European Journal of Scientific Research
ISSN 1450-216X Vol. 87 No 1 September, 2012, pp.100-108

http://www.europeanjournalofscientificresearch.com

Assessment of Antisickling Properties of Extracts of Plants used in the Traditional Treatment of Sickle Cell Disease in Benin

C. T. Avaligbe
Laboratoire de Pharmacognosie et des Huiles Essentielles (LAPHE)
Faculté des Sciences et Techniques (FAST), Université d'Abomey-Calavi (UAC)
01 BP: 918 ISBA Champ de Foire, Cotonou Benin

J. D. Gbenou
Laboratoire de Pharmacognosie et des Huiles Essentielles (LAPHE)
Faculté des Sciences et Techniques (FAST), Université d'Abomey-Calavi (UAC)
01 BP: 918 ISBA Champ de Foire, Cotonou Benin

D. S. Salomé Kpoviessi
Laboratoire de Pharmacognosie et des Huiles Essentielles (LAPHE)
Faculté des Sciences et Techniques (FAST), Université d'Abomey-Calavi (UAC)
01 BP: 918 ISBA Champ de Foire, Cotonou Benin
E-mail: kpovsalome@yahoo.fr
Tel: 00 229 97883927

F. Gbaguidi
Laboratoire de Pharmacognosie et des Huiles Essentielles (LAPHE)
Faculté des Sciences et Techniques (FAST), Université d'Abomey-Calavi (UAC)
01 BP: 918 ISBA Champ de Foire, Cotonou Benin

M. Moudachirou
Laboratoire de Pharmacognosie et des Huiles Essentielles (LAPHE)
Faculté des Sciences et Techniques (FAST), Université d'Abomey-Calavi (UAC)
01 BP: 918 ISBA Champ de Foire, Cotonou Benin

G. C. Accrombessi
Department of Chemistry, Faculty of Sciences and Technologies
University of Abomey-Calavi, Laboratory of Physic and Synthesis Organic Chemistry (LaCOPS), 01 PB: 526 Cotonou, Benin

M. Gbeassor
Laboratoire de Physiologie Animale et de Pharmacologie
Faculté des Sciences, Université de Lomé, Togo

Abstract

The purpose of this study is to value the in vitro anti-sickling activity of aqueous extracts of six plants used in the treatment of the sickle cell disease in Benin: *Morinda*

lucida, Uvaria chamae, Lonchocarpus cyanescens, Croton zambesicus*, Raphiostylis beninensis* and *Xylopia aethiopica*. A decrease in the rate of sickling was observed after incubation of erythrocytes with plant extracts and 2% sodium metabisulfite as compared to incubation with 0.9% NaCl. The results of the study about the inhibitory properties of the SS erythrocytes sickling by the aqueous extract of the mixture of *Raphiostylis beninensis, Croton zambesicus, Lonchocarpus cyanescens, Uvaria chamae, Morinda lucida* and *Xylopia aethiopica* show that it has an obvious antisickling effect in vitro according to our experimental conditions (sickling inhibition rate > 50%). It is the same with aqueous extracts of *Uvaria chamae, Morinda lucida* and *Xylopia aethiopica* which inhibit more than 45% of SS erythrocytes sickling. Antisickling effects of those last plants on AS and SS erythrocytes are otherwise highly correlated ("r " is respectively equal to 0,88; 0,88 and 0,66). The extract *of Raphiostylis beninensis, Croton zambesicus* and *Lonchocarpus cyanescens* have relatively less effect (inhibition rate < 21%) particularly on SS erythrocytes sickling and a correlation coefficient "r" respectively equal to -0,2353; 0,3959 and 0,723. Our results point out that these six plants have, at different doses, an obvious antisickling effect in vitro and support the interest of their use in the traditional treatment of sickle cell disease.

Keywords: Sickle cell disease-Sickling-Morinda lucida-Uvaria chamae-Lonchocarpus cyanescens-Croton zambesicus-Raphiostylis beninensis-Xylopia aethiopica

1. Introduction

Hereditary autosomal recessive affection, sickle cell disease is a pathology characterized by an abnormal structure of hemoglobin. This condition is illustrated by a deformation of the sickle shaped erythrocytes which results in haemolytic anemia and blood vessels blockage, behind the major causes of death in sickle cell disease. (Latoundji et al, 1991 ; Bunn, 1997).

