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# Antisickling and antibacterial activities of *Garcinia punctata* Oliv. (Clusiaceae) and *Tetradenia riparia* (Hochst.) Codd (Lamiaceae) from Democratic Republic of the Congo

Koto-te-Nyiwa Ngbolua[1, 3, *], Pius T. Mpiana[2], Fabrice B. Mwanza[1], Damien S.T. Tshibangu[2], Dorothée D. Tshilanda[2], Masengo C. Ashande[3], Virima Mudogo[2], Robijaona Baholy[4], Pierre Ruphin Fatiany[5]

[1] Department of Biology, Faculty of Science, University of Kinshasa, P.O. Box 190 Kinshasa XI, Democratic Republic of the Congo
[2] Department of Chemistry, Faculty of Science, University of Kinshasa, P.O. Box 190 Kinshasa XI, Democratic Republic of the Congo
[3] Scientific Committee for Research, Conservation and the Development of Biodiversity, Faculty of Science, University of Kinshasa, Democratic Republic of the Congo
[4] Department of Chemical Engineering, Polytechnic High School, P.O. Box 1500, University of Antananarivo, 101-Antananarivo, Madagascar
[5] Malagasy Institute of Applied Research, Avarabohitra –Itaosy lot AVB 77, P. O. Box 3833, 102-Antananarivo Madagascar
*Corresponding author: Prof. Dr. Koto-te-Nyiwa Ngbolua, E-mail: jpngbolua@unikin.ac.cd
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## ABSTRACT

Sickle cell Disease (SCD) is a health public problem in Democratic Republic of the Congo (DRC). The aim the present study was to evaluate both the antisickling and antibacterial activities of *Garcinia punctata* and *Tetradenia riparia* and the modes of antisickling action of organic acids extracted from the two selected plants.Results revealed that the erythrocytes (RBCs) normalization rates were >75% for all plant extracts. While the rates of sickle RBCs hemolysis inhibition were 64 and 57% respectively for organic acids extracted from *G. punctata* and *T. riparia*. Organic acids extract displayed also an increase in osmotic fragility of sickle RBCs, an inhibition of the polymerization of hemoglobin S into tactoids (anti-gelling effect) and an inhibition/reduction of MetHb S formation in aqueous solution. Anthocyanins and organic acids extracts displayed also interesting antibacterial activities. *E coli* ATCC 25 922 was the most sensitive strain towards anthocyanins and organic acids extracts of *G. punctata* and *T. riparia* (MIC = MBC = 31.25 µg/ml).On the other hand, the anthocyanin extracts of these two plant species displayed bacteriostatic effect towards Staphylococcus strains. These bacteria are implicated in septicemia and osteomyelitis in SCD patients. Medicinal plant extracts displaying at the same time antibacterial, antisickling and anti-hemolytic effects could be useful in the management of Sickle cell disease.

**Keyword:** *Sickle cell disease, bacterial infections, anthocyanins, organic acids extracts, Democratic Republic of the Congo*

## INTRODUCTION:

Several tropical diseases cause million deaths in the world, and particularly in Africa. Among these is the sickle cell disease (SCD). It is a genetic disease caused by a base substitution in the gene of human β-globin. This disorder results in the replacement of β6 glutamic acid by valine and reduces the solubility of sickle cell hemoglobin (Hb S) at oxygen low pressure. In hypoxic conditions, the Hb S molecules aggregate and form long crystalline intracellular polymers which induce the sickling of erythrocytes. Each year, about 300,000 children are born in the world with pathological hemoglobin of which 70% are affected by SCD and most of them die before the age of five years when they do not receive regular medical health care [1-3]. Africa remains the most affected continent with a high prevalence in the Western and Central parts. In the Democratic Republic of the Congo (DRC), approximately 2% of the populations are sicklers [4-6]. Most of the proposed therapies for SCD appear to be unsatisfactory. This is the case of bone marrow transplantation which is expensive for African poor population and some featal heamoglobin synthesis stimulants such as hydroxyurea which were reported to be toxic for a long time use [1].

