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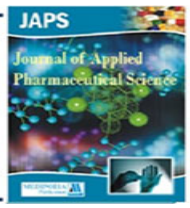

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Antihemolytic Properties of Extracts of Six Plants Used in the Traditional Treatment of Sickle Cell Disease in Benin

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ABSTRACT

This study is focused on the *in vitro* evaluation of the hemolysis inhibitory activity of aqueous extracts of six plants used in the traditional treatment of sickle cell disease in Benin: *Morinda lucida, Uvaria chamae, Lonchocarpus cyanescens, Croton zambesicus, Raphiostylis beninensis* and *Xylopia aethiopica*. AS and SS red blood cells are subjected to hyposmotic impact with decreasing concentrations of NaCl solution. All the aqueous extracts of the six plants showed a better contribution in erythrocyte osmotic resistance from the concentration of 5 mg/mL to 1 mg/mL, except the extract at 5 mg/mL of *Raphiostylis beninensis* that caused hemolysis of both red blood cells AS and SS. The extract at 1 mg/mL of *Raphiostylis beninensis* and the extracts at 5mg/mL of *Xylopia aethiopica* and *Croton zambesicus* showed a high hemolysis inhibition of red blood cells AS and SS. The extracts of *Lonchocarpus cyanescens* showed moderate hemolysis inhibition of *SS* red blood cells while extracts of *Uvaria chamae* inhibited highly the hemolysis of AS red blood cells. As for the roots of *Morinda lucida*, only the extract at 5mg/mL highly inhibited the hemolysis of the AS red blood cells. This protocol seems appropriate to work with both AS and SS blood because the comparative effects of each tested extract on hemolysis of AS and SS blood showed a good correlation coefficient of Pearson (1 or -1). All the plants tested in this work showed, at different doses, an *in vitro* anti-sickling effect and this explains partially their use in the traditional treatment of sickle cell disease.

Keywords: Sickle cell disease, antihemolysis, *Morinda lucida, Uvaria chamae, Lonchocarpus cyanescens, Croton zambesicus, Raphiostylis beninensis, Xylopia aethiopica*

INTRODUCTION

Hereditary autosomal recessive affection, sickle cell disease is a pathology characterized by an abnormal structure of hemoglobin (Wajcman and Ladie, 1981). This pathological condition is illustrated by a deformation of the sickle shaped erythrocytes which results in hemolytic anemia and blood vessels blockage. Those factors are behind the major causes of death of the people suffering from sickle cell disease. (Latoundji *et al.*, 1991; Bunn, 1997).

Despite its ubiquitous nature, sickle cell disease is an illness that affects especially black people (Lehmann, 1974). The prevalence rate is about 2% in Africa with life expectancy below 20 years against 0.02% 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 cost of the treatment of sickle cell disease per year at three times the health budget which is the equivalent of one tenth of the national budget. The search for an effective and less costly plant protection is then compulsory. A number of plant products have been implicated in the management of several human ailments including sickling and sickle cell disease (Uphof, 1968; Soforowa *et al.,* 1971; Willis, 1973; Soforowa, 1975; Iwu, 1985; Ekeke *et al.,* 1990; Elekwa *et al.,* 2005). They could lead to the discovery of an efficient and less costly treatment for this disease. In Benin, a traditional recipe combining in a single decoction of the roots of *Uvaria chamae*, *Lonchocarpus cyanescens*, *Morinda lucida*, the leaves of *Croton* zambesicus, the fruits of *Xylopia aethiopica* and the stems of *Raphiostylis beninensis* is used as a traditional cure against sickle cell disease. In this work, we focused on the evaluation of the antihaemolytic properties the roots of *Uvaria chamae*, *Lonchocarpus cyanescens*, *Morinda lucida*, the leaves of *Croton* zambesicus, the fruits of *Xylopia aethiopica* and the stems of *Raphiostylis beninensis*.