Despite its ubiquitous nature, sickle cell disease is an illness that affects especially black people. The prevalence is 2% on average in Africa with life expectancy below 20 years against 0.02% on average in other continents (Galacteros, 2000). In Africa, less than 50% of homozygous people affected by sickle cell disease reach 5 years old and in Benin less than 18% reach adulthood (Latoundji et al., 1991). Despite this sinister epidemiology, there is currently in the market, no specific drug that can reduce the mortality associated with this disease.

In Benin, Zohoun et al. (1992) evaluated the amount of the treatment of sickle cell disease per year at three times the health budget that is one tenth of the national budget.

In the light of the forgoing, the search for an effective and less costly plant protection is inevitable. To this end, we have evaluated the antihaemolytic properties of a traditional recipe combining in a single decoction the roots of *Uvaria chamae*, *Lonchocarpus cyanescens*, *Morinda lucida*, leaves of *Croton* zambesicus, fruits of *Xylopia aethiopica* and stems of *Raphiostylis beninensis*.

2. Materials and Methods

2.1. Plant Material

It is constituted of the roots *Morinda lucida, Uvaria chamae*, *Raphiostylis beninensis* stems, leaves of *Croton* zambesicus and fruits of *Xylopia aethiopica.* Different plant samples were authenticated at the National Herbarium of Benin located at the University of Abomey-Calavi (UAC) and have been registered under number 008-09/HNB/FAST/UAC.

2.2. Biological Material

Blood samples for hemoglobin AS and SS phenotypes are collected in tubes containing EDTA, from voluntary patients who have given their consent in writing and signed note.

2.3. Chemical

For the sickling induction, we used the sodium metabisulfite ($Na_2S_2O_5$) at 2%.

For the preparation of the plant extracts solutions at the different concentrations, we used a solution of NaCl to 0,9%.

2.3.1. Phytochemical Screening

Each powder obtained was analyzed according to various staining reactions and precipitation to determine the qualitative chemical composition of the different plants studied. The different classes of active ingredient have been identified by the method of Houghton and Raman (1998).

2.3.2. Preparation of Hot Aqueous Extracts

The technique used is the decoction's one. We introduced 50 grams of powdered leaves or fruit or 100 grams for the roots and stems in a round bottom flask. Then we added 500ml of distilled water (1L for roots and stems). The mixture is heated to boiling for one hour and poured into a beaker and then filtered. The filtrate is evaporated in large part to a rotavapor and then placed under vacuum in an oven at 40°C. Finally, we proceeded to the grinding of the final solid extract obtained. The fine powder thus obtain has been kept refrigerated at 4°C in small colored flasks.

2.3.3. In Vitro Study of Sickling Inhibition

Recent studies on several antisickiling plant showed that the sickling inhibition is not only dependent on time but also the concentration of the extract solution. The sickling rate is maximum after two hours for SS erythrocytes and 24 hours for AS erythrocytes while the sickling inhibition rate is increased when the concentration of extract is greater or equal to 15 mg / mL (Joppa et al, 2008; Elekwa et al, 2004).

So we mixed 0.5 mL of freshly drawn blood and 0.5 mL of each plant extract with concentration 15 mg/mL. After having homogenized them for a few seconds, we add 0.5 mL of 2% sodium metabisulfite.

As for the reference solution, the 9g/L NaCl solution is used instead of the extract.

After the mixture homogenized again, we put a drop of preparation between slide and coverslip. The slides are incubated at room temperature in a petri box whose bottom is covered with filter paper soaked in water to prevent drying.

Reading the slides was made under an optical microscope ZEISS brand, after 24 hours incubation for AS blood and 2 hours for SS blood. For each sample, we conducted five slides. Three areas of each of these slides mounted under an optical microscope at X40 enlargement, were photographed using a digital camera SONY (12.1 megapixels) set at A3 size max.

It was then proceeded to count the number of sickle cells relative to the total number of erythrocytes. Any cell that has lost its original form discoidal is considered as cell disease. The rate of inhibition of sickling obtained by the extract is determined by counting the percentage of cells in the presence of sickling extract, that of the reference solution.

