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Phytochemistry and in vitro Anti-sickling activity of *Senna Occidentalis* L. (Fabaceae)

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ABSTRACT

Introduction

Sickle cell disease is an inherited genetic disorder characterized by the presence of abnormal hemoglobin, leading to the deformation of red blood cells and serious complications. It is a major public health problem in many countries of inter-tropical Africa. In the Democratic Republic of the Congo, over a million people (2%) are affected by this hemoglobinopathy.

Purpose

This study aimed to scientifically validate the anti-sickle cell activity of aqueous extracts of *S. occindentalis* seeds and to identify the chemical constituents responsible for this activity.

Methods

In this study, we used *S. occidentalis* seeds harvested at Ilebo in Central Kasai Province, while the blood samples used were taken from sickle-cell patients. The phytochemical composition was determined according to the standard method described previously by Iteku et al. and Nkasa et al. The Emmel test was carried out according to the standard protocol described previously by Bongo et al.

Results

The results obtained in this study showed that the seeds of this plant are rich in secondary metabolites such as total polyphenols (flavonoids, anthocyanins, leuco-athocyanins, tannins, and saponins), di-terpenes, alkaloids, and bound quinones. However, these seeds do not contain triterpenoids and steroids. Total seed extracts from this plant showed significant anti-sickle cell activity.

Conclusion

This study identified a medicinal plant used by the sickle cell disease community.

INTRODUCTION

Sickle cell disease is a hereditary genetic disease characterized by the presence of abnormal hemoglobin, leading to the deformation of the red blood cells and causing serious complications (Masengo et *al.*, 2021; Bongo et *al.*, 2017). It is the leading genetic disease in terms of the number of sufferers and represents a major public health problem in many countries of inter-tropical Africa. It affects more than 50 million people worldwide. It is estimated that more than 300,000 babies are born with this hemoglobinopathy every year (Gbolo et al., 2023; Gulbis et al., 2005).

In the Democratic Republic of Congo, over a million people (2%) are affected by this hemoglobinopathy (Gbolo et al., 2023; Mpiana et al., 2007). Several therapeutic actions have been developed to combat sickle cell disease. Gene therapy is being carried out on several samples. It offers a glimmer of hope but is not accessible to everyone. Blood transfusions are recommended in the event of a crisis. Polytransfusion slows down hemoglobin S synthesis, and reduces the frequency of vaso-occlusive crises and superinfections, but presents risks, including HIV, hepatitis, and blood incompatibilities. The administration of hydroxyurea, a molecule that increases fetal hemoglobin production and improves erythrocyte phenotype. It also has a disadvantage: toxicity occurs with prolonged use (Masengo et *al.*, 2021). Attempts to alleviate the suffering of sickle cell sufferers include the use of medicinal plants (Inkoto et *al.*, 2021; Panzu et *al.*, 2020). However, the folkloric use of plant species has led scientists to carry out studies on plants cited in traditional medicine as being able to treat SS anemia. The aim is to validate and popularize these plants. Many of these studies have demonstrated anti sickle cell activity in vitro (Bongo et al., 2018; Tshilanda et al., 2016; Ngbolua et al., 2014; Kitadi et al., 2022; Kitadi et al., 2019; Mpiana et al., 2012; Ogoda et al., 2002).

This research has revealed that the anti-sickle cell activity of these plants is due to the presence of active principles. The present study aims to assess the in vitro antifalcemic effect of *S. occidentalis*, a medicinal plant widely used in traditional medicine for its therapeutic properties. To this end, we will formulate hypotheses to determine whether *S. occidentalis* extracts have beneficial effects on red blood cell deformation in individuals with sickle cell disease. In parallel, we will carry out an in-depth analysis of the plant's phytochemistry to identify the bioactive compounds potentially responsible for the observed antifalcemic activity.

This study aims to scientifically validate the anti-sickle cell activity of aqueous extracts of *S. occindentalis* seeds, as well as to identify the chemical constituents responsible for this activity. The results of this research could contribute to the development of promising new therapeutic approaches for the management of sickle cell disease, improving patients' quality of life and opening new prospects in the field of herbal medicine.