The search for affordable and accessible medicines displaying various modes of action for the management of SCD, mainly from plant resources is a priority agenda in Africa where the disease is endemic. Indeed, medicinal plants are potential source of antisickling agents [7, 8]. Their use in the management of SCD is more affordable for Africa because the continent boasts wide arrange of biological resources. In DRC which is one of the biodiversity hotspots of the world, several evidence based medicine investigations have been conducted on medicinal plant species and interesting antisickling agents were identified and characterized [9-12]. A bio-guided selection of some botanical taxons conducted us to develop a therapeutic complement based plant extracts so called Drepanoalpha® which displayed both *in vitro* and *in vivo* antisickling activities [13, 14]. A new approach in the search of antisickling compounds is known as zoo-pharmacognosy (animal self-medicative behavior) [15, 16]. Indeed, there is a convergence of use between great apes and human ones concerning the use of certain plant species especially those which aid animal in the control of infectious diseases like malaria by inhibiting erythrocytes hemolysis. The strong similarities in plant selection criteria among the African great apes (AGA) in response to diseases attack and the use convergence of some plant species by AGA and humans to treat such illnesses constitutes an evidence of the origin of the African Traditional

Medicine. Thus, our earliest hominid ancestors may have exhibited some similarities in plant selection criteria with both extant apes and modern humans [17].

Since great apes are infected by malaria parasite but they cannot develop malaria disease, we recently hypothesized that great apes plant foods could protect sickle erythrocyte against hemolysis by inhibiting the polymerization of hemoglobin within sickle RBCs because of the overlapping geographic distribution of the two diseases [16, 18-20]. This approach allowed us to select *Garcinia punctata* a plant species consumed by AGA while *Tetradenia riparia* was selected on the basis of it wide traditional use. Indeed, in African Traditional Medicine, the same plant species can treat several diseases at the same time. These plants, despite the fact that they are not directly used in traditional medicine against SCD are still very useful as they could help to relief or reduce complications associated with SCD such as severe bacterial infections which constitute the leading cause of death in SCD children [21]. In the present study, we hypothesized that *G. punctata* and *T. riparia* which are traditionally used for the treatment of various ailments (like malaria and microbial infections respectively) in Africa [22] could contain secondary metabolites acting as antisickling and/or antibacterial agents. This study was therefore designed with the aim of investigating the *in vitro* antisickling and direct antibacterial activities of the antocyanins and organic acids extracted from *G. punctata* and *T. riparia*. The two selected chemical groups were previously reported for their *in vitro* antisickling activity [4-6, 9, 10, 18, 23-25].

## 2. MATERIALS AND METHODS

### *2.1. Plant material collection and identification*

The tested plant materials: leaves (*Tetradenia riparia:* fig. 1a) and stem barks (*Garcina punctata:* fig.1b) used in the present study were collected respectively in Kinshasa city and Equateur Province (Democratic Republic of the Congo) during a field work in 2015 and were authenticated by Mr B.L. Nlandu of the INERA (Institut National d'Etudes et Recherches Agronomiques). Voucher specimens no. H. Breyne 4157 (*G. punctata*) and H. Breyne 3970 (*T. riparia*) are on deposit at the herbarium of the Department of Biology (Faculty of Science, University of Kinshasa, Democratic Republic of the Congo).

**Figure 1a.** ***Tetradenia riparia***

**Figure 1b.** ***Garcinia punctata***

### *2.2. Extraction and chemical screening*

The dried and powdered plant material (10 g) was repeatedly extracted by cold percolation with 95% ethanol (EtOH) and water (100 mL x 2) for 48 hours. Chemical screening was performed on the aqueous and organic extracts to investigate the presence of alkaloids, saponins, total polyphenols, flavonoids, tannins, anthocyanis, leuco-anthocyanins, quinones, terpenes and steroids according to standard protocol [26]. Fractions were filtered and concentrated to dryness under reduced pressure using a rotary evaporator. Extraction of anthocyanins was done using 100 g of dried powdered plant material following an established protocol [2, 9]. Anthocyanins extracts were then defatted by n-hexane and all extracts were stored at +4 °C. Organic acids were extracted according to the protocol of Ouattara et al. [27] with minor modification. Briefly, the powdered stem barks or leaves of tested plants (50 g) were macerated with 100 mL of methanol-$H_2O$ (50/50) and then percolated with 400 mL of the same solvent at room temperature. The extract was concentrated under reduced pressure until 100 mL. The aqueous solution was basified to pH 9 with $Na_2CO_3$ and repeatedly extracted with ether. The aqueous solution was then acidified with 4% acetic acid. The resulting acidic (pH 3) solution was repeatedly extracted by ethyl acetate. The solution were dried over $Na_2SO_4$ and concentrated to give organic acids crude extract.