3. Statistical Analysis

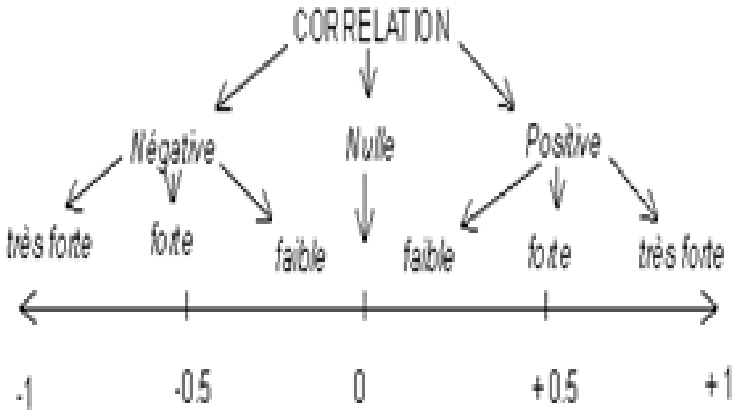
3.1. Student Test T

To study the sickling activity, we compared the average number of sickle cells, obtained in the presence of different extracts with the reference solution. For this, we have inserted in the spreadsheet Microsoft Excel 2007 five values of the number of sickle cells obtained from five slides of the reference solution and those in the presence of each extract. This test was used to determine whether the antisickling activity of the different samples extracts is statistically different from the reference solution. The probability threshold p used is 0.05.

3.2. Bravais-Pearson's Correlation Coefficient R

This coefficient is used to detect the presence or absence of a linear connection between two continuous quantitative characters. It varies between -1 and +1 and its interpretation is as follows:

Figure 1: Interpretation of Pearson's correlation coefficient r



In the context of our study, it's used to determine whether there is a correlation between the effects of each of the extracts on the erythrocytes AS and SS sickling.

4. Results and Discussions

4.1. Slides Photograph

Different slides made for each extract (15 mg/mL) plants were photographed and presented through figures from 2 to 7. Arrows indicate in each photograph the cells sickled or considered as such.

4.1.1. Raphiostylis Beninensis Extract

Figure 2a point several cells being sickled but figure 2b shows the cells of which sickled shape is more or less clear. The extract of Raphiostylis beninensis stems inhibits better erythrocytes AS (26.47%) sickling than red blood cells SS one (20.5%).

Figure 2a: AS slide Photograph

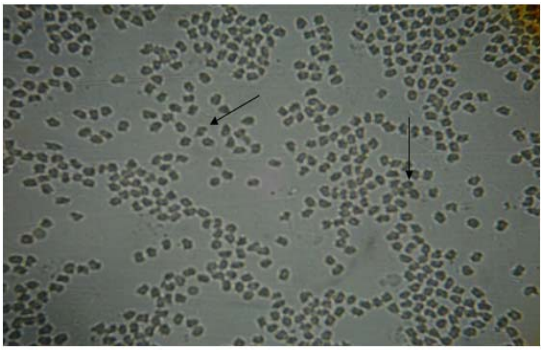
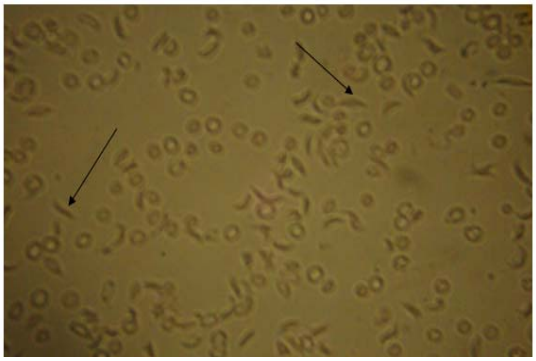


Figure 2b: SS slide Photograph



4.1.2. Xylopia Aethiopica Extract

Figure 3a shows few cells in the process of sickling and figure 3b point several cells whose sickled shape is more or less clear.

The extract from fruit of *Xylopia aethiopica* inhibits better AS red blood cells sickling (75.7%) than the SS erythrocytes one (45%).

