfite (2 %) and phenylalanine solution at different concentrations (0.3125; 0.625; 1.25; 2.5; 5 and 10 mg/mL). All these tubes were conditioned in a dark room protected from light.

Thereafter, every 30 min, a drop of each tube was deposited between blade and coverslip, followed by microscopic observation at x40. This enabled morphological analysis of erythrocytes and sickle cell counting for 120 min.

Abnormal red blood cells (sickle cells) were counted. The antisickling activity of each plant extract solution at different concentrations was then expressed as a percentage of sickle cells formed in the presence of the extracts, compared to the number of sickle cells formed by the negative control according the formula below:

$$AA = \frac{P0-PI}{P0} \mathbf{x}\ \mathbf{100}$$

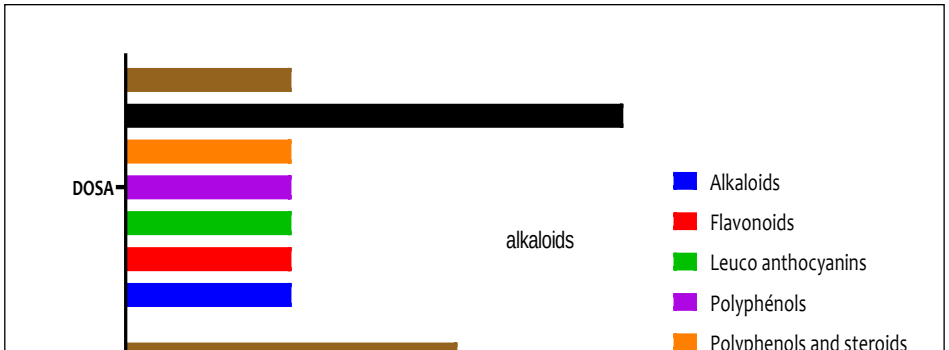
3. RESULTS AND DISCUSSION

3.1 Results

3.1.1Triphytochemical study of extracts

Triphytochemical study of plant extracts revealed for:

- DRC extract, an average presence of alkaloids and steroids. Catechic tannins were moderately abundant (score 2). Saponins are abundant (score 3). Gallic tannins, polyphenols, flavonoids, leuco-anthocyanins and quinones are absent.
- DOSA extract, all the chemical compounds studied were present on average (score 1), with the exception of gall tannins and quinones, which were absent (score 0) (Figure 1).



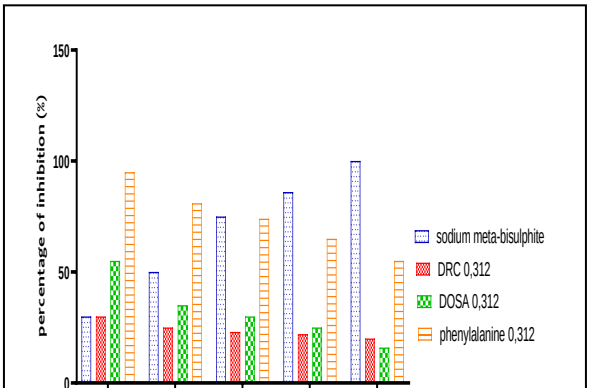
Legend Score 1: Low Score 2: Medium Score 3: Abundant

Fig. 1. Phytochemical screening of aqueous extracts of *Rhynchosporacorymbosa* and *Olaxsubscorpioïdea*

3.1.2 Falciformation inhibition test

Sickle cell count results for 120 min and every 30 min at 10 mg/mL to 0.3125 mg/mL concentrations of DRC and DOSA extracts allowed histograms to be made on the evolution of the percentage of inhibition of sickle red blood cells (Figure 2).

Comparison of the shapes of the different histograms shows that the histograms of the extract samples (DRC or DOSA) are generally decreasing. There is a sharp drop in the first thirty minutes and then an increasingly shallow followed by an ever-smaller drop until 120th min. These histograms show the same pattern as the positive control (phenylalanine) contrary to the histogram of sodium metabisulfite (negative control) which is crossing (Figure 2). On the other hand, at concentrations of 0.625 to 10 mg/mL, the histograms of the extracts (DRC and DOSA) appear faintly, then disappear when concentrations become increasingly high (Figure 2).