### 2.3. Biological testing

#### *2.3.1. Antisickling and hemolytic experiments*

- EMMEL test

The samples of blood used to assess the antisickling activity of the selected plant extracts were taken from known SCD patients attending the "Centre de Médecine Mixte et d'Anémie SS" located in Kinshasa, DR Congo. None of the patients had been transfused recently with Hb AA blood and all antisickling experiments were carried out with freshly collected blood. In order to confirm their SS nature, the above-mentioned blood samples were first characterized by Haemoglobin electrophoresis on cellulose acetate gel, as previously reported [4]. They were found to be SS blood and were then stored at +4 °C in a refrigerator. An informed consent was obtained from SCD blood donors and all the research procedures have received the approval of Department of Biology Ethics Committee (Réf. no. CDB/FSC/MMJ/039/MM/2015). An aliquot of Hb S-blood was diluted with 150 mM phosphate buffered saline ($NaH_2PO_4$ 30 mM, $Na_2HPO_4$ 120 mM, NaCl 150 mM) and mixed with an equivalent volume of 2% sodium metabisulfite. A drop from the mixture was spotted on a microscope slide in the presence or absence of anthocyanins or organic acids extracts and covered with a cover slip. Paraffin was applied to seal the edges of the cover completely to exclude air (Hypoxia). The red blood cells (RBCs) were analyzed by a computer assisted image analysis software (Motic Images 2000, version 1.3; Motic China Group Co LTD).

- Hypoxic induced hemolysis assay

RBCs were washed twice in physiological saline (NaCl 0,9 %, 1:5 v/v) by centrifugation at 3000 rpm for 10 min, re-suspended in phosphate buffer (150 mM, pH 7,4) containing 2% sodium metabisulfite and incubated in the absence (control) or presence of organic acids extracts (50 µg/ml in basified NaCl 0,9%) at 37 °C for 60 min. At fixed time points, aliquots of the blood samples were removed and centrifuged at 3000 rpm at ambient temperature for 5 min. The absorbance of the supernatant was

measured at 540 nm and was expressed as the degree of haemolysis. The rate of hemolysis inhibition versus time was calculated as previously reported [5].

- Osmotic fragility test

Fragility of RBCs was determined by placing the cells in graded series of hypotonic saline solutions buffered at pH 7.4 with 150 mM phosphate according to protocol of Mpiana et al. [2]. Concentrations ranged from 0.2% to 0.8% NaCl were made to 10 ml (final volume). A 10 µl of washed sickle RBCs sample were added to 1990 µl of each hypotonic saline solution and immediately mixed by inverting several times. The tubes were allowed to stand for 150 min at room temperature. For the effect of the organic acids extracts, 10 µl of extract (30 mg/mL) were added to 1980 µL of each hypotonic saline solution. A 10 µL of RBCs were added and the mixture treated as described earlier. The number of RBCs not lysed/saline concentration was determined by the use of photonic microscope (OLYMPUS×21) and a hemacytometer (Neubauer's cell). Hemolysis was calculated using the following equation: Number of RBCs after 150 min×100/Number of RBCs inoculated (0 min).

- ITANO test

This test was carried out as previously reported [5]. Briefly, RBCs were washed twice in physiological saline solution (NaCl 0.9%) by centrifugation at 3000 rpm for 10 min, re-suspended in hypotonic medium. After that, the hemolysate of RBCs was centrifuged and an equivalent volume of 2% metabisulfite was added to supernatant. It was then incubated at ambient temperature for 45 min. At fixed time points aliquots (50 µl) of the 2% sodium metabisulfite pre-treated hemolysate were diluted with 500 µl of phosphate buffer (pH 7.5) containing $(NH_4)_2SO_4$ 30%, Saponine 1% and $K_2HPO_4$ 1.2% . 50 µl of organic acids extracts were added to the test sample, mixed and incubated for 10 min. The equivalent volume of phosphate buffered saline (PBS) was added to the control sample instead of the plant extract. At predetermined time intervals, aliquots of test or control samples were removed and centrifuged at 3500 rpm at ambient temperature for 5 min. The absorbance of the supernatant was measured at 700 nm. The solubility of the deoxygenated sickle cell hemoglobin was expressed as the decrease of the optical density at 700 nm.

