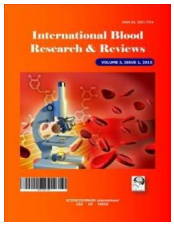

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In vitro Anti-erythrocyte Sickling Effect of Lunularic Acid of Natural Origin

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Authors' contributions

This work was carried out in collaboration between all authors. Authors R. Herintsoa and R. Hajatiana isolated and characterized lunularic acid, author KNN designed and conducted biological experiments; authors KNN, PTM and VM wrote the first draft and authors DSTT and DDT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antisickling activity of lunularic acid.
Study Design: Biological experiment *in vitro* (Emmet test), evaluation of cell morphological parameters such as radius, perimeter and surface.
Place and Duration of Study: University of Kinshasa, Democratic Republic of the Congo, from February 2015 to June 2015.
Methodology: The antisickling activity is carried out *in vitro* in isotonic (NaCl 0.9%) and hypoxic conditions ($Na_2S_2O_5$ 2%) using Sickle red blood cells (RBCs) as model system. The RBCs phenotype were analyzed using a computer assisted image analysis program (Motic Images 2000, version 1.3; Motic China Group Co LTD) and statistical data analyses were processed using Microcal Origin 8.5 Pro package software.

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Results: The biological testing revealed that lunularic acid has antisickling activity (Normalization rate > 95% at 25 µg/ml). This phenolic acid acts by reducing the perimeter of sickle RBCs and by increasing their surface. The treated SS RBCs demonstrated a remarkable similarity to normal blood cells values of morphological parameters.
Conclusion: The naturally occurring lunularic acid could serve as promising lead compound candidate for improving the quality life of SCD patients.

Keywords: Sickle cell disease; nutraceuticals; lunularic acid; antisickling activity.

1. INTRODUCTION

Sickle cell disease (SCD) is a genetic disease characterized by a chronic hemolytic anemia [1]. Strategies to treat this pathology focus on prophylactic measures for alleviating the painful crises and preventing adverse effects. Due to the high cost of SCD management, scientific research in endemic regions has been directed toward tropical flora bio-prospection in order to solve health challenges caused by this hemoglobinopathy as a priority agenda for some African research centers [2]. Recently our research group shows that many medicinal plant species traditionally used in Democratic Republic of the Congo (DRC) to manage SCD had *in vitro* antisickling activity and that this activity is mainly due to anthocyanins [3-8]. These compounds are acylated by phenolic acids in the cell vacuole and may have considerable utility as nutraceuticals in the management of sickle cell disease [2,9]. Indeed, the risk of toxicity or side effect of allopathic drugs has shift more emphasis on the safe use of nutraceuticals or "functional foods". They are defined as non-drug substances produced in an extracted form and administered orally to a patient with the intent of improving the health and well being of humans. Such products may range from isolated nutrients, dietary supplements, and diets to herbal products or phytochemicals such as phenolics [10,11]. Medicinal plants containing phytochemicals like benzoic acid derivatives were reported to exhibit antisickling properties [12,13]. Plant species containing such related compounds could serve as ingredient in formulating a phytomedicine against SCD. Drepanoalpha®, a combination of medicinal plants based food made using the same rationale, is a promising and potent functional food for people suffering from SCD scientifically validated in Democratic Republic of the Congo [14,15].

The present study was performed with the aim of evaluating the antisickling activity of a phenolic acid namely dihydro-stilben carboxylic acid or lunularic acid from *Noronhia divaricata* Perr. (Family Oleaceae). This study is the first report of the antisickling activity of this lead compound.

2. MATERIALS AND METHODS

2.1 Lunularic Acid

Lunularic acid sample was previously isolated from *Noronhia divaricata*, a medicinal plant species endemic to Madagascar by our collaborative research group based in Antananarivo, Republic of Madagascar. This plant is traditionally used to treat malaria in the southern part of the island [16]. The physicochemical and spectroscopic data of this acid are given as follow: NMR^{1}H 200MHz, CD Cl_3) δ (ppm): 2.79-3.00(m, 2H, CH_2 CH_2), 3.21-3.37(m, 2H, CH_2 CH_2), 6.62-6.77(m, 4H, H arom.), 6.98(t, J=8.0Hz, 2H, H aromatic), 7.16(t, J=8.0Hz, 1H, H aromatic). NMR^{13}C 100MHz, Acetone-d_6) δ (ppm): 37.9; 39.2; 112.6; 115.4; 115.8; 129.7; 133.3; 134.4; 145.9; 155.8; 163.2; 173.2. Melting point=199-200°C, Principal absorption bands IR (cm^{-1}): 3360-2350; 1685; 1581; 1471; 1266 [2].