MATERIALS AND METHODS

Plant material

Fresh roots of *Uvaria chamae*, *Lonchocarpus cyanescens*, *Morinda lucida*, leaves of *Croton* zambesicus, fruits of *Xylopia aethiopica* and stems of *Raphiostylis beninensis* were collected (dry season) in Abomey-Calavi (south of the Republic of Benin). Different plant samples were identified by the National Herbarium of the University of Abomey-Calavi (Benin) and vouchers specimens (n° 008-09/HNB/FAST/UAC) were deposited at this National Herbarium.

Biological material

Blood samples for hemoglobin AS and SS phenotypes are collected in sterile tubes containing EDTA, from volunteer patients who have given their consent by writing and signing a letter in the Area Hospital of Menontin (Cotonou, Republic of Benin). The different blood samples were genotyped using standard electrophoretic procedure. The blood samples were stored at about 4°c and used within 24 hours of collection.

Chemical

Sodium chloride from PROLABO (Paris, France) was used to prepare the various hypotonic salt solutions and isotonic with blood. Fehling's solution (A and B), sulphuric acid (H_2SO_4), ammoniac, chloroform, ferric chloride, acid alcohol, Mayer's reagent, Draggendorff's reagent, glacial acetic acid were used for the phytochemical screening.

Hot aqueous extracts of plant materials

The extracts used in this work were obtained by decoction. The plant materials (roots of *Uvaria chamae*, *Lonchocarpus cyanescens*, *Morinda lucida*, leaves of *Croton* zambesicus, fruits of *Xylopia aethiopica* and stems of *Raphiostylis beninensis*) were air-dried at room temperature (26°C) for 2 weeks, after which it was grinded to a uniform powder. 50 g of powdered leaves or fruits (100 g of powdered roots or stems) were added to 500 ml of distilled water (1L for roots and stems). The mixture was heated to boiling for one hour and filtered, first through a Whatmann filter paper N°. 42 (125 mm) and then through cotton wool. The ectracts were concentrated using a rotary evaporator with the water bath set at 45°C and then placed under vacuum in an oven at 40°C. Finally, the obtained solid extract was grinded. The fine powder thus obtained was kept refrigerated at 4°C in sterilized colored flasks.

Phytochemical screening

Each powder obtained was analyzed according to various staining and precipitation reactions to determine the qualitative chemical composition of the different studied plant materials. The different classes of active compounds have been identified by standard procedures (Houghton and Raman,1998; Ayoola *et al.,* 2008).

Determination of erythrocyte osmotic fragility

This followed the procedure developed by Parpart et al (1947) as modified by Elekwa et al (2003). In a series of nine tubes were mixed successively: 4.5mL of NaCl (at 9, 7,6,5,4,3,2,1 and 0 g/L), 0.5mL of each type of blood. In another series of nine tubes only 0.5 ml of NaCl at 9 g/l was replaced by the extract to be tested according to the dose. The different tube contents were re-mixed and centrifuged for 5 minutes at 1,500 r.p.m by a centrifuge brand Compact Star CS4. The absorbance (or optical density) of the supernatant was read with a spectrophotometer DR 2800 brand at 540 nm using 0.9 g/L NaCl tube as blank. This procedure was repeated five times and the average was determined for each dose of each extract and for each the different blood genotype (hemoglobin AS and SS).

From measurements of optical density, the rate of hemolysis inhibition of the different extracts was determined with the following formula:

$$\% \text{ Hemolysis inhibition} = 100 \times \frac{DO_0 - DOi}{DO_0}$$

DOi: Absorbance of sample extract; DO_0: Optical density of the control solution.

The profiles of hemolysis inhibition as compared to concentrations of NaCl, obtained from the previously calculated values, were used to determine a characteristic point: CIH_{50}. That

is the concentration of NaCl corresponding to the inhibition of lysis of 50% erythrocytes. The more, the concentration of NaCl decreases, the more the hemolysis is emphasized. Therefore the CIH_{50} is so weak that the plant is more protective against the hemolysis. In order to compare the effect of the extracts on the hemolysis of both phenotypes AS and SS, the Pearson's correlation coefficient (r) was determined (Joppa *et al.*, 2008).

RESULTS ET DISCUSSIONS

Phytochemical screening

The Phytochemical screening helps to identify the groups of chemicals and revealed some differences in the constituents of the six plants tested (Table 1).