- Met-hemoglobin reducing test

The effect of organic acids extracts (OAE) on met-haemoglobin formation was carried out as follow: 20 µl of OAE (50 µg/ml) were introduced into separate test tubes. This was followed by the addition of 5 ml of distilled water and 20 µl of whole sickle blood sample. The mixture was allowed to stand for 60 min at room temperature, after which, the absorbance was read at 540 nm (haemoglobin) and 630 nm (met-haemoglobin) using a UV-vis spectrophotometer. The haemoglobin and met-haemoglobin percentages were calculated according to the formula of Davidson and Henry [28].

### *2.3.2. Antibacterial activity*

- **Microbial strains**

The activity of the plant samples was tested toward *Staphylococcus aureus* (*S. aureus* ATCC 25923, MRSA ATCC 1625, MSSA ATCC 5668) and *Escherichia coli* (*E. coli* ATCC 25922) strains. The tested strains were obtained from the American Type Culture Collection (ATCC, Rockville MD, USA).

**Determination of Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

The MIC and MBC were determined by broth micro-dilution method as reported in our previously research work [25, 29, 30]. The inocula of used microorganisms were prepared from 24 hours old broth cultures. The absorbance was read at 600 nm and adjusted with sterile physiological solution (0.9% NaCl) to match that of a 0.5 McFarland standard solution ($10^8$cells/ml). The prepared microbial suspension was diluted 1/100 to achieve $10^6$ CFU/ml. Stock solutions of the plant extracts were prepared in Tween 80 (Fisher chemicals) (3 mg/300 µl) and diluted to 2.7 ml with Mueller Hinton Broth (MHB) (Conda, Madrid, Spain) to achieve a Twee 80 final concentration of 0.1%. This solution was transferred in 96-wells plates (200 µl/well) and two-fold serially diluted with MHB to give final concentrations ranging from 1000 to 3,906 µg/ml.

An aliquot (10 µl) of a $10^6$ CFU/ml overnight culture was added to wells of a sterile 96-well micro-plate titer. The positive control wells contained MHB+ bacteria suspension without plant extract while negative control wells contained MHB only. The MIC was determined as the lowest plant extract concentration at which no growth were observed after 24 hours. MTT (30 µl) in aqueous solution (0.01%) was used to evaluate the micro-organism viability. For minimum bactericidal (MBC) determination, 10 µl was taken from each well of complete inhibition of bacterial growth after incubation and spot inoculated on freshly prepared trypticase soy agar (TSA) plates and incubated for 72 hours at 37 °C. The concentration at which no growth was observed on subculture was determined as the MBC.

### *2.3.3. Statistical analysis*

Triplicate analyses were run for each test and statistical data analyses were processed using Microcal Origin 8.5 Pro package software. The difference between the mean values was tested for statistical significance using Student's paired t-test. A value of $P<0.05$ was considered statistically significant.

## 3. RESULTS AND DISCUSSION

### *3.1. Chemical screening*

The results of chemical screening of *G. punctata* and *T. riparia* are presented in Table 1.

| **Chemical groups** | **Medicinal plant species (used parts)** | |
|---|---|---|
| | *G. punctata* (Stem bark) | *T. riparia* (Leaves) |
| Total polyphenols | + | + |
| Anthocyanins | + | ± |
| Leuco-anthocyanins | + | ± |
| Flavonoids | + | - |
| Tannins | + | + |
| Quinones | + | - |
| Alkaloïds | + | + |
| Saponins | + | + |

(Legend: +: presence; -: absence; ±: trace, n=3 independent determinations)

From the Table 1, it is deduced that the stems bark of *G. puntacta* contain total polyphenols, anthocyanins, leuco-anthocyanins, flavonoids, tannins, quinines, alkaloids and saponins. Although, the leaves of *Tetradenia riparia* contain total polyphenols, tannins, alkaloids and saponins while favonoids and quinones were absent. Secondary metabolites such as anthocyanins and leuco-anthocyanins are in trace state in the leaves of *T. riparia.* The natural products which are present in the two investigated medicinal plants were reported in the literature for their various

pharmacological properties like antimicrobial, antisickling and antioxidant activities thus justifying their use as medicine in African Traditional Medicine [9, 31-33]. The presence of the sickling inhibitory phyto-markers like phenolic compounds likes anthocyanins in these botanical taxons conducted us to investigate their antisickling activity and those of organic acids. This study follows previous observations made by Ngbolua et al. [25] who showed that anthocyanins and organic acids extracts possess antisickling and antibacterial activities. A combination of medicinal plants containing such bioactive compounds could be the best therapeutic option in designing a phytomedicine against SCD.