METHODS

Materials

In this study, the *S. occidentalis* seeds used were harvested at Ilebo in the province of Kasaï centrale, while the blood was obtained from sickle cell patients seen in consultation at the Centre Mixte d'Anémiemia SS, located at Yolo-Sud, in the commune of Kalamu, city-province of Kinshasa. To be included in the study, blood had to come from patients whose sickle cell status had been proven by the hemoglobin electrophoresis technique. This test was performed on freshly collected blood. The samples were stored in a refrigerator at plus or minus 4°C. But before storage, it should be noted that to 1.6 mL of SS blood, 0.4 mL of 0.14 sodium citrate solution was added as an anticoagulant.

Methods

Chemical screening

Chemical screening was carried out using the classic method described previously by Iteku et *al.* (2020) and Nkasa et *al.* (2020).

Evaluation of anti-sickle cell activity

The Emmel test was performed according to the standard protocol as previously described by Bongo et al. (2017). The percentage of falciformation inhibition was calculated as follows (Kotue et *al.*, 2019): $SI = \frac{FO-FN}{FO}x100$.

Where, SI: percentage of falciformation inhibition, Fo: percentage of falciformation of the mixture [blood+ $Na_2S_2O_5$] (negative control), and Fn: percentage of falciformation of the mixture [SS blood + extract or tested compound + $Na_2S_20_5$]. The percentage of inhibition was

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used to calculate the normalization rate for each concentration according to the following formula: R(%) = SIn - Sio. Where (R) is the normalization rate (%), (SI0) is the initial percentage of sickling inhibition (control), and (SIn) is the maximum percentage of sickling inhibition obtained with different concentrations (15, 625 µg/mL, 31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL). Dose-response curves were obtained using Origin 8.5 software.

RESULTS AND DISCUSSION

Phytochemical screening

Table 1 gives the results of the chemical screening performed on the aqueous and organic extracts of *S. occidentalis* seeds.

Table 1:
Results of chemical screening performed on aqueous and organic extracts of *S. occidentalis* seeds

N°	Chemical groups	Results
1	Polyphenols	+
2	Flavonoids	+
3	Anthocyanins	+
4	Leuco-anthocyanins	+
5	Catechic tannins	+
6	Gallic tannins	+
7	Linked quinones	+
8	Alkaloids	+
9	Triterpenoids	-
10	Steroids	-
11	Diterpenoids	+
12	Saponins	+

Legend: +: Presence of desired compound; -: Absence of desired compound

Table 2 shows that *S. occidentalis* seeds are rich in secondary metabolites. A total of eight secondary metabolites were identified in *S. occidentalis* seeds: total polyphenols (flavonoids, anthocyanins, leuco-athocyanins, tannins, and saponins), di-terpenes, alkaloids, and bound quinones. However, these seeds do not contain triterpenoids and steroids. These results are like those of Nde et *al.* (2021), who found numerous phytochemical constituents such as flavonoids, saponins, alkaloids, tannins, terpenes, and glycosides in this plant. The presence of compounds such as anthocyanins would justify the use of this plant in traditional Congolese medicine against SS anemia, as these compounds are known for their anti-sickle cell properties (Tshilanda et *al.*, 2014; Kambale et *al.*, 2013; Mpiana et *al.*, 2007).

Antifalcemic activity

Induction of sickle cell disease

Table 2 shows the results of red cell sickle cell induction.

Table 2:
Induction of red cell sickling

Patient	Sample	Total number of red blood cells	Number of sickle cells	Percentage of falciformation	Results
01	SS-Ci40	359	336	93,5	Positive
02	SS-AN7	250	250	100	Positive
03	SS-Ci5	321	312	97,1	Positive
04	SS-15	278	274	98,5	Positive
05	SS-AN6	241	240	99,5	Positive
06	SS-C7	304	304	100	Positive
07	SS-D13	272	272	100	Positive
08	SS-D11	344	331	96,2	Positive
09	SS-84	277	272	98,1	Positive
10	SS-AN41	178	178	100	Positive
11	SS-AN17	290	157	54,1	Positive
12	SS-AN22	310	175	56,4	Positive
13	SS-25	299	141	47,1	Negative
14	SS-Ci9	306	152	49,6	Negative
15	SS-AN26	199	31	15,5	Negative
16	SS-C4	211	43	20,3	Negative
17	SS-AN25	271	129	47,6	Negative
18	SS-AN16	221	155	48,2	Negative
19	SS-16	379	271	71,5	Negative
20	SS-D15	287	207	72,1	Negative

The **Table** shows that out of 20 samples from homozygous patients treated with $Na_2S_20_5$ 2% to check their ability to falciform under hypoxic conditions, 6 samples showed a low falciformation rate (falciformation rate below 50%). These patients could be on hydroxyurea and/or antioxidant therapy.