Table. 1: Results of phytochemical screening.

Chemical groupes	X.a.	R.b.	C.z.	L.c	M.i.	U.c.
Alkaloids	-	-	-	+	-	++
Gallic tannins	++	-	-	-	-	++
Catechic tannins	+	-	++	-	-	++
Triterpenoids	-	-	-	-	-	+
Steroids	-	-	-	-	+	-
Cardenolides	-	-	-	-	-	-
Mucilages	+	-	-	+	-	-
Reducing sugar	++	-	++	-	+	++
Quinonic derivatives	-	-	-	-	++	++
Saponins	++	++	-	-	++	-
Flavonoids	-	-	-	-	-	++
Athracenic free	+	-	-	-	-	-
Athracenic c-heterosids	-	-	+	-	-	-
Anthocyanes	-	-	-	-	+	-
Leucoanthocianes	++	+	-	-	-	++
Cyanogenetic glycosides	-	-	-	-	-	-
Essentials oils	+++	-	+	-	+/-	+

+++ : clear presence ; ++ : large presence ; + : present ; - : absent ; +/- : traces X.a. : *Xylopia aethiopica* ; R.b. : *Raphiostylis beninensis* ; C.z. : *Croton zambesicus* ; L.c. : *Lonchocarpus cyanescens* ; M.l. : *Morinda lucida* ; U.c. : *Uvaria chamae* ; Anthr. : Anthracéniques.

The presence of tannins and saponins in the fruit of *Xylopia aethiopica* (Table 1) confirms the work of Yemoa (2008) who has moreover highlighted the presence of alkaloids, triterpenoids and steroids in aqueous extracts of these fruits. The works of Block (2000) showed that the leaves, stems and fruits of *Croton zambesicus* Burch exclusively contain terpenes and steroids, groups of chemicals that have been highlighted during our phytochemical analysis. The phytochemical studies carried out by Okwu and Iroabuchi (2009) on the ethanolic extracts of roots of *Uvaria chamae* P. Beauv revealed the presence of bioactive compounds consisting of flavonoids, alkaloids, tannins, saponins and phenolic compounds. Apart from saponins, all the other groups of compounds were revealed by the current phytochemical screening. Their absence could be justified by the nature of the solvent used and/or their low concentration in the roots of *Uvaria chamae*. The phytochemical analysis carried out by Lasisi *et al.*, (2011), on the ethyl acetate extract of *Raphiostylis beninensis* revealed the presence of alkaloids, flavonoids, saponins and tannins. Similarly, the methanol extracts of *Raphiostylis beninensis* contain alkaloids, flavonoids, saponins and tannins. These previous works confirm the abundant presence of saponins in the stems of studied *Raphiostylis beninensis*. However, the absence of alkaloids, flavonoids and tannins in aqueous extracts could be explained by the nature of the used solvent. In addition, Adjanohoun and Souza (2002) reported that the active compound present in the leaves of *Lonchocarpus cyanescens* was glycyrrhizin which is a steroid. The phytochemical screening is a qualitative chemical analysis based on coloration or precipitation reactions. So, the differences of results could be explained by the lack of specification on the sensitivity and detection limits of testing. These differences also reflect the phenomenon of chemotype often encountered in the plants

Determination of hemolysis inhibitory concentration 50% (CIH_{50})

The results of different optical density measurements were used to calculate the inhibition percentage of hemolysis for each of the extracts as shown in figures 1 to 5 below.

The protective effect on hemolysis by aqueous extract of the stems of *Raphiostylis beninensis* was inversely proportional to the concentration of the extracts (Figure 1). The negative inhibition rate of 5 mg/mL extracts showed their hemolytic effects on erythrocytes AS and SS. These hemolytic effects were due to the presence of saponosides (Bruneton, 2009). In contrast, extracts at 1 mg/mL have a very positive effect on the stabilization of the erythrocyte membrane, particularly of red blood cells AS.