### *3.2. Antisickling activity*

The figures 2a, b, c and d give the phenotypes of SS blood alone (a) or treated with anthocyanins (b and c) and organic acids (d and e) extracted from *G. punctata* and *T. riparia*.

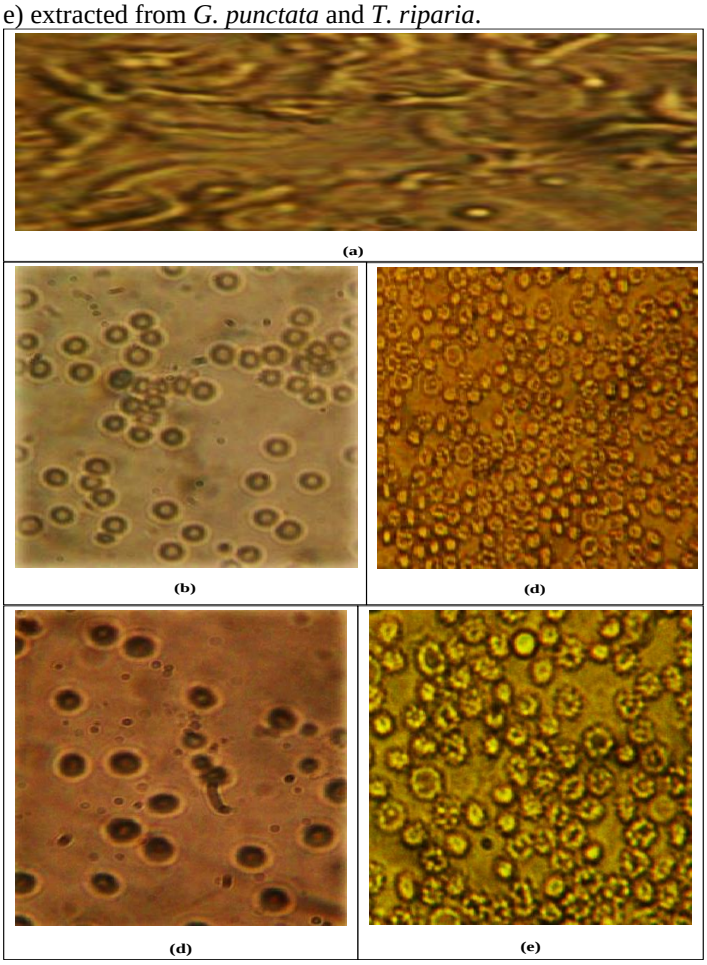


Figure 2: Phenotype of SS red blood cells (SS RBCs) [NaCl 0,9% ; Na2S2O5 2%; X500]: (a): untreated SS RBCs (control); (b) SS RBCs treated with antthocyanins extract of G. punctata (50 µg/ml); (c): SS RBCs treated with anthocyanins extract of T. riparia (50 µg/ml); (d): SS RBCs treated with organic acids extract of G. punctata (50 µg/ml); (e): SS RBCs treated with organic acids extract of T. riparia (50 µg/ml).

Figure 2 shows that in the hypoxic conditions, the microscopic field of the negative control (a) contains sickle-shaped RBCs confirming thus the SS nature of the used blood. Mixed together with anthocyanins and organic acids extracts (b-e), the sickled RBCs are recovered normal-shape in such conditions. At the concentration of 50 µg/ml, the sickled erythrocytes normalization rates were > 75% for all of the tested extracts as revealed in figure 3.