Antifalcemic effects on SS erythrocytes

The results of the antifalcemic test on erythrocytes are shown in Table 3 below.

Table 3:
Antifalcemic effects on SS erythrocytes (60 min contact with Na2S205 2%, sodium cromoglycate, and 24 h contact with *S. occidentalis* extracts under hypoxic conditions)

Blood sample	Negative control				Sodium Cromoglycate				Aqueous extracts of *S. occidentalis* (250µg/mL)			
	NT	ND	F	Ro	NT	ND	F	Ro	NT	ND	F	Ro
SS-AN7	250	250	100	0	244	17	6,9	93,1	238	12	5,0	95
SS-15	278	274	98,5	0	224	13	5,8	94,1	261	16	6,1	93,8
SS-AN6	241	240	99,5	0	236	23	9,7	90,2	234	14	5,9	94,0
SS-Ci5	321	312	97,1	0	306	30	9,8	89,9	298	18	6,0	93,8
SS-C7	304	304	100	0	284	22	7,7	92,3	286	17	5,4	94,1
SS-D11	344	331	96,2	0	316	20	6,3	93,4	290	19	6,5	93,[illegible]
SS-D13	272	272	100	0	253	17	6,7	93,3	253	15	5,9	94,[illegible]
SS-84	277	272	98,1	0	261	19	7,2	92,6	261	14	5,3	94,[illegible]
SS-AN41	178	178	100	0	168	8	4,7	95,3	164	12	7,3	92,[illegible]
SS-40	322	313	97,2	0	282	29	10,2	89,5	299	20	6,6	93,2

Legend: NT: Total number of red blood cells, N.D: Number of sickle cells, F: Percentage of sickle cell induction, Ro: Reversibility.

Table 3 shows that the negative control has a normalization rate of 0%, while the aqueous extracts of *S. occidentalis* seeds have antifalcemic properties. This indicates that extracts of this plant species can be applied to sickle-cell patients.

Table 4 gives the antifalcemic effects of *S. occidentalis* extract on SS erythrocytes, at different concentrations (µg/mL).

Table 4:
Antifalcemic effects of *S. occidentalis* extract on SS erythrocytes, at different concentrations (µg/mL)

Concentrations	NT	ND	F	Si	R(%)
15,625	286	286	100	0	-6,5
31,25	285	279	97,8	0,71	-5,7
62,5	283	136	48,0	51,2	44,2
125	280	30	10,7	88,9	52,4
250	279	0	0	100	93,5
500	269	0	0	100	93,5
1000	266	0	0	100	93,5

Legend: N.T: Total number of red blood cells, N.D: Number of sickle cells, F: Percentage of sickle cells, Si: Percentage of sickle cell inhibition, R(%): Normalization rate of SS erythrocytes.

Table 4 shows that *S. occidentalis* extracts have a high antifalcemic activity at concentrations of 125µg/mL and above. At higher concentrations, this activity rises to 93.5% and remains constant. At concentrations below 125µg/mL, however, the activity is uninteresting (<50%).

Figure 1 gives the evolution of sickle cell normalization rates as a function of *S. occidentalis* extract concentrations.

Figure 1:
Sickle cell normalization rate as a function of S. occidentalis aqueous extract concentrations