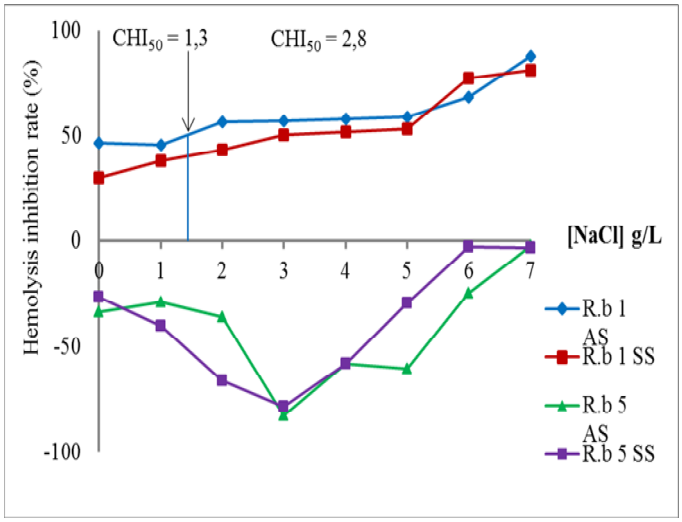

Fig 1: Curves of inhibition of hemolysis of SS and AS erythrocytes by Raphiostylis beninensis extracts; Rb 1: extract at 1 mg/ml Raphiostylis beninensis; Rb 5: extract at 5 mg/ml Raphiostylis beninensis; CIH50: concentration of NaCl corresponding to 50% of hemolysis.

The protective effect of the aqueous extract of *Xylopia aethiopica* on hemolysis was observed in both phenotypes (Figure 2). This effect was highly significant with the extracts at 5 mg/mL and the hemolysis inhibition was higher than 30% whatever the extract concentration and the phenotype.

The extract of the leaves of C*roton zambesicus* has a protective effect on hemolysis in both phenotypes. This effect is more pronounced with the extract at 5 mg/mL, particularly in AS red blood cells they inhibit the haemolysis of more than 50% regardless of the concentration of NaCl. The value of CIH_{50} therefore could not be determined.

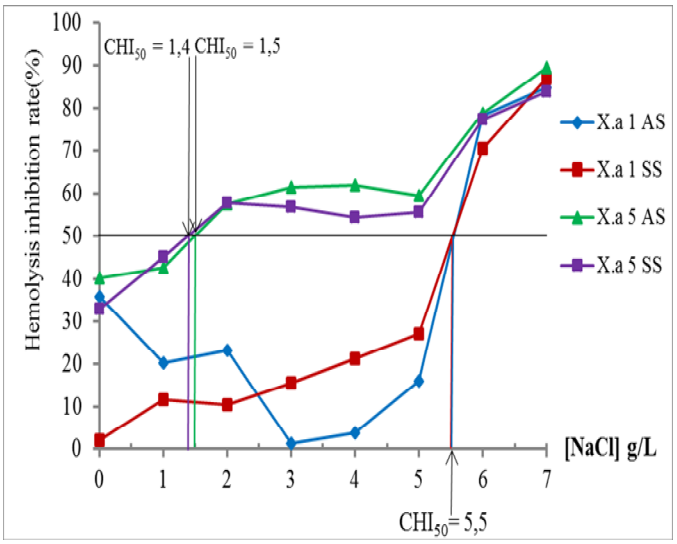

Fig. 2: Curves of inhibition of SS and AS erythrocytes hemolysis by Xylopia aethiopica extracts, Xa 1: extract at 1 mg/mL of Xylopia aethiopica, Xa 5: extract at 5 mg/mL of Xylopia aethiopica ; CIH50: concentration of NaCl corresponding to 50% of hemolysis.