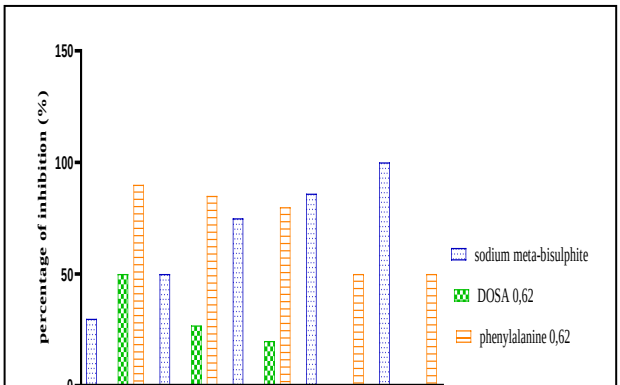


Fig. 2. In vitro effects of phenylalanine and extracts (DRC and DOSA) at different concentrations on falciformation inhibition versus sodium meta-bisulfite.

3.1.3 Percentage inhibition of falciformation versus negative control (sodium meta-bisulfite)

The percentages of sickle cell inhibition for the negative control showed that the sickle cell rate increased from 30 to 50, 75, 86 and 100 % respectively at T_0, T_{30}, T_{60}, T_{90} and T_{120} min.

As for the DRC and DOSA extracts, for concentrations ranging from 10 to 0.625 mg/mL, no blood cells could be observed instead a gelatin cluster visible under the microscope at 120th min (Table 1).

On the other hand, at a concentration of 0.3125 mg/mL for both extracts (DRC and DOSA), cells could be observed. Their percentage evolves with time from 70 to 80 % for the DRC extract and from 45 to 84 % for the DOSA extract (Table 1).

In the case of the positive control (phenylalanine), cells can be observed at all concentrations and the number of normal cells varies from one concentration to another. Their percentage is proportional to the concentration up to 120th min. (Table 2).

Overall, these results show that DRC and DOSA extracts at different concentrations have an activity on the in vitro inhibition of red blood cell falciformation over time (Table 1).

Table 1. Percentage inhibition of DRC falciformation

	Concentrations of extracts (DRC and DOSA) (mg/mL)						
	DRC	DOSA	DRC and DOSA				
Time (min)	[0.3125]	[0.3125]	[0.625]	[1.25]	[2.50]	[5]	[10]
T_0	70 %	45 %	-	-	-	-	-
T_{30}	75 %	65 %	-	-	-	-	-
T_{60}	77 %	70 %	-	-	-	-	-
T_{90}	79 %	75 %	-	-	-	-	-
T_{120}	80 %	84 %	-	-	-	-	-

Legend: -: Absence of cells in the field of observation

Table 2. Percentage inhibition of phenylalanine falciformation (positive control)

	Concentrations of phenylalanine (mg/mL)					
Time (min)	[0.3125]	[0.625]	[1.25]	[2.50]	[5]	[10]
T_0	5 %	10 %	15 %	12 %	18 %	32 %
T_{30}	19 %	15 %	20 %	11 %	12 %	30 %
T_{60}	26 %	20 %	30 %	26 %	35 %	40 %
T_{90}	35 %	50 %	40 %	45 %	48 %	50 %
T_{120}	45 %	50 %	55 %	58 %	70 %	75 %

3.2 Discussion

The aim of the present study was to evaluate the *in vitro* anti-sickle cell activity of two medicinal plant species, *Rhynchosporacorymbosa* and *Olaxsubscorpioïdea*, on the blood of SS-type sickle cell subjects. Decoction was chosen as the method of extraction because of its widespread use in the preparation of herbal medicines solicited at 65.38 % or even 88.2 % according to studies by Bla*et al.* 2015 [11]. With regard to the various chemical compounds in plant extracts, it should be noted that several factors could influence them. Indeed, according to Sofowora, 1985, the composition of a plant in secondary metabolites, responsible for the bioactive properties of plants, varies depending on the geographical location, the organ harvested plant, the period, the time of sampling and the storage conditions [12].