The samples of the isolated compound (lunularic acid) and crude extract of *Noronthia divarica* (ethanol) were kindly furnished by Professor Rafatro Herintsoa of the "Institut Malgache de Recherches Appliquées, Madagascar".

2.2 Biological Experiments

2.2.1 Biological material

Blood samples used to evaluate the antisickling activity of the compounds in this study were taken from known SCD adolescent patients attending the "Centre de Médecine Mixte et d'Anémie *SS*" and "Centre Hospitalier Monkole", both located in Kinshasa area, DRC. None of the patients had been transfused recently with Hb AA blood. 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 Hemoglobin electrophoresis on cellulose acetate gel, as previously reported [1-9]. They were found to be *SS* blood and were then stored at±4ºC in a refrigerator. An informed consent was obtained from all the patients participating in the study.

2.2.2 Antisickling assay

Sickle cell 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 isolate compound/crude extract and covered with a cover slip. Paraffin was applied to seal the edges of the cover completely to exclude air (Hypoxia). Duplicate analyses were run for each drug (crude extract and isolated compound). The RBCs were analyzed using a computer assisted image analysis program (Motic Images 2000, version 1.3; Motic Chine Group Co LTD) and statistical data analyses were processed using Microcal Origin 8.5 Pro package software [17,18].

3. RESULTS AND DISCUSSION

Figs. 1 (a-c) show respectively the micro-photography of SS blood alone in a NaCl 0.9% solution (control, Fig. 1a) and the SS blood incubated with crude extract of *Noronia divaricata* (ND) and lunularic acid isolated from ND (Figs. 1b & c).