Figure 3a : AS slide Photograph

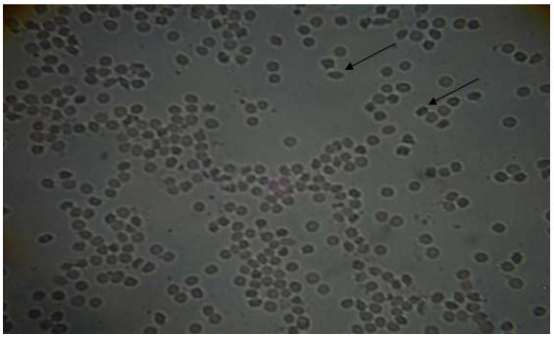
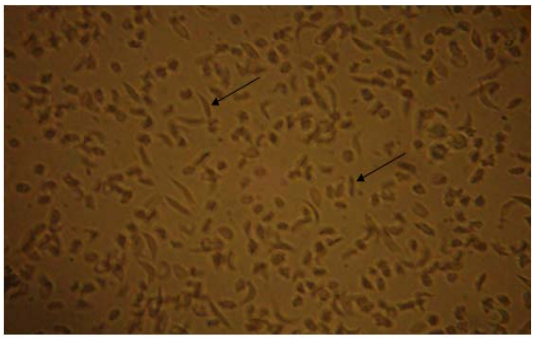


Figure 3b: SS slide Photograph



4.1.3. Croton Zambesicus Extract

Figure 4a shows many cells being sickled and figure 4b points a large number of clear sickled cells. *Croton zambesicus* leaves extract inhibit much better AS sickling erythrocytes (47.44%) than the SS erythrocytes one (12.78%).

Figure 4a: AS slide Photograph

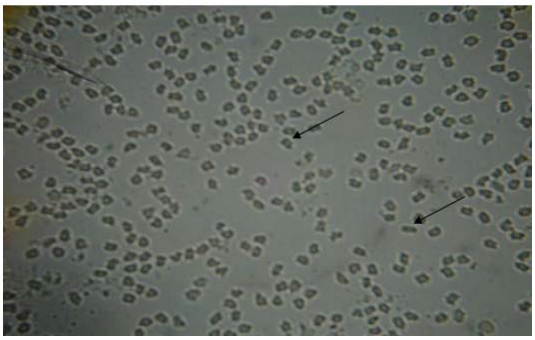
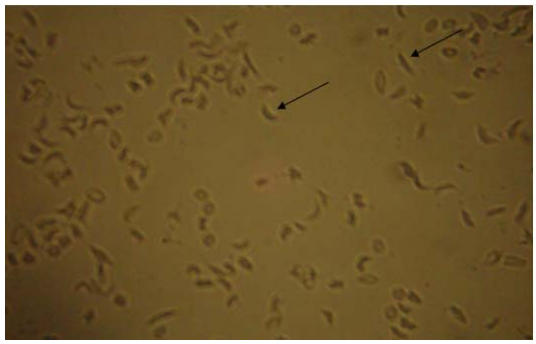


Figure 4b: SS slide Photograph



4.1.4. Uvaria Chamae Extract

Figure 5a shows many cells in the process of sickling and figure 5b shows very few sickled cells. The extract of the roots of *Uvaria chamae* inhibits better SS sickling erythrocytes (55.9%) the AS erythrocytes one (26.6%).

Figure 5a: AS slide Photograph

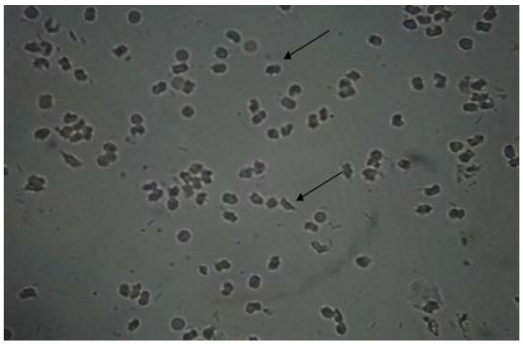
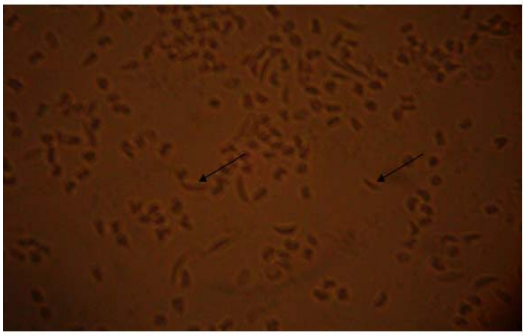


Figure 5b: SS slide Photograph



4.1.5. Morinda lucida Extract

Figures 6a and 6b show that there are very few sickled cells in process of sickling. The root extract of *Morinda lucida* inhibits better AS sickling erythrocytes (63.38%) than the SS red cells one (57.99%).