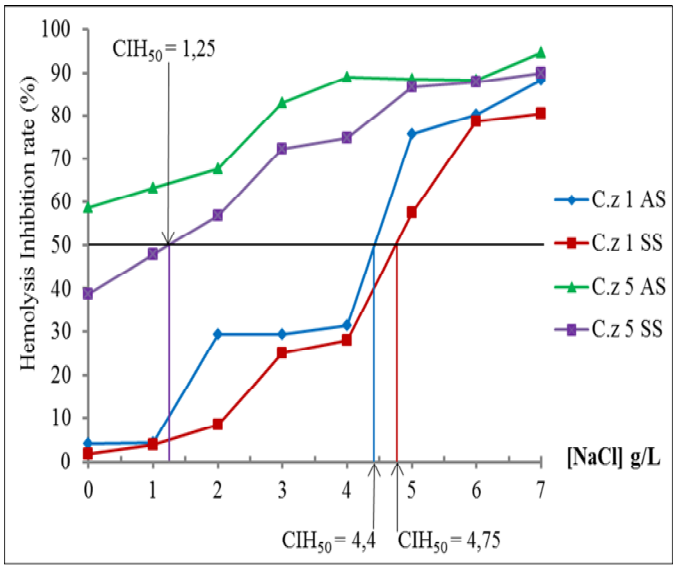

Fig. 3: Curves of inhibition of AS and SS erythrocyte hemolysis by leaf extracts of Croton zambesicus; Cz 1: extract at 1 mg/ml of Croton zambesicus; Cz 5: extract at 5 mg/ml of Croton zambesicus ; CIH50: concentration of NaCl corresponding to 50% of hemolysis.

The extract of the roots of *Uvaria chamae* offers good osmotic resistance to red blood cells with an AS degree of inhibition greater than 27%. As for SS red blood cells, the activity of these extracts was relatively low (figure 4).

Despite the wealth of the roots of *Uvaria chamae* in large group of chemical compounds, its antihémolytique activity is low. This could be a problem since the dose of extract at 5 mg/mL has an inhibitory effect of hemolysis slightly higher than that of the extract to 1 mg/mL.

The curves (Figure 5) are virtually overlapped for the extract to 1 mg/mL. Its effect is the same for both phenotypes. On the contrary, regarding the extract at 5 mg/mL there is a large difference between the profiles of hemolysis inhibition for both phenotypes, this reflects the more protective effect of AS red blood cells by the extracts at 5 mg/mL.

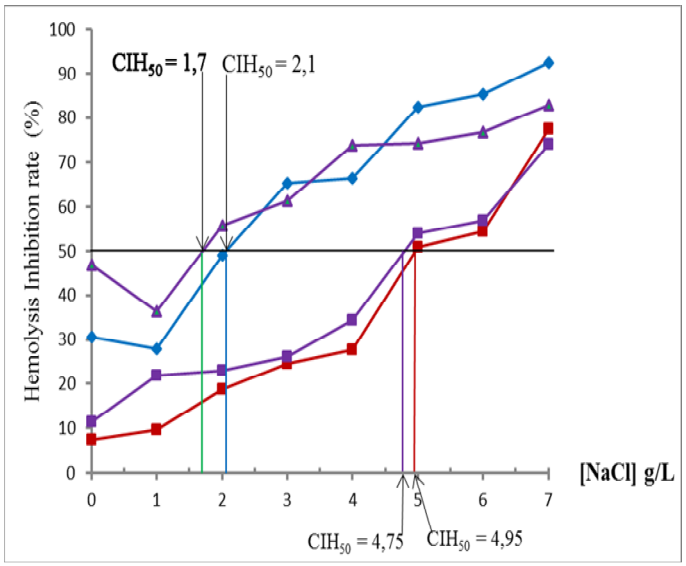

Fig. 4: Curves of inhibition of AS and SS erythrocyte hemolysis by extracts from roots of Uvaria chamae; Uc 1: extract to 1 mg/ml of Uvaria chamae; Uc 5: extract at 5 mg/ml of Uvaria chamae.