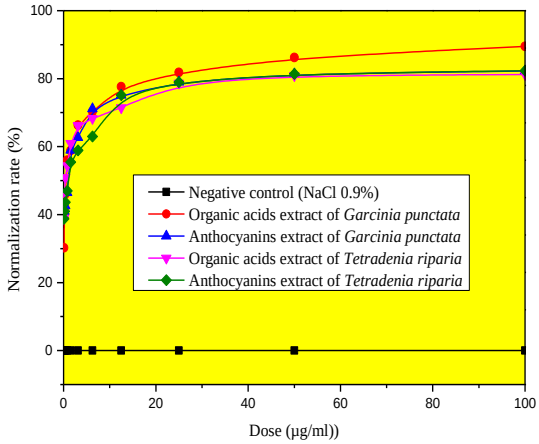


Figure 3: Evolution of the normalization rate of sickle erythrocytes with dose extracts from selected medicinal plant species (NaCl 0.9%; $Na_2S_2O_5$ 2%; X500). %Normalization rate=[number of drepanocytes of untreated SS blood (control)-number of drepanocytes of treated SS blood (sample)/ number of drepanocytes of untreated SS blood] ×100; (n=3 independent experiments).

The results of the present study show that anthocyanins and organic acids could constitute, among others compounds, the antisickling principles of both *Garcinia punctata* and *Tetradenia riparia* confirming thus those already reported results on anthocyanins and organic acids from other plant species used in Congolese folk medicine for the management of SCD [23-25, 29]. As antisickling agents, anthocyanins were reported to have the ability to interact with proteins [2, 5]. The interaction of these compounds with the sickle hemoglobin could chemically compete with the polymerization of this abnormal hemoglobin thus preventing the sickling of sickle erythrocytes and their dehydration.

In addition, anthocyanins (for which intestinal catabolism was reported to give phenolic acids) are also known for their antioxidant properties and could affect the $Fe^{3+}/Fe^{2+}$ higher ratio in sickle cells and the stability of erythrocytes membrane by preventing the oxidation of membranes phospholipids. It is well known that in vacuole of the plants, anthocyanins are acetylated by the organic acids. The latter seem to play a key role in the antisickling activity of anthocyanins as revealed by the present research and previous works and are chemically very stable than anthocyanins [9]. As naturally occurring antisickling secondary metabolites, we evaluated the modes of action of organic acids extracts. Thus, according to [2] and [5], the modes of action of organic acids extracts were investigated by evaluating their effects on the hemolysis of Sickle red blood cells (RBCs) in isotonic condition, the hemoglobin (Hb) S polymerization (Itano test), the erythrocyte membrane stability (osmotic fragility test) and the percentage of methemoglobin S in aqueous solution. The fragility of RBCs was evaluated by placing cells in graded series of hypotonic saline solutions (ranging from 0.2% to 0.8% NaCl) buffered with phosphate at pH 7.4. Extracts were considered as active if the rates of sickle RBCs hemolysis of treated cells is greater than that of the untreated sickle erythrocytes and means that sickle RBCs are rehydrated. The effect of organic acids extracts on *G. punctata* and *T. riparia* on hypoxic induced membrane damage of sickle RBCs were evaluated by comparing % of hemolysis of untreated and treated sickle RBCs in isotonic

medium (NaCl 0,9%) by monitoring the optical density of released Hb S at 540 nm at different times. Figure 4 shows the rate of sickle RBCs hemolysis inhibition in the course of time and reveals that after 60 min of incubation, the rates of inhibition are 64 and 57% respectively for organic acids extracted from *G. punctata* and *T. riparia*. The anti-hemolytic activity is an important feature for an antisickling agent since it has been known so far that chronic anemia is the most frequent SCD symptom.

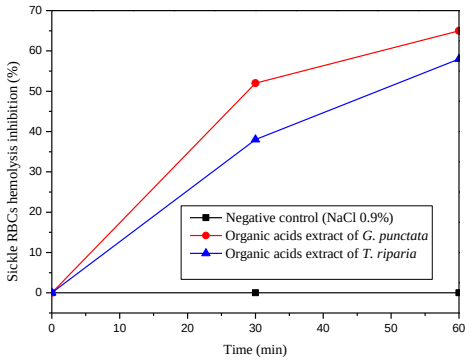


Figure 4. *In vitro* effect of organic acids extracts (50 µg/ml) from *G. punctata* and *T. riparia* on the hemolysis of Sickle red blood cells (RBCs) (NaCl 0.9%; 2% Sodium metabisulfite) (n=3 independent experiments).

The effect of organic acids extracts of *G. punctata* and *T. riparia* on the sickle RBCs membrane stability were evaluated by comparing % of hemolysis of untreated and treated sickle RBCs in graded hypotonic saline concentration (osmotic fragility test: figure 5).