Figure 6a : AS slide Photograph

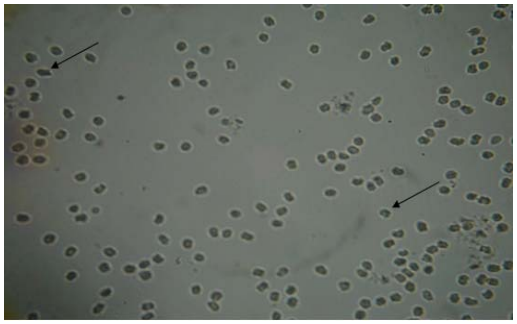
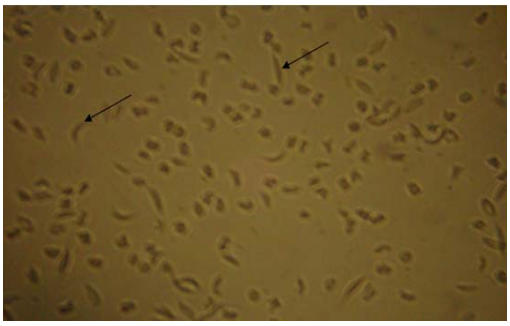


Figure 6b: SS slide Photograph



4.1.6. Lonchocarpus Cyanescens Extract

Figure 8a shows very few cells in the process of sickling and figure 8b points several sickled cells. The root extract of *Lonchocarpus cyanescens* inhibits much better AS sickling erythrocytes (65.97%) than the SS erythrocytes one (18%).

Figure 7a: AS slide Photograph

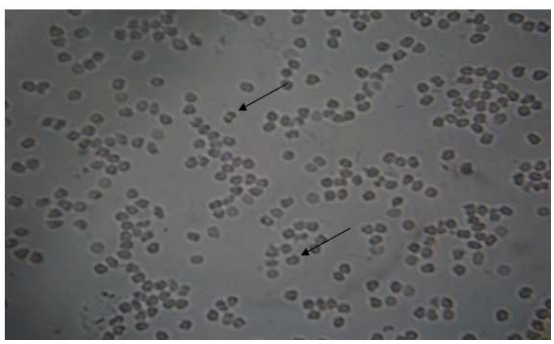
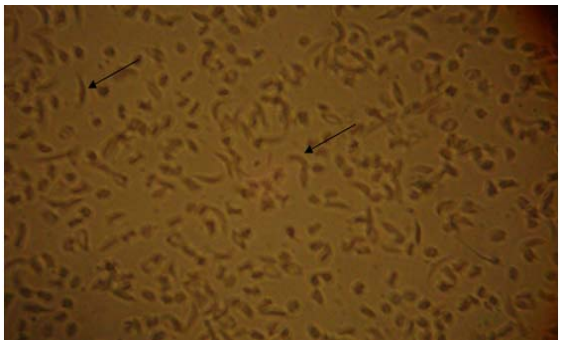


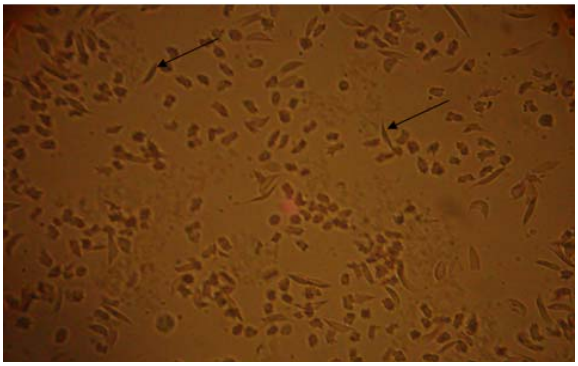
Figure 7b: SS slide Photograph



4.2. Mixture of Six Plants Extract

The antisickling activity of the extract of mixture of six plants was investigated only on SS erythrocytes due to the lack of *Lonchocarpus cyanescens* extract. Figure 8 shows that there are more cells being sickled than sickled cells. The sickling inhibition rate (50.89%) of that extract is higher than the one of *Raphiostylis beninensis* (20.5%), *Lonchocarpus cyanescens* (18.05%), Croton zambesicus (12.78%) and *Xylopia aethiopica* (45%) tested separately. However, extracts of *Morinda lucida* and *Uvaria chamae* have a rate of inhibition (respectively 55.89 and 57.99%) greater than the one of the extract of the mixture of six plants. These results show that *Uvaria chamae* and *Morinda lucida* are more active when isolated than be in association with other plants. This could be explained by molecular interactions that partially inhibit their antisickling potential.