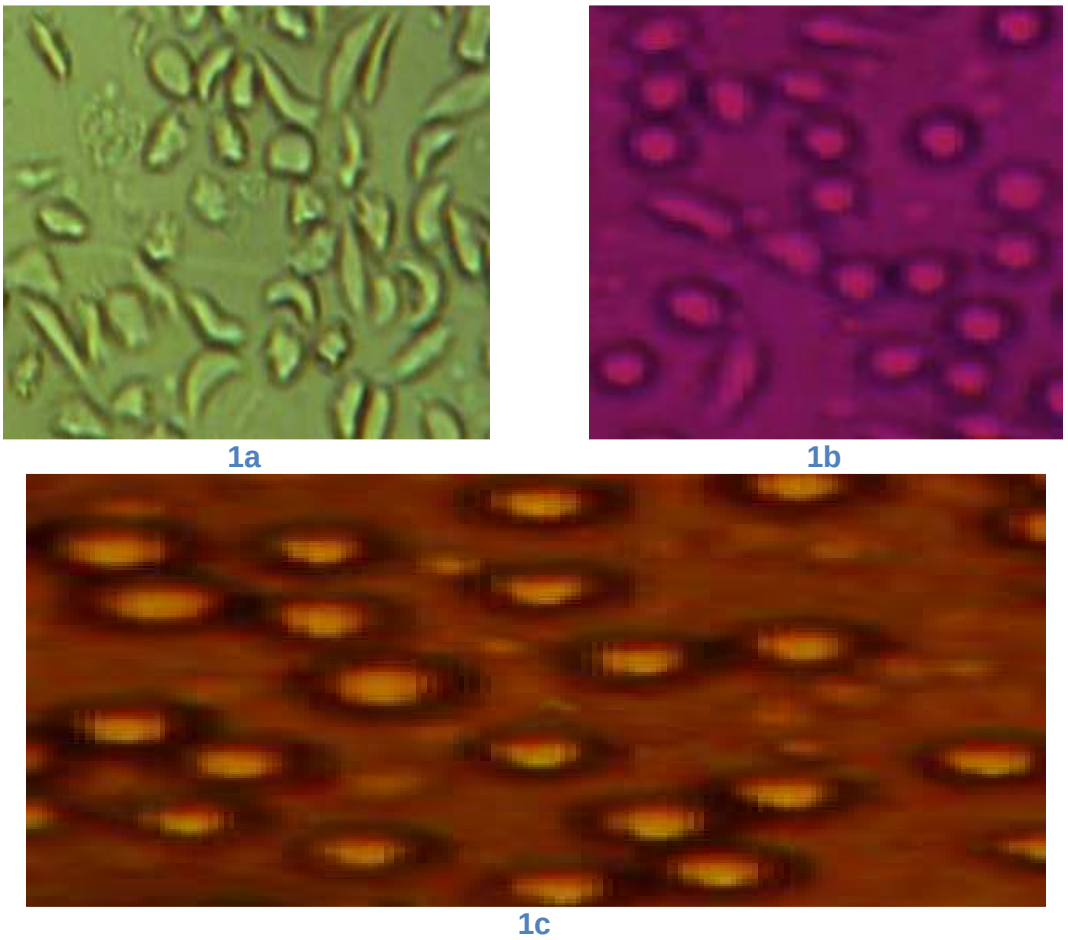

Fig. 1. Microphotography of SS blood alone (control, 1a) and SS blood incubated with tested drugs (1b: ethanolic crude extract: 25 µg/ml; 1c: lunularic acid: 25 µg/ml). (x500) [NaCl 0.9%; $Na_2S_2O_5$ 2%]

Fig. 1 shows that the control (1a) contains in majority sickle-shaped erythrocytes, confirming the SS nature of the blood. Mixed together with crude extract or lunularic acid (Fig. 1 b & c), the majority of erythrocytes are reversed normal-shape. Normalization of sickle red cells is much better with the isolated compound. This indicates that lunularic acid has antisickling activity (Normalization rate > 95%), thus confirming result already reported [9,17].

The treated SS RBCs demonstrated a remarkable similarity to normal blood cells values of morphological parameters as revealed in the Table 1.

Table 1. Average values of radius, perimeter and surface of erythrocytes before and after treatment with lunularic acid (LA) of *Noronhia divaricata*

Measured parameters	Untreated *SS* RBCs	*SS* RBCs (+LA)
Radius (µm)	0.00±0.00	3.35±0.28
Perimeter (µm)	32.16±1.71	19.75±1.12
Surface (μm^2)	20.45±1.54	32.98±1.56

As it can be seen in Table 1, the used computer software package/program did not give the average radius for drepanocytes, as sickled cells of untreated SS blood are not circular. The average radius appeared after treatment of SS RBCs with lunularic acid (25 µg/ml), lead into the re-appearance of the normal and classical biconcave form of RBCs. We can also note that, lunularic acid acts by reducing the perimeter of sickle RBCs and by increasing their surface. Statistical treatment revealed a significant difference between the average values of both the perimeter and the surface of the untreated and treated erythrocytes ($p<0.05$), thus confirming the antisickling effect of lunularic acid. Indeed, in hypoxic conditions ($Na_2S_2O_5$ 2%]), the red blood cells (RBCs) were observed to change from the sickled shape to normal biconcave cells. These values are consistent with previously reported data [18].

The maximal normalization rate or minimal concentration of normalization (MCN) of lunularic acid was determined. Fig. 2 shows the dose dependent antisickling activity of lunularic acid extracted from *Noronhia divaricata* (concentration ranging from 0.781 to 50 µg/ml).

The normalization rate of sickled cells in the presence of lunularic acid increase with the concentration and reached a maximum and constant value at 25 µg/mL (MCN). This corresponds to a normalization rate > 95%. The antisickling activity of this acid is dose dependent. A molecular docking study using Hansch lipophilicity pi (π) and Hammett electronic sigma (σ) constants for predicting the antisickling effect of phenolic acid derivatives revealed that this bioactivity is due to the strong electron donating groups attached on the benzene ring and thus to their anti-oxidative properties [12,13]. As SCD is a chronic and oxidative stress based disease, the use of lunularic acid as nutraceuticals would be a good approach instead of giving pharmaceutical products to sicklers during all their life.