Thus, triphytochemical analysis revealed the presence of secondary metabolic groups including alkaloids, catechic tannins, polyterpene sterols and saponins in the decoction of *Rhynchosporacorymbosa* (DRC), while that of *Olaxsubscorpioïdea* (DOSA) rather revealed polyphenols, flavonoids, leuco-anthocyanins, alkaloids, saponins and steroids [13].

These chemical groups have been shown to have biological activities including antioxidant properties that could activate immune defense and protect erythrocytes against protein oxidation and membrane lipid peroxidation [14,15]. Saponosides thought have protective activities on blood vessels (veins and capillaries), oedematous and hormonal activity [16].

In particular, flavonoid compounds are active in maintaining good blood circulation and inhibit the tendency of small blood cells or platelets to clump together and form blood clots [17]. Indeed, the work by Mpiana *et al.* 2010 revealed that the antisickling activity of the majority of Congolese medicinal plants was attributed to this chemical group [18]. They are also involved in inhibiting the deleterious effects of reactive oxygen species produced during sickle cell disease. Flavonoids such as myricetin, quercetin and rutin have been shown to have protective effects against reactive oxygen species [19,20]. They also improve endothelial function by activating nitric oxide synthesis; a vaso-relaxant wich may therefore play an important role in the treatment of sickle cell disease. Indeed, vaso-relaxation leads to improved blood circulation, thus preventing the onset of vaso-occlusive crises [21].

Overall, these results show that the DRC and DOSA extracts at different concentrations have an activity on the inhibition *in vitro* of red blood cell sickle formation as a function of concentration and time.

Thus, analysis of extract results showed that their activity was comparable to that of phenylalanine (positive control). Results observed with concentrations above 0.312 mg/mL showed that these extracts induced hemolysis. These results are in line with those of Soro *et al.* 2021 [13]. In addition, *Olaxsubscorpioïdea* extract showed lethal effects on *Artemia salina* shrimp larvae at concentrations above 0.469 mg/mL [22].

Moreover, at the DRC extract concentration of 0.3125 mg/mL, cells can be observed and their percentage changes over time. This rate of sickle-cell cells is 20 % at 120th min (or 80 % inhibition).

As for the DOSA extract at the same concentration (0.3125 mg/mL), the sickle cell rate was 16 % (or 84 % inhibition). DOSA extract at 0.3125 mg/mL showed better sickle cell inhibition activity than DRC extract. The decrease in red blood cell count by hemolysis reveals that plant extracts may contain harmful phytochemicals. Saponins have been implicated as being highly toxic, causing hemolysis of red blood cells and eventually destroying them [23]. This decrease could also be linked to the possible presence in these extracts of hemolysin, a saponoside substance with a hemolytic effect [24].

Moreover, the gaits of the histograms of the extracts (DRC and DOSA) identical to that of the phenylalanine (positive control) contrary to the histogram of the sodium metabisulfite (negative control) which is increasing, confirms that these two species of plants (*Rhynchosporacorymbosa* and *Olaxsubscorpioïdea*) would have an activity on the inhibition of the falciformation of red blood cells of genotype SS.

4. CONCLUSION

Extracts of *Rhynchosporacorymbos* and *Olaxsubscorpioïdea* species are rich in various chemical groups such as alkaloids, polyphenols, flavonoids, tannins, steroids and saponosides. Some compounds in these chemical groups are thought to be responsible for the anti-sickle cell activity. They are used in the treatment of sickle-cell anemia. At a concentration of 0.312 mg/mL of DRC and DOSA extracts, the percentage of falciformation inhibition is 80 % and 84 % respectively. Both extracts have demonstrated activity on the *in vitro* inhibition of SS-genotype human blood sickle cells. This is the best concentration for sickle cell inhibition, with high activity for the DOSA extract. Thus, the use of *Rhynchosporacorymbosa* and *Olaxsubscorpioïdea* in the treatment of sickle cell disease is therefore justified.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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