Figure 8: Photograph of SS blade



4.3. Sickled Erythrocytes Counting

The middle rate of sickled erythrocytes and sickling inhibition rate are consigned in the Table 1 and illustrated by the figure 9.

Table 1: Percentage of sickled erythrocytes and sickling inhibition rate

Extracts to 15 mg/mL	MRSC		InF		"r"
	AS	SS	AS	SS	
R.beninensis	55,85	34,95	26,47	20,5	-0,2353
X.aethiopica	6,65	34,95	75,67	45	0,661
U.chamae	59,69	24,07	22,63	55,89	0,8790
M.lucida	18,94	21,97	63,38	57,99	0,8832
L.cyanescens	16,35	61,90	65,97	18,05	0,7230
C.zambesicus	34,88	67,17	47,44	12,78	0,3959
Mélange	-	29,07	-	50,89	-

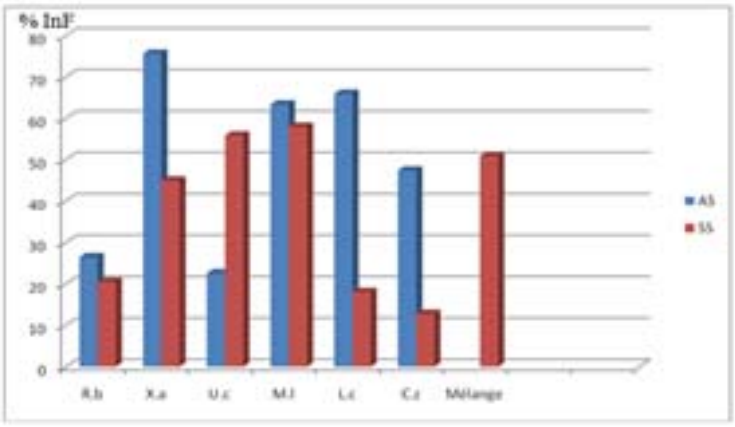
MRSC : Middle rate of sickled erythrocytes; SIR : sickling inhibition rate ; "r" : Pearson's corrélation coefficient.

The decrease of AS and SS erythrocytes sickling rate compared with their reference solution is significant for all extracts ($p < 0,05$).

The maximal inhibitory effect of AS erythrocytes sickling is gotten with the aqueous extract of Xylopia aethiopica's fruits. They inhibit the sickling of more than 75% of AS erythrocytes against 45% for the SS AS erythrocytes. These fruits constituting aromatic spices (Guissou et al, 1995), their daily use as substitutes of pepper in the family cooking would be highly recommended for the prevention of the blood vessel-blockage crises.

After deduction, the percentage of sickling inhibition (% InF) by different extracts is presented as follow:

Figure 9: Percentage of AS and SS erythrocytes sickling



All plant extracts, except Uvaria chamae roots, inhibit AS erythrocytes sickling more than those of SS phenotype.

However, the inhibitory effect of AS and SS erythrocytes sickling is highly correlated (|r| tend to 1) for all extracts with the exception of extracts Raphiostylis beninensis stems and the leaves of Croton zambesicus with which the AS/SS correlations is low (respectively -0,24 and 0,4).

The best rate of SS erythrocytes sickling inhibition (57,98%) is obtained with the aqueous extract of the roots of Morinda lucida. This result is significantly different from the one of Joppa et al. (2008) who got, with half-ethanolic extracts of the same plant at the same dose, an inhibition of 86,19% of SS erythrocytes sickling. The correlation coefficient AS/SS is 0,89. This difference of inhibition rate is explained by the type of the extraction solvent used.

Moreover, Mpiana et al. (2008) showed that the ethanolic extract of Morinda lucida leaves, either used alone or associated to the stems bark of this same plant, inhibit the sickling of more than 50% of SS erythrocytes. In the opposite, the aqueous extract of these same leaves have no effect on the sickling normalization.