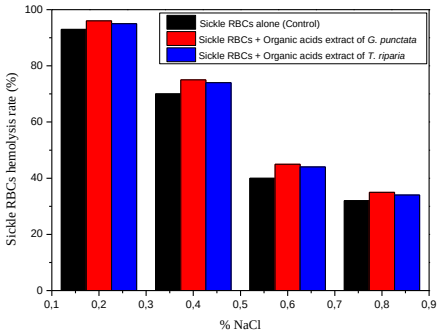


Figure 5. Hemolysis rate (%) of untreated sickle RBCs (control) and treated sickle RBCs (+ 50 µg/ml of OAE) with the saline concentration (n=3 independent experiments).

From figure 5, it can be noted that, the hemolysis decreases with the hypotonic saline concentrations. The figure reveals also that the rate of sickle RBCs hemolysis of treated cells is greater than that of the untreated sickle erythrocytes (control).

This mean that organic acids extract (OAE) causes an increase in osmotic fragility of sickle RBCs ie they enhance the ability of sickle RBCs to take up water without lyses. Such stabilizing effect has been also reported for anthocyanins extracts by our research group [2].

Figure 6 show the inhibitory effect of organic acids extracts from *G. punctata* and *T. riparia* on the aggregation of deoxy-Hb S (The evolution of absorbance of tactoids polymer which is formed in hemoglobin aqueous solution).

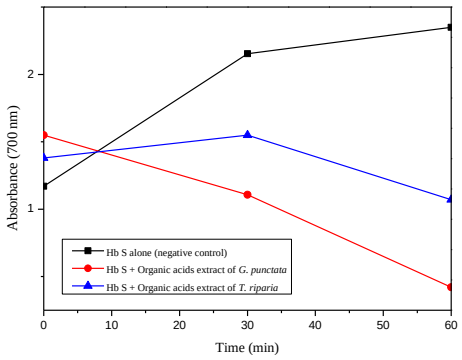


Figure 6. *In vitro* effect of organic acids extracts (50 µg/ml) of *G. punctata* and *T. riparia* on the aggregation of deoxy-Hb S (Phosphate buffered saline 150 mmol/l; pH 7.4; 2% Sodium metabisulfite, wave length: 700 nm), (n=3 independent experiments).

The figure 6 indicates that, in the untreated hemoglobin aqueous solution (control) the absorbance at wave length of 700 nm increase with the time as a result of the loss of hemoglobin solubility in hypoxic conditions created by 2% Sodium metabisulfite. However, after addition of the organic acids extract, we can observe an absorbance decrease at 700 nm in the course of time. These results indicate that organic acids extracted from *G. punctata* and *T. riparia* inhibit the polymerization of hemoglobin S into tactoids (anti-gelling effect).

Figure 7 gives the profile of met-hemoglobin S reducing effects of organic acids extracted from *G. punctata* and *T. riparia.*

Figure 7 shows the decrease of %MetHb S in aqueous solution in the presence of organic acids extracted from *G. punctata* and *T. riparia* indicating the inhibition/reduction of MetHb S formation in aqueous solution. This interesting property of organic acids extracts may play key role in SCD management. Indeed, as reducing agent, such bioactive secondary metabolites could prevent *in vivo* oxidative reactions, often by inhibiting the conversion of hemoglobin into met-hemoglobin (unsuitable form).

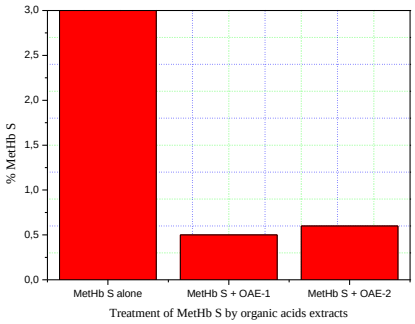


Figure 7. Methemoglobin S reducing effects of organic acids extracted from *G. punctata* and *T. riparia* (50 µg/ml); (n=3 independent experiments).

### *3.3. Antibacterial activity*

Due to the high cost of modern therapy for SCD, plant extracts displaying at the same time antibacterial and antisickling activities could be useful in the management of this hereditary blood disorder. Although, it was reported that biodegradation of anthocyanins by human gut microflora lead into smaller phenolic acids (end-products) which are more stable [34]. The antibacterial activity of anthocyanins and organic acids extracts from the selected botanical taxons against *S. aureus* and *E. coli* strains was evaluated and results are shown in Table 2.