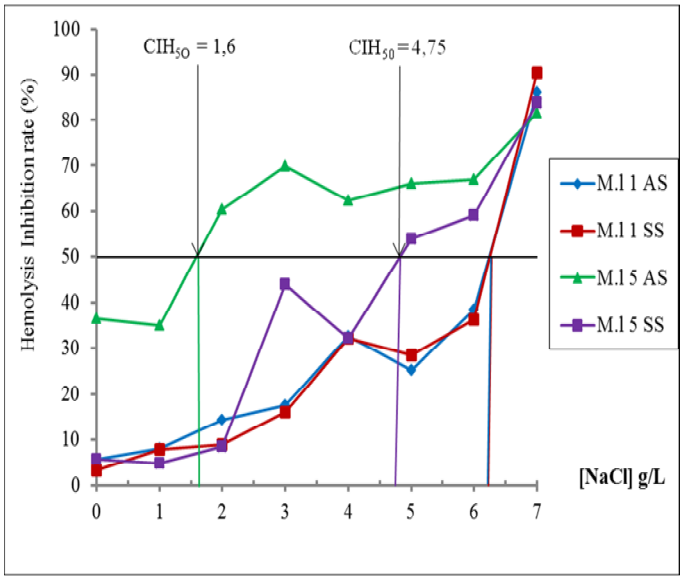

Fig. 5: Curves of inhibition of AS and SS erythrocyte hemolysis by the root extracts of Morinda lucida; MI 1: extract at 1 mg/mL of Morinda lucida, Ml 5: extract at 5 mg/mL of Morinda lucida; CIH50: NaCl concentration of 50% of hemolysis.

The extract of the roots of *Lonchocarpus cyanescens* protects red blood cells against hemolysis in proportion to the concentration of the latter (Figure 6). This plant is commonly used for its dyeing properties due to its rich indigo leaves. However, experiments on the brains of rats by Oluwasunmibare (2010) have shown that aqueous extracts (1 mg/ml) of these leaves have a low inhibitory activity (16.8%) of monoamine oxidase (MAO).

The current results on aqueous extracts of the roots of *Lonchocarpus cyanescens* must be continued to highlight on the one hand, the optimal dose inhibiting hemolysis and on the other hand active molecules present in the root.

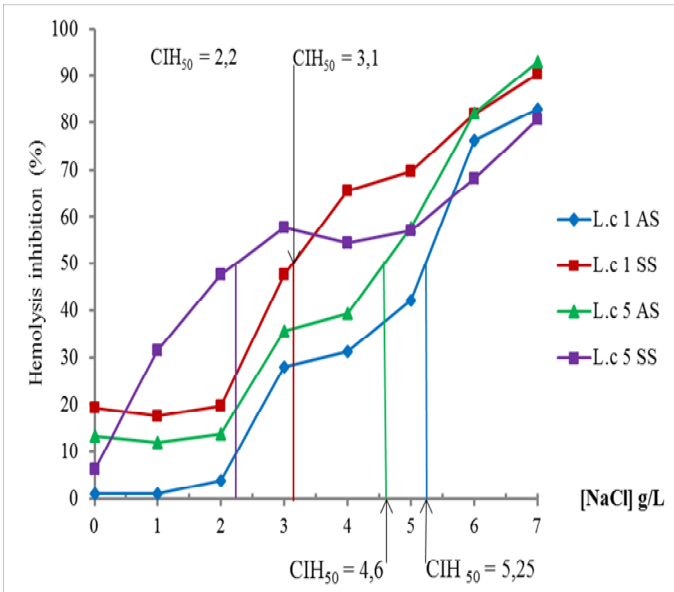

Fig. 6: Curves of inhibition of AS and SS erythrocyte hemolysis by the root extract of Lonchocarpus cyanescens L.c1: extract at 1 mg/ml of Lonchocarpus cyanescens, L.c5: extract at 5 mg/ml of Lonchocarpus cyanescens; CIH50: concentration of NaCl corresponding to 50% of hemolysis.

Potential inhibitory activity of different extracts of hemolysis

The different concentrations of NaCl corresponding to 50% hemolysis inhibition (CIH_{50}), determined from figures 1 to 6, enabled us to determine and compare the potential activity of each of the extracts (Table 2).

Table. 2: Summary of CIH_{50} and comparison of the potential inhibitory hemolytic activity of different extracts.