From all studied plants, three are especially active on SS erythrocytes sickling. It concerns Morinda lucida, Uvaria chamae and Xylopia aethiopica. These three plants and the mixture of the six plants present some significant rates of sickling inhibition (> 40%). Indeed, Ohnishi et al. (2001) got with Alium sativum at 6 mg/mL an inhibition of 30% "dens cells" formation induced by 500 mM of urea.

Iyamu and al. (2002) reached 50% of inhibition with 5 mg/mL of Niprisan® (composed of seeds of Piper guineense, stems of Pterocarpus osun, fruits of Eugenia caryophyllum and leaves of Sorghum bicolor).

Ahmed et al. (1997) have gotten inhibitions going from 12 to 21% with the calcic inhibitors.

5. Conclusion

Sickle cell disease is an illness of public health, particularly in sub-Saharan Africa. The use of medicinal plants for prevention or treatment of sickle cell crises is very common.

The results of the study of sickling inhibitory properties of the aqueous extracts of Uvaria chamae, Lonchocarpus cyanescens, Morinda lucida, Croton zambesicus, Xylopia aethiopica and Raphiostylis beninensis show that the six plants have an antisickling clear effect in vitro under our experimental conditions. Except for Uvaria chamae extract, they protect AS erythrocytes better than the SS erythrocytes sickling.

Then, our results prove the interest of the use of these six plants in the traditional treatment of sickle cell disease.

References

[1] Adjanohoun, E.J.; Adjakidjè, V.; Ahyi, M.R.A.; Aké Assi, L.; Akoègninou, A.; d'Almeida, J.; Apovo, F.; Boukef, K.; Chadare, M.; Cusset, G.; Dramane, K.; Eyme, J.; Gassita, J.N.; Gbaguidi, N.; Goudote, E.; Guinko, S.; Houngnon, P.L.I.; Keita, A.; Kiniffo, H.V.; Kone-Bamba, D.; Musampa Nseyya, A.; Saadou, M.; Sodogandji, T.; De Souza, S.; Tchabi, A.; Zinsou Dossa, C.; Zohoun, T., 1989. Contribution aux études ethnobotaniques et floristiques en République Populaire du Bénin. Agence de Coopération Culturelle et Technique, Paris, France, pp 895.

[2] Adjanohoun, E., De SOUZA, S., 2002. "Guide pratique de Phytothérapie", *Bulletin Régional d'Information du CENPREBAF*, 4, pp.78.

[3] Begue, P., Assimadi, K., 1984. Diagnostic de la drépanocytose et de ses complications. In : La Maladie drépanocytaire - Ed. Labo Sandoz, pp78.

[4] Iyamu, E.W., Turner, E.A., Asakura, T., 2002. In vitro effect of Niprisan (Nix-0699): a naturally occurring potent antisickling agent, *British Journal Haematology*, 118, pp. 337-343.

[5] Ahmed, J.H., Al-Diwan, M.S., Jawad, A.M., 1997. "Effect of methoxyverapamil, diltiazem, morphine and their combination on the formation of irreversibly sickled cells: an in vitro study", *East Medit Health J*, 3(2), pp. 301-309.

[6] Joppa, K.M., Vovor A., Eklu-Gadegbeku K., Agbonon A., Aklikokou K., Gbeassor M., 2008. Effet de Morinda lucida Benth.(Rubiaceae) et de Newbouldia laevis P.Beauv.(Bignoniaceae) sur la falciformation, *Med. Trop*, 68, pp. 251-256.

[7] Mpiana, P.T., Tshibangu, D.S.T., Shetonde, O.M., Ngbolua, K.N., 2007. In vitro antidrepanocytary activity (anti-sickle cell anaemia) of some Congolese plants. *Phytomedicine*, 14, pp. 192-195.

[8] Ohnishi, S.T., Ohnishi, T., Ogunmola, G.B., 2001. Green tea extract and aged garlic extract inhibit anion transport and sickle cell dehydration in vitro", *Blood Cells Mol Dis*, 27, pp. 148-155.

[9] Zohoun, I.S., Mérault G., Reinette P., Rosa J., 1992. Politique de santé et drépanocytose. *Rev. Prat.*, 42 pp 1873-1877