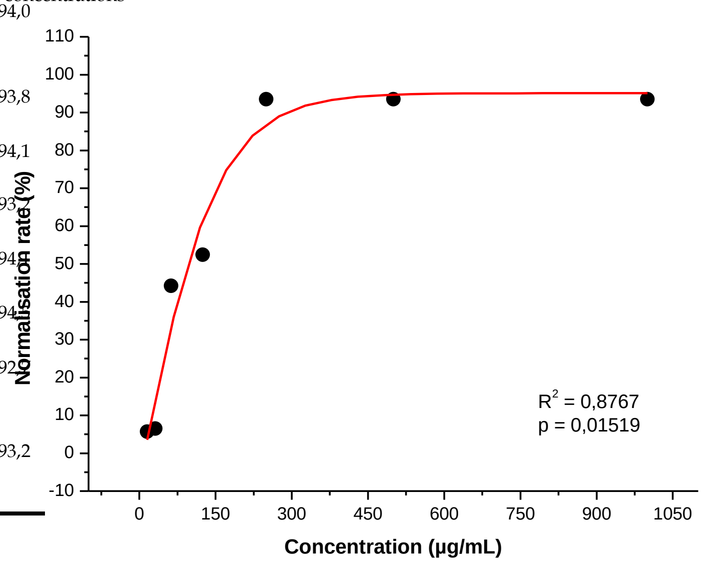

Figure 1 shows that the normalization rate increases with concentration, and reaches a fixed value above a certain concentration, known as the minimum normalization concentration. This is 125µg/mL for the total extract of *S. occidentalis*. It is much higher than those of the plants studied by Mpiana et *al.* (2007). These were : *A. cordifolia* (CMN: 0.097 µg/mL and TN: 93%), *A. digitata* (CMN: 0.43 µg/mL and TN: 80%), *A. betzichiana* (CMN: 0.12 µg/mL and TN: 90%), *B. pentadrum* (CMN: 0.81 µg/mL and NT: 78%), *F. capensis* (CMN: 04 µg/mL and NT: 89%), *H. angustifolia* (CMN: 0.42 µg/mL and NT: 90%), *O. basilicum* (CMN: 0.13 µg/mL and NT: 86%), *Z. macronata* (CMN: 0.7 µg/mL and TN: 87%), *C. senegalensis* (CMN: 0.15 µg/mL and TN: 90%), *H. crepitans* (CMN: 0.18 µg/mL and TN: 90%), *D. brazzal* (CMN: 0.11 µg/mL and TN: 86%).

Figure 2:
Antisickling test Microscopy: untreated erythrocytes (A); Erythrocytes treated with aqueous extract 125 µg/mL (B) of S. occidentalis (magnification X500).

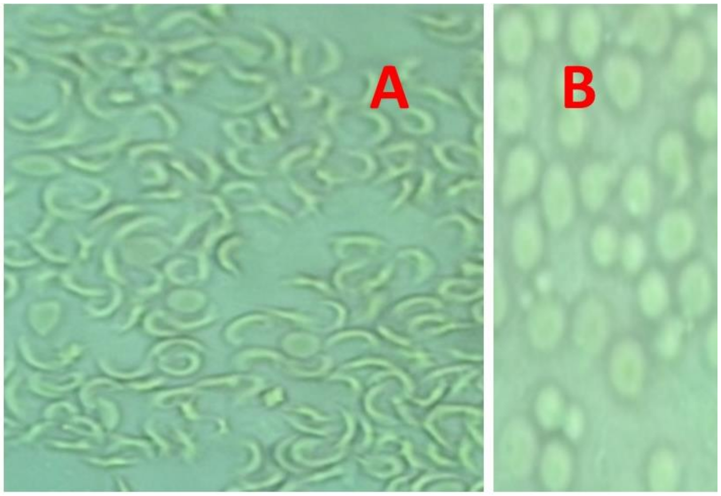

Figure 2(A) shows falciform red blood cells, effectively indicating that this is SS blood. On the other hand, Figure 2(B) shows the ability of the aqueous extract of *S. occidentalis* to modify the shape of SS red blood cells in vitro, transforming them from sickle-shaped (abnormal) to biconcave (normal), when placed under the same hypoxic conditions as the control in the presence of physiological solution. These results confirm previous work (Mpiana et *al.*, 2007). These results show that our drugs prevent the various complications associated with sickle cell disease. This anti-sickle cell activity would be due to anthocyanins (Table 1), as anthocyanins have.

CONCLUSION

This study aimed to scientifically validate the anti-sickle cell activity of aqueous extracts of *S. occindentalis* seeds, as well as to identify the chemical constituents responsible for this activity. The results obtained in this study show that the seeds of this plant are rich in secondary metabolites such as flavonoids, anthocyanins, leuco-athocyanins, tannins, saponins, di-terpenes, alkaloids, and bound quinones. However, they do not contain triterpenoids or steroids. The total seed extracts of this plant have significant anti-sickle cell activity. In addition, this study identified a medicinal plant used by Lele traditional healers in Ilebo territory, Kasai province, for the treatment of sickle cell disease.

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Ethical Approval: Nil required

Conflicts of Interest: None declared.

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