Traitements		CIH_{50}		Hemolysis stabilisation		Pearson coefficient r
Dose	Extract	AS	SS	AS	SS	
1 mg/mL	X.a	6,5	5,5	+	+	0,86431144
	R.b	**1,3**	**2,8**	**++++**	**+++**	**0,96539255**
	L.c	5,20	3,1	+	++	0,95540696
	U.c	2	4,75	++++	+	0,99161333
	C.z	4,4	4,75	+	+	0,96297556
	M.l	6,2	6,25	+	+	0,95947729
5 mg/mL	**X.a**	**2,55**	**1,3**	**++++**	**++++**	**-0,80627893**
	R.b	Néant	Néant	-	-	0,93070652
	L.c	4,5	2,2	+	+++	0,95540696
	U.c	1,65	4,5	++++	+	-0,9384318
	C.z	**Néant**	**1,25**	******	**++++**	**0,96352346**
	M.l	1,6	4,75	++++	+	0,99708149

-: Hemolysis; +: weak inhibition (CIH_{50}> 4mg/mL) + +: Inhibition moderate (3 <CIH_{50} ≤ 4) + + +: Good inhibition (2 <CIH_{50} ≤ 3) + + + +: Very good inhibition (≤ CIH_{50} 2mg/ml) **: Excellent inhibition (stabilization> 50%);

It is clear from the analysis of this table that the extract of the leaves of *Croton zambesicus* at 5 mg/mL presents the best profile of hemolysis inhibition, followed by the fruits of *Xylopia aethiopica* at 5 mg/mL and extract of *Raphiostylis beninensis* stems at 1 mg/mL. This shows that the contribution of different plants to osmotic erythrocyte resistance is not only dose dependent but also changes depending on the hemoglobin phenotype.

All "r" correlation coefficients calculated tend towards 1 or -1, which allows saying that the extracts have therefore the same effects on the hemolysis of both phenotypes except that the inhibitory effect is generally more significant on the red blood cells AS (Joppa *et al.*, 2008). For the Study of red cell osmotic fragility, these results show that with this protocol, instead of working with the SS blood that is difficult to find, we can use AS blood. Indeed, for red blood cell AS, inhibition is already significant at low concentration for R*aphiostylis beninensis* and *Uvaria chamae* ($CIH_{50} \leq$ 2mg/ml). It would be wise to increase the concentration of plant extract to get the same effect with blood SS.

It must be noted that most studies on antidrepanocytary plants are limited to the evaluation of antisickling properties. However the results obtained with different aqueous extracts at 5 mg / mL and overall are relatively very significant compared to the work of Elekwa (2004). He got under the same conditions, with 19.8 mg/ml aqueous extract of *Zanthoxylum zanthoxyloides*, on AS and SS red blood cells, some CIH_{50} respectively equivalent to 3.75 and 3.20, corresponding to moderate inhibitions of hemolysis according to our classification (Table 2).

CONCLUSION

The study of the stabilizing properties of hemolysis aqueous extracts of *Uvaria chamae*, *Lonchocarpus cyanescens*, *Morinda lucida*, *Xylopia aethiopica* and *Croton zambesicus* and the extract of 1mg/mL *Raphiostylis beninensis* showed that the six plants have an *in-vitro* antihaemolytic effect. They contribute more to erythrocyte osmotic resistance at 5 mg/mL than at 1 mg/mL. In addition, there is a good correlation (|r|> 0.8) between the stabilization of AS red blood cells and the SS ones.

The aqueous extract at 5 mg/mL of *Raphiostylis beninensis* stems causes hemolysis of red blood cells AS and SS. Therefore, the use of this plant in decoction proposed by the traditional healers must be strictly controlled or banned because of its dose-dependent effect.

REFERENCES

Adjanohoun, E., De Souza, S. Guide pratique de Phytothérapie. *Bulletin Régional d'Information du CENPREBAF*. 2002; 4 : 78.

Ayoola G.A., Coker H.A.B., Adesegun S.A., Adepoju-Bello A.A., Obaweya K., Ezennia E.C., Atagbayila T.O. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. *Trop. J. Pharm. Res.* 2008 ; 7(3) 1019-1024.

Block, S., Quetin-Leclerq, J. Fractionnement biocontrôlé d'un extrait de *Croton zambesicius*. Mémoire DEA Pharmacognosie. Université Catholique de Louvain (2000).