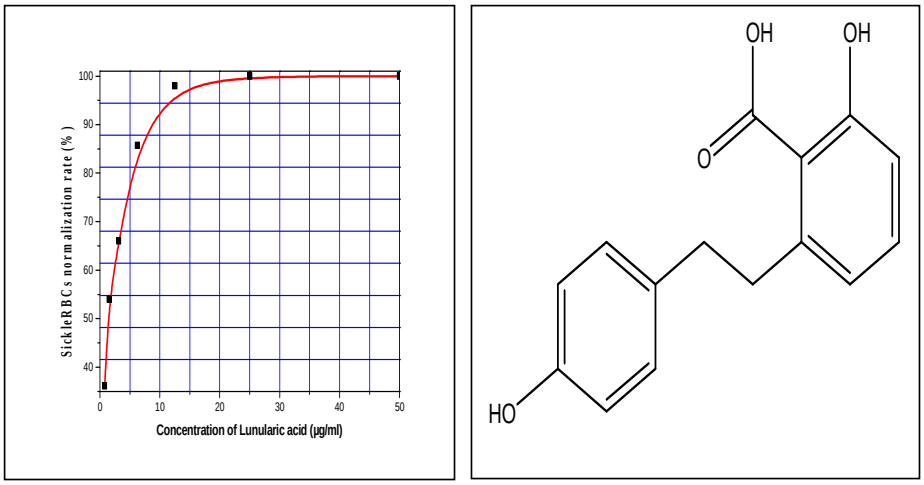

Fig. 2. Evolution of normalization rate of drepanocytes (Sickled RBCs)) in the presence of lunularic acid at different concentrations (NaCl 0.9%; $Na_2S_2O_5$ 2%)

4. CONCLUSION

The present research depicts the antisickling effect of lunularic acid, as lead compound candidate for improving the quality life of sicklers. It is therefore necessary to evaluate the interaction of this phenolic acid with sickle erythrocytes membrane and hemoglobin S in order to elucidate it precise modes of action. It is also necessary to synthesize, formulate and optimize lunularic acid loaded capsules as antisickling drug candidate using an appropriate gel base/vehicle for large scale utilization and clinical trials.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tshilanda DD, Onyamboko DNV, Babady PB, Ngbolua KN, Tshibangu DST, Dibwe EF, Mpiana PT. Anti-sickling Activity of Ursolic Acid Isolated from the Leaves of *Ocimum gratissimum* L. (Lamiaceae). Nat. Prod. Bioprospect; 2015.
DOI: 10.1007/s13659-015-0070-6.
2. Ngbolua KN. Evaluation de l'activité anti-drépanocytaire et antipaludique de quelques taxons végétaux de la République Démocratique du Congo et de Madagascar, Thèse de Doctorat: Université de Kinshasa, République Démocratique du Congo; 2012.
DOI: 10.13140/RG.2.1.3513.3606.
3. Yuma PM, Mpiana PT, Bokota MT, Wakenge IB, Muanishay CL, Gbolo BZ, Mathina DMG, Tshibangu DST, Ngbolua KN. Etude de l'activité anti-falcémiante et de la thermo et photo-dégradation des anthocyanes de *Centella asiatica*, *Thomandersia hensii* et *Maesopsis eminii*. Int. J. Biol. Chem. Sci. 2013;7(5):1892-1901.
4. Mpiana PT, Misakabu FM, Yuma PM, Tshibangu DST, Ngbolua KN, Muanyishay CL, Gbolo BZ, Misengabu NM, Kayembe JS. Antisickling activity and physico-chemical stability of anthocyanin extracts from *Ipomoea batatas* leaves. Journal of Life Medicine. 2014;2(1):25-31.
5. Mpiana PT, Bokota MT, Ndjele MBL, Mudogo V, Tshibangu DST, Ngbolua KN, Atibu EK, Kwembe JTK, Makelele LK. Antisickling activity of three species of Justicia from Kisangani (DR Congo): *Justicia tenella, J. gendarusa* and *J. insularis*. International Journal of Biological and Chemical Sciences. 2010;4(6):1953-1961.
6. Mpiana PT, Tshilanda DD, Onyamboko DV, Mwanangombo DT, Tsalu PV, Misengabu NK, Tshibangu DST, Ngbolua KN. *In vitro* Antisickling Activity of Anthocyanins from *Ocimum canun* (Lamiacea). J. of Advancement in Medical and Life Sciences. 2015;V3I2.
DOI: 10.15297/JALS.V3I2.01.
7. Ngbolua KN, Tshibangu DST, Mpiana PT, Mihigo SO, Mavakala BK, Ashande MC, Muanyishay LC. Anti-sickling and antibacterial activities of some extracts from *Gardenia ternifolia subsp. jovis-tonantis* (Welw.) Verdc. (Rubiaceae) and *Uapaca heudelotii* Baill. (Phyllanthaceae). Journal of Advances in Medical and Pharmaceutical Sciences. 2015;2(1): 10-19.
8. Mpiana PT, Mudogo V, Tshibangu DST, Ngbolua KN, Mangwala PK, Atibu EK, Kakule MK, Makelele LK, Bokota MT. Antisickling activity and thermo-degradation of an anthocyanin fraction from *Ocimum basilicum* L. (LAMIACEAE). Comp. Bio. Nat. Pro. Effects, Safety & Clinical (Pt II). 2010;3:278-287.
9. Ngbolua KN, Bishola TT, Mpiana PT, Mudogo V, Tshibangu DST, Ngombe KN, Tshilanda DD, Baholy R. *In vitro* antisickling and free radical scavenging activities of *Pentaclethra macrophylla* Benth. (Fabaceae). Journal of Advancement in Medical and Life Sciences 2014;V1I2.
DOI: 10.15297/JALS.V1I2.03.
10. Kalra EK. Nutraceuticals--definition and introduction. AAPS Pharm Sci. 2003;5:E25.

11. Gurfateh S, Anu K, Raminderjit K, Hari K. Pleiotropic effects of nutraceuticals. International Journal of Development Research. 2015;5(07):5015-5018.
12. Pierre LL, Moses NM, Peter CM. Structure Activity Relationship (SAR) of some benzoic acid derivatives from plant origin that exhibit anti- sickling properties *in vitro*- (Review). Nat Prod Chem Res. 2015;3: 176.
Doi: 10.4172/2329-6836.1000176.
13. Fasanmade AA, Olaniyi AA, Ab-Yisak W. Mathematical models of antisickling activities of benzoic acid derivatives on red blood cells of sicklers. Afr J Med Med Sci. 1994;23:369-377.
14. Ngbolua KN, Mpiana PT, Tshibangu DST, Mazasa PP, Gbolo BZ, Atibu EK, Kadima JN, Kasali FM. *In vitro* anti-sickling and radical scavenging activities of a poly-herbal formula (Drepanoalpha®) in Sickle cell erythrocyte and acute toxicity study in Wistar albino rats. European Journal of Medicinal Plants. 2014;4(10):1251-1267.
15. Ngbolua KN, Mpiana PT. The possible role of a Congolese polyherbal formula (Drepanoalpha®) as source of epigenetic modulators in sickle cell disease: A hypothesis. J. of Advancement in Medical and Life Sciences. 2014;V2I1.
DOI: 10.15297/JALS.V2I1.02.
16. Rakotoarimanana H, Rafatro H, Jeannoda V, Raoelison G, Robijoana RB, Ratsimamanga-Urveg S. Composé antiplasmodial isolé du Tsilaitra (*Noronhia divaricata* Perr., Oleaceae). Ethno-pharmacologia. 2008;41:71-73.
17. Mpiana PT, Ngbolua KN, Mudogo V, Tshibangu DST, Atibu EK, Tshilanda DD, Misengabu NM. Antisickle erythrocytes haemolysis properties and inhibitory effect of anthocyanins extracts of *Trema orientalis* (ULMACEAE) on the aggregation of human deoxyhemoglobin S *in vitro.* Journal of Medical Sciences. 2011;11(3): 129-137.
18. Mpiana PT, Ngbolua KN, Bokota MT, Kasonga TK, Atibu EK, Mudogo V. *In vitro* effects of anthocyanins extracts from *Justicia secunda* VAHL on the solubility of hemoglobin *s* and membrane stability of sickle erythrocytes, blood transfusion. 2010;8:248-254.

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