Table 2: Antibacterial effect of selected plant extracts (data from three experiments in quadruplicate)

| Plant extracts | Concentration (µg/ml) | | | | | | | | | MIC/MBC (µg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1000 | 500 | 250 | 125 | 62,5 | 31,25 | 15,625 | 7,813 | 3,906 | |
| *MRSA* ATCC 1625 | | | | | | | | | | |
| OAE-1 | - | - | - | - | - | + | + | + | + | 62,5/62,5 |
| ACE-1 | - | - | - | + | + | + | + | + | + | 250/>1000 |
| OAE-2 | - | - | - | - | - | + | + | + | + | 62,5/62,5 |
| ACE-2 | - | - | - | - | - | + | + | + | + | 62,5/125 |
| *MSSA* ATCC 5668 | | | | | | | | | | |
| OAE-1 | - | - | - | - | + | + | + | + | + | 125/125 |
| ACE-1 | - | - | + | + | + | + | + | + | + | 500/>1000 |
| OAE-2 | - | - | - | - | + | + | + | + | + | 125/125 |
| ACE-2 | - | - | - | - | + | + | + | + | + | 125/>1000 |
| *Escherichia coli* ATCC 25922 | | | | | | | | | | |
| OAE-1 | - | - | - | - | - | - | + | + | + | 31,25/31,25 |
| ACE-1 | - | - | - | - | - | - | + | + | + | 31,25/62,5 |
| OAE-2 | - | - | - | - | - | - | + | + | + | 31,25/31,25 |
| ACE-2 | - | - | - | - | - | - | + | + | + | 31,25/31,25 |
| *Staphylococcus aureus* ATCC 25923 | | | | | | | | | | |
| OAE-1 | - | - | - | - | + | + | + | + | + | 125/125 |
| ACE-1 | - | - | + | + | + | + | + | + | + | 500/>1000 |
| OAE-2 | - | - | - | - | + | + | + | + | + | 125/125 |
| ACE-2 | - | - | - | - | + | + | + | + | + | 125/>1000 |

(Legend: OAE-1: Organic acids extract of *Garcinia punctata;* ACE-1: Anthocyanins extract of *Garcinia punctata*; OAE-2: Organic acids extract of *Tetradenia riparia*; ACE-2: Anthocyanins extract of *Tetradenia riparia*; +: bacterial growth; -: inhibition of bacterial growth; MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin- susceptible *Staphylococcus aureus*); ATCC: American Type Cell Collection), (n=3 independent experiments).

The results from the table 2 showed that anthocyanins and organic acids extracted from *G. punctata* and *T. riparia* had both interesting antibacterial activities. Indeed, the antibacterial activity of a plant extract is considered significant when the MICs are below 100 µg/ml, moderate when $100 \leq MIC \leq 625$ µg/ml and weak when MIC are above 625 µg/ml [35]. Consequently, it is deduced from the table 2 that *E coli* ATCC 25 922 is the most sensitive strain towards anthocyanins and organic acids extracts of *G. punctata* and *T. riparia*. One can observe that the MIC = MBC = 31.25 µg/ml (bactericidal effect).On the other hand, the anthocyanin extracts of these two plant species displayed bacteriostatic effect towards all tested strains of Staphylococcus (*Staphylococcus aureus* ATCC 25923, MRSA ATCC 1625 and MSSA ATCC 5668). The bactericidal activity of the plants extracts on gram negative bacteria is a rare event in pharmaceutical microbiology. This is the first time to report the antisickling activity of *Tetradenia riparia*. Such plant species (as well as *Garcinia punctata*) displaying both antibacterial and antisickling, anti-hemolytic, sickle RBCs membrane stabilizing and met-hemoglobin reducing effects could be useful in the management of this hemoglobinopathy.

## CONCLUSION AND SUGGESTIONS

In the present study, we evaluated the chemical composition of *Garcinia punctata* and *Tetradenia riparia* and the antisickling and antibacterial effects of anthocyanins and organic acids extracted from the above mentioned plant species and suggests that extracts from these plants could be a source of naturally occurring

antisickling and antibacterial agents. This scientific based evidence supports the possibility of using *G. punctata* and *T. riparia* extracts as an affordable medicine for managing SCD and allied diseases in DRC. It is therefore necessary to screen these extracts for their *in vitro* and *in vivo* toxicological effects in order to guarantee their harmlessness.

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