Bruneton J. Pharmacognosie, Phytochimie, Plantes Médicinales (4ème édition). Tec et Doc : (2009) 1120.

Bunn H.F. Pathogenesis and treatment of sickle cell disease. *N. Engl. J. Med.* 1997; 337:762-769.

Ekeke G.I. and Shode F.O. Phenilamine is the predominant antisickling agent in Cajanus cajan seed extract. Planta Medica. 1990; 56:41-43.

Elekwa, I., Monanu M.O., Anosike E. O. Effects of aqueous extracts of *Zanthoxylum macrophylla* roots on membrane stability of human erythrocytes of different genotypes. Nigerian Society for Experimental Biology, 2004 ; (7) 7-12.

Elekwa, I., Monanu M.O., Anosike E. O. Effects of aqueous extracts of *Garcinia kola* seeds on membrane stability of HbAA, HbAS and HbSS human erythrocytes. Global J. of Medical Sciences. 2003; 2(2):97-101.

Galacteros, F., La Drépanocytose. Encyclopédie Orphanet (2000).

Houghton P.J., Raman A. Laboratory handbook for the fractionation of natural extracts, Ed Chapman and Hall, New York, (1998) 208.

Iwu M.M. Anti-hepatotoxic constituents of Garcinia kola seeds. Experientia. 1985; 42:699-700.

Joppa K.M., 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. Mad Trop. 2008; 68:251-256.

Lasisi, A. A., Folarin O. M., Dare E. O., Akinloye O. A. and Fisuyi M. O. Phytochemical, antibacterial and cytotoxic evaluation of *Raphiostylis beninensis* [hook f. ex planch] stem bark extracts. International Journal of Pharma and Bio Sciences, 2011; 2 (3): 489-495.

Latoundji, S., Anani L. Ablet E. & Zohoun I. Morbidité et mortalité drépanocytaire au Bénin. *Méd Afr Noire*, 1991 ; 38 : 571-576.

Lehmann, H., Hunstman, R.G. Man's haemoglobins- Revised edition. North Holland Publ. co, Amsterdam, Oxford(1974).

Okwu, D., Iroabuchi, F. Phytochemical Composition and Biological Activities of *Uvaria chamae* and *Clerodendoron splendens*. E-Journal of Chemistry. 2009; 6 (2) 553-560.

Oluwasunmibare, O.S. In-vitro study of the effects of aqueous extracts of *Olax subscorpioidea* and *Lonchocarpus cyanescens* on monoamine oxidase activity from rat brain. Thesis PhD No 06/1044, Department of Biochemistry, University of Agriculture, Ogun state (2010).

Parpart, A.K., Lorenz P.B. Parpart E.R., Gregg J.R. and Chase A.M. The osmotic resistance (fragility) of human red cells. *J. Clin. Invest*. 1947; 26:636-638.

Soforowa E. A. Isolation and characterization of an anti-sickling agent from Fagara zanthoxyloides. Lloydia. 1975; 38: 169-171.

Soforowa E.A. and Isaacs-Sodeye W. A. Reversal of sickling and crenation in erythrocytes by the root extract of Fagara zanthoxyloides. Biochemistry. 1971;10: 2617-2624.

Uphof J.C.T. A dictionary of economic plants. Whelan & Wesley Ltd., Herts (1968) 101.

Wajcman, H., Labie, D. Aspects de la biologie de la drépanocytose. Annuaire Médecine Interne. 1981; 132 (8) 568-594.

Willis J.C. A dictionary of flowering plants (8th ed.). Revised by H.K. Airs-Show. Cambridge University Press, London (1973) 250.

YEMOA, A.L., Gbenou J.D., Johnson R.C., Djego J.G., Sinsou C., Moudachirou M., Quetin-Leclercq J., Bigot A., Portaels F. Identification et étude phytochimique de plantes utilisées dans le traitement traditionnel de l'ulcère de Buruli au BENIN. Ethnopharmacologia 2008 ; 142 :48-55.

ZOHOUN, I.S., Mérault G., Reinette P., Rosa J. Politique de santé et drépanocytose. *Rev. Prat.* 1992 ; 42 : 1873-1877