



## Drepanoalpha: An Overview on the Quality Control Process and Standardization Feature of an Antisickling Herbal Drug from Democratic Republic of the Congo

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### ABSTRACT

Sickle cell disease (SCD) is an autosomal recessive type of hemoglobin disorder. Nowadays, the main challenge is how to improve the clinical aspects of life quality of the patients with low toxicity and safer therapy from indigenous knowledge. Democratic Republic of the Congo is one of the biodiversity rich countries in the world and can play the lead role in production and commercialization of standardized antisickling formulations like Drepanoalpha<sup>®</sup> worldwide. In this research paper, various methods of phytochemical standardization, such as TLC, HPTLC, HPLC, UV-Vis, IR, MS and their hyphenated techniques for profiling and quantification of markers compounds like phenolics (anthocyanins, flavonoids), phenolic and/or triterpenic acids in Drepanoalpha<sup>®</sup>, a polyherbal medicine produced through a bio-guided based plant selection after eight years of intensive advanced laboratory research, are proposed for quality control and standardization feature.

**Keywords:** Sickle cell disease, Traditional Medicine, Medicinal plants, Drepanoalpha<sup>®</sup>, standardization, Democratic Republic of the Congo

### INTRODUCTION

Sickle Cell Disease (SCD) or sickle cell anaemia (SCA) is due to the polymerization of abnormal haemoglobin S when oxygen tension decreases. This lead to the erythrocytes shape modification and anaemia [1, 2].

Clinical symptoms of SCD are associated to the polymerization of unstable haemoglobin S under hypoxic conditions. These symptoms include erythrocytes shape modification as well as anaemia [3]. Except the bone marrow transplant, SCD has currently no widely available cure. However, the treatment is based on the inhibition of the naturally occurring pathophysiological events of the disease: anemia, vaso-occlusives crises, infections, pain, pulmonary hypertention, etc. [4, 5]. In regions where SCD is endemic, herbal medicines are widely used to relieve the symptoms of the disease. Clinical improvement has been observed in SCD patients undergoing treatment with medicinal plants [6].

Indeed, in Africa, Traditional medicine (TM) remains still the principal recourse for a major part of the population towards the disease for reasons of a socio-cultural, socio-economic and medical purpose [7]. For ten years, our research team has undertaken scientific studies on the validation of biological activity of the medicinal plants used in Traditional Medicine against Sickle cell disease (SCD), with an aim of improving the medical cover of the country. For the promotion of TM, our investigations lead to the formulation of an herbal drug for the management of SCD in DRC. This herbal drug, also called Improved Traditional Medicine (ITM) results from Congolese TM receipts. It has a quantified posology, its effectiveness is scientifically proven and its limits of toxicity are known.

Drepanoalpha<sup>®</sup> constitutes a great opportunity making it possible to obtain adequate therapeutic responses, thus joining to a scientific rigor (safety, effectiveness, quality) an economic and cultural accessibility of the populations.

### STRATEGY OF RESEARCH AND DEVELOPMENT OF THE IMPROVED TRADITIONAL MEDICINES

The strategy of valorization of biological resources for the production of improved traditional medicines (ITM) implies a solid traditional knowledge with the help of traditional practitioners and the herbalists. After a correct identification of the plant based receipt, the following scientific procedures are undertaken:

- ❖ Study of the toxicity limits of the plant extracts to confirm harmlessness;
- ❖ Study of the pharmacological activity in order to confirm the therapeutic effectiveness;
- ❖ Phytochemical study of the plant extracts for a better knowledge of the chemical composition and possible useful active principles for the quality control of the future herbal drugs;
- ❖ Study of the adequate formulation of the herbal drugs;
- ❖ Clinical evaluation of the herbal drugs;
- ❖ Product engineering of the ITM and its marketing.

### ANTISICKLING BIOASSAYS METHODS

When searching for active antisickling plants/extracts/compounds, it is necessary to have relatively simple biological tests available in order to validate the bioactivity

of the chosen plant species. Recent findings in biochemistry and cell biology of SCD have indicated that three main targets are of chemotherapeutic relevance. These include erythrocyte membrane, unstable haemoglobin S and free radicals which are spontaneously produced within red blood cells (RBCs) [8]. It is possible therefore to act directly on each target involved in the pathophysiology of SCD. For example, plant extract which inhibit the sickling of RBCs or the polymerization of deoxy form of haemoglobin S in hypoxic conditions and free radicals scavengers will alleviate all the clinical symptoms of SCD by preventing cell dehydration, cell haemolysis and haemoglobin oxidative damage. In our bio-prospecting program, a RBCs sickling inhibitory test (Emmel test) has been adapted and used successfully to test systematically a library of Congolese plants for their reported antisickling properties. The testing procedure consists of exposing sickled erythrocytes to plant extracts and the number of the normalized RBCs is evaluated microscopically. A 6x zoom CANNON-type digital camera is used to convert the photonic micrograph image into a digital image, which is then digitalized with the help of MOTIC Images 2000 ver. 1.3 software [9-29].

An extract/compound is considered to possess: very high activity (+++) if normalization > 70%; high activity (++) if 50 < normalization < 70%; weak activity (+) if 10 < normalization < 50%; no activity (-) if normalization < 10% [30]. The modes of action are investigated by evaluating the effects of extracts/compounds on inhibition of Hb S polymerization and erythrocyte membrane stability using Itano and osmotic fragility tests respectively [31]. The solubility of the deoxygenated sickle cell hemoglobin (Itano test) is expressed as the increase of the optical density at 540 nm after its treatment with active extracts/compounds. Fragility of RBCs is evaluated by placing cells in graded series of hypotonic saline solutions (from 0.2% to 0.8% NaCl) buffered with phosphate at pH 7.4. The number of RBCs not lysed by saline concentration is determined microscopically. Extract/compound is considered as active if the mean corpuscular fragility (which is the concentration of saline causing 50% haemolysis of the RBCs) value of treated cells is greater than that of the untreated sickle erythrocytes and means that RBCs are rehydrated.

#### ANTISICKLING MOLECULES/COMPOUNDS OF NATURAL ORIGIN FROM DRC FLORA

The major phytochemicals of pharmacological relevance in the management of SCD isolated by our Laboratory include phenolics (lunularic acid) [29, 30], polyphenolics (anthocyanins) [8-14, 18-29, 31], pentacyclic triterpenoids (oleanoic acid, maslinic acid, ursolic acid and betulinic acid) [32, 33] and esters (butyl stearate) [34]. It is observed that some plant species which have not been displayed any antisickling properties are still very useful as they could help to relief pains and reduce inflammation and other complications associated with SCD. However, most of the active principles have antioxidant activity. Thus, the roles of antioxidants in SCD management were investigated *in vitro* using DPPH radical scavenging assay and met-hemoglobin profiling assay. Plant phenolics and polyphenolics displayed interesting antioxidant activities, and may play key role in SCD management. Indeed, as reducing agent, such bioactive secondary metabolites could prevent *in vivo* oxidative reactions, often by scavenging free radicals before they can damage cells. The Antisickling molecules of natural origin from Democratic Republic of the Congo flora are listed in figure 1.

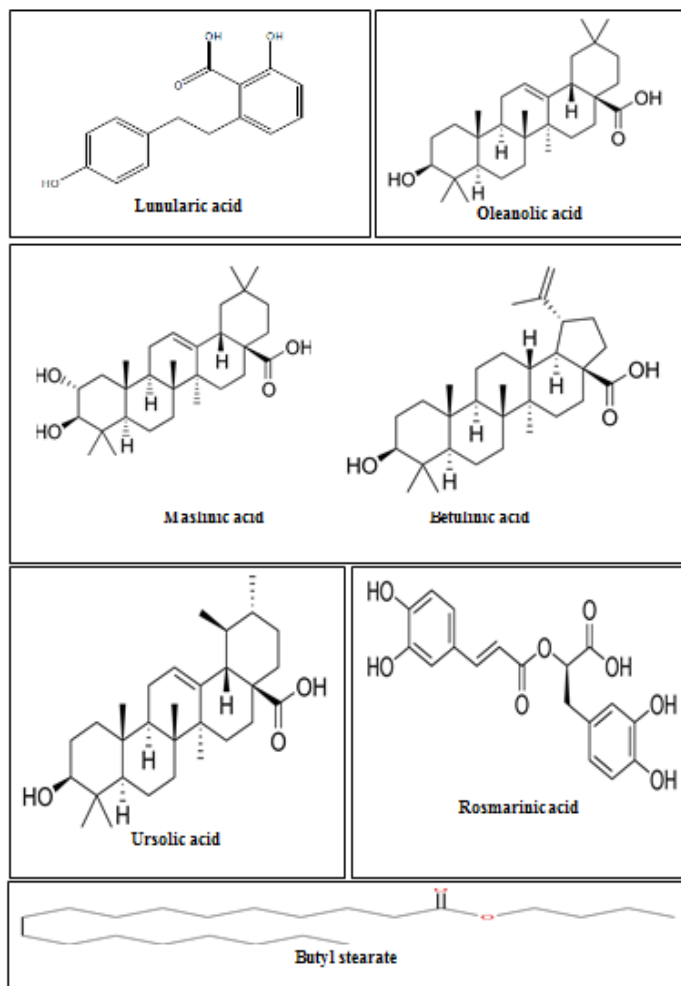


Figure 1: Antisickling molecules of natural origin from Democratic Republic of the Congo flora

#### DREPANOALPHA®

Drepanoalpa® is a polyherbal medicine produced through a bio-guided based plant selection after eight years of intensive advanced laboratory research by the research team of Professor Pius T. Mpiana at the University of Kinshasa, Democratic Republic of the Congo. Drepanoalpa® possess a significant antisickling (normalization rate >80%) and antioxidant activities *in vitro* ( $ED_{50} = 0.604 \pm 0.028 \mu\text{g/mL}$ ) and have the medium lethal dose ( $LD_{50}$ ) higher than 4000 mg/kg (in Wistar rat model). The product has non-toxic effect on immune cells and blood clotting factors. This poly-herbal formulation increases the hemoglobin rate in the rats (500-4000 mg/kg bodyweight) and preserves the histological architecture of the liver cells at the dose of 4000 mg/kg bodyweight (fig. 3b). The aqueous and alcoholic extracts of Drepanoalpa® contain phenolic compounds especially anthocyanins (fig. 2) as the biologically active constituents among others [35].

In human clinical use, this nutraceutical is taken three times daily for the treatment of SCD. Drepanoalpa® reduces the frequency of crises in SCD and improves the general state treated patients. The oral administration of Drepanoalpa® does not induce any signs of toxicity (no side effects). All the treated patients have normal phenotype if compared them to normal individuals and have not made any more crises for more than six months last. Non-recourse to blood transfusion is done for a long period after cessation of medication. The preliminary multicentre clinical trial of

Drepanoalpa<sup>®</sup> indicates that this poly-herbal formulation increases also the hemoglobin rate in both Wistar rat (table 1) and human. However, the heterogeneity of drug response was observed among treated SCD patients [17]. The herbal drug Drepanoalpa<sup>®</sup> contains proteins (16.6 g/100g) and micro-nutrients such as Fe (9.0 mg/100g), Mg (1.4 mg/100g), Ca (4.8 mg/100g) Zn, Mn, K, P and vitamin C (calculated energetic value: 1482 kJ/100g). Iron is required for the production of hemoglobin and has the ability to improve bone marrow functions thus increasing erythropoiesis.

Vitamin C is a very important anti-oxidant which plays an important role in absorption of iron. It also prevents formation of insoluble and unabsorbable iron compounds which in effect oppose the anti-iron activity of phytate and tannin components of the herbal drug.

The presence of natural antioxidant compounds such as anthocyanins and organic acids in Drepanoalpa<sup>®</sup> could protect red blood cell membranes of sicklers from free radicals and oxidative damage of hemoglobin. These compounds could induce hemoglobin fetal synthesis and immune-boosting effect by increasing the leukocyte production (table 2).

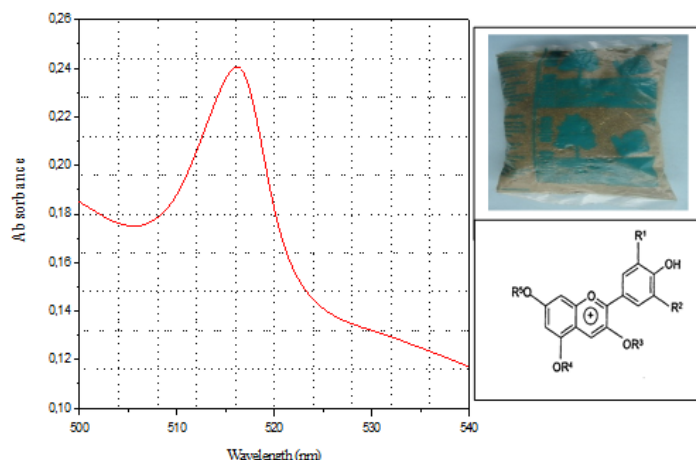


Figure 2: UV-Vis Spectrum of anthocyanin extract from Drepanoalpa<sup>®</sup> (MeOH-HCl 0.1 N 1% mixture).

Tableau 1 : Effect of Drepanoalpa<sup>®</sup> on Wistar albino rat biochemical markers

Biomarkers	Group control	Dose of Drepanoalpa <sup>®</sup> aqueous lyophilized extract (mg/kg)				Sign.
		500	1000	2000	4000	
ALT (UI/L)	21.41±2.82	24.50±1.64	16.15±1.20	13.95±1.20	25.20±1.54	NS
AST (UI/L)	35.00±1.40	30.70±8.62	26.90±1.00	24.90±4.80	16.62±3.31	↓S
ALP (UI/L)	85.00±6.87	60.55±3.26	60.00±2.62	38.00±4.24	48.50±3.77	↓S
Creatinine (mg/dL)	0.56±0.08	0.62±0.04	0.68±0.05	0.65±0.03	0.55±0.02	NS
Urea (mg/dL)	32.25±5.16	28.80±0.84	33.05±3.84	26.10±0.98	30.75±4.45	NS
Total protein (g/dL)	6.15±0.63	6.70±0.42	6.70±0.46	6.45±0.07	6.65±0.07	NS
Albumin (mg/dL)	3.91± 0.12	3.85±0.27	4.14±0.28	3.85±0.77	4.25±0.07	NS
Globulin (mg/dL)	2.25±0.63	2.85±0.35	2.45±0.21	2.61±0.84	2.40±0.14	NS
Albumin/Globulin	1.74±0.19	1.35±0.14	1.64±0.25	1.62±0.82	1.77±0.12	NS

Mean values ±SD (n=3); ↑S significant increase of values; ↓S significant decrease of values (p<0.05)

Tableau 2 : Effect of Drepanoalpa<sup>®</sup> on Wistar albino rat hematological markers

Blood parameters	Group control	Dose of Drepanoalpa <sup>®</sup> aqueous lyophilized extract (mg/kg)				Sign.
		500	1000	2000	4000	
WBC (x 10 <sup>3</sup> /μL)	5.44±0.62	4.51±0.88	4.87±3.31	6.67±3.70	6.85±4.53	↑S
RBC (x 10 <sup>6</sup> /μL)	4.79±2.27	7.86±0.07	8.29±0.13	8.53±0.50	8.39±0.24	↑S
Hb (g/dL)	9.10±3.67	14.00±0.14	14.2±0.42	15.50±1.13	14.65±0.91	↑S
HCT (%)	27.35±2.23	43.70±0.98	45.00±0.56	47.35±2.47	45.75±1.90	↑S
MCV (fL)	57.55±1.76	55.60±1.83	54.90±2.40	55.50±0.42	54.50±0.70	NS
MCH (pg)	19.35±1.48	17.80±0.10	17.30±0.09	18.15±0.21	17.45±0.63	NS
MCHC (g/dL)	33.65±1.62	32.05±1.06	31.55±1.34	32.70±0.70	32.02±0.70	NS
PLT (x 10 <sup>3</sup> /μL)	366.00±145.70	649.00±110.30	799.00±223.40	806.50±12.00	979.50±61.50	↑S
IDR-SD (fL)	30.35±1.48	28.90±0.42	28.25±1.06	26.55±0.35	28.55±0.21	↓S
IDR-CV (%)	15.70±1.13	17.90±0.42	17.35±1.76	17.70±1.27	18.40±0.03	↑S
PDI (fL)	9.85±0.91	9.70±0.14	8.90±0.98	8.60±0.28	8.65±0.21	NS
MPV (fL)	8.80±0.56	8.80±0.28	8.20±0.70	7.85±0.07	8.20±0.14	NS
P-RGC (%)	17.55±4.17	18.20±2.96	12.85±4.31	10.15±1.06	12.90±1.13	↓S
PCT (%)	0.32±0.14	0.56±0.07	0.66±0.24	0.63±0.01	0.79±0.03	↑S

Mean values ±SD (n=3); ↑S significant increase of values; ↓S significant decrease of values (p<0.05)

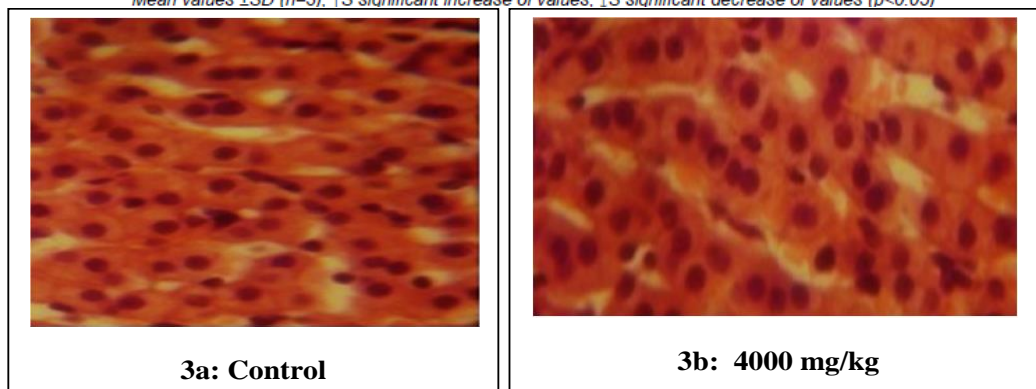


Figure 3: Evidence of no toxic effect of Drepanoalpa<sup>®</sup> on the histological architecture of the liver tissue of Wistar rat (expressed as sinusoidal cells with well-preserved cytoplasm and prominent nucleus)

## AUTHENTICATION AND QUALITY CONTROL METHODS FOR HERBAL MEDICINES

The authentication steps for herbal medicines concern area of the collection, parts of the plant collection, the regional situation, as phyto-morphology, botanical identity, microscopic and histological analysis (characteristic features of cell walls, cell contents, starch grains, calcium oxalate crystals, hairs, fibers, vessels etc). Several studies of the histological parameters are list of palisade ratio, vein islet number, vein termination, stomatal number, stomatal index, trichomes, stomata, quantitative microscopy, taxonomic identity, foreign matter. Loss on drying, swelling index, foaming index, ash values and extractive values, Chromatographic and spectroscopic evaluation (fingerprints pattern). The authentication includes also determination of heavy metals, pesticide residues, microbial contamination and radioactive contamination [36, 37].

### CHEMICAL FINGERPRINTS

**Chromatographic fingerprints:** TLC (Thin layer chromatography)/HPTLC (High performance thin layer chromatography), HPLC (High performance liquid chromatography), GC (Gas chromatography), CE (Capillary electrophoresis).

**Spectral fingerprints:** UV, IR, MS, X-ray and their hyphenated techniques.

### BIOLOGICAL FINGERPRINTS

These techniques refer mainly to genomics fingerprints. Since genetic composition is unique for each individual, DNA methods for herbal medicines identification are less affected by age, physiological conditions, environmental factors, harvest, storage and processing methods.

## HERBAL MEDICINE (LIKE DREPANOALPHA®) STANDARDIZATION

### DEFINITION

Standardization involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations.

When the active principles are unknown, marker substances should be established for analytical purposes and standardization. Marker substances are chemically defined constituents of an herbal drug that are important for the quality of the finished product. Ideally, the chemical markers chosen would also be the compounds that are responsible for the pharmacological effects in the body. There are two types of standardization. In the first category, “true” standardization, a definite phytochemical or group of constituents is known to have activity. The other type of standardization is based on the guarantee of the manufacturers for the presence of a certain percentage of marker compounds which are not indicators of therapeutic activity or quality of the herb [37, 38].

### STANDARDIZATION STEPS

**Macro and microscopic examination:** For Identification of right variety and search of adulterants.

**Foreign organic matter:** This involves removal of matter other than source plant to get the drug in pure form.

**Ash values:** These are criteria to judge the identity and purity of crude drug – Total ash, sulphated ash, water soluble ash and acid insoluble ash etc.

**Moisture content:** Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture suggests better stability against degradation of product.

**Extractive values:** These are indicative weights of the extractable chemical constituents of crude drug under different solvents environment.

**Crude fiber:** This helps to determine the woody material component, and it is a criterion for judging purity.

**Qualitative chemical evaluation:** This covers identification and characterization of crude drug with respect to phytochemical constituent. It employs different analytical technique to detect and isolate the active constituents. Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification, constituents of pharmaceutical importance.

**Chromatographic examination:** Include identification of crude drug based on the use of major chemical constituents as markers.

**Quantitative chemical evaluation:** To estimate the amount of the major classes of constituents.

**Toxicological studies:** This helps to determine the pesticide residues, potentially toxic elements, safety studies in animals like LD<sub>50</sub> and Microbial assay to establish the absence or presence of potentially harmful microorganisms.

A schematic representation of herbal drug standardization is given in figure 3.

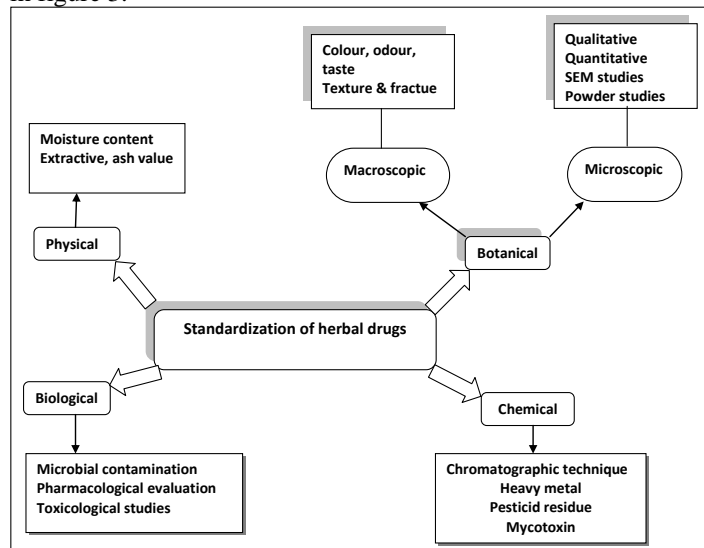


Figure 3. Schematic representation of herbal drug standardization [38]

## FACTORS AFFECTING THE QUALITY OF HERBAL MEDICINE

Different batches of the same herbal medicine may differ in quality due to a number of factors such as:

- ❖ Inter- or intra-species variation: The variation in constituents is mostly genetically controlled and may be related to the country of origin.
- ❖ Environmental factors: The quality of an herbal ingredient can be affected by environmental factor like climate, altitude and other conditions under which it was cultivated.
- ❖ Time of harvesting: For some herbs the optimum time of harvesting should be specified as it is known that the concentrations of constituents in a plant can vary during the growing cycle or even during the course of a day.
- ❖ Plant part used: Active constituents usually vary between plant parts and it is not uncommon for an herbal ingredient to be adulterated with parts of the plant not normally utilized.

- ❖ Post-harvesting processing treatments can greatly affect the quality of an herbal ingredient. Inappropriate storage after harvesting can result in microbial contamination, and processes such as drying may result in a loss of thermo-labile active constituents like anthocyanins [36].

## CONCLUSION

Democratic Republic of the Congo is one of the biodiversity rich countries in the world and can play the lead role in production of standardized antisickling formulations like Drepanoalpha<sup>®</sup>. It is therefore necessary that this herbal drug can be evaluated and analyzed by modern techniques of standardization (TLC, HPTLC, HPLC, UV-Vis, IR, MS and their hyphenated techniques) using phenolics (anthocyanins, flavonoids), phenolic and/or terpenoid acids as markers of antisickling activity.

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## REFERENCES

- O.F. Kunle, H.O. Egharevba, 2013. Chemical constituents and biological activity of medicinal plants used for the management of sickle cell disease - A review. *Journal of Medicinal Plants Research* 7(48): 3452-3476.
- P.M. Yuma, P.T. Mpiana, M.T. Bokota, I.B. Wakenge, C.L. Muanishay, B.Z. Gbolo, G.M. Di Mathina, D.S.T. Tshibangu, K.N. Ngbolua (2013). Etude de l'activité anti-falcémiant et de la thermo et photo-dégradation des anthocyanes de *Centella asiatica*, *Thomandersia hensii* et *Maesopsis eminii*. *Int. J. Biol. Chem. Sci.* 7(5): 1892-1901.
- J. Okpuzor, O. Adebesein, H. Ogbunugafor, I. Qmadi, 2008. The potential of medicinal plants in sickle cell disease control: a review. *International Journal of Biomedical and Health Sciences* 4(2):47-55.
- P.T.Mpiana, D.S.T. Tshibangu, O.M. Shetonde, K.N.Ngbolua, 2007. *In vitro* antidrepanocytary activity (anti-sickle cell anaemia) of some Congolese plants. *Phytomedicine* 14: 192-195.
- J. Villagra, S. Shiva, L.A. Hunter, R.F. Machado, M.T. Gladwin, G.J. Kato, 2007. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. *Blood* 110: 2166-2172.
- K.N. Ngbolua, P.T. Mpiana, D.S.T.Tshibangu, Z.B. Gbolo, 2015. Bioactivity of Medicinal Plants Traditionally Used for the Management of Sickle cell disease in Democratic Republic of the Congo: State of the Art and Future Directions. In: *Sickle Cell Disease: Genetics, Management and Prognosis*. NOVA PUBLISHERS, New York, USA, pp: 79-94.
- P.R. Fatiany, R. Baholy, E. Randrianarivo, A. Raharisolalao, M.T. Martin, K.N. Ngbolua, 2014. Isolation and structural elucidation of cytotoxic compounds from *Diospyros quercina* (Baill.) endemic to Madagascar. *Asian Pacific Journal of Tropical Biomedicine* 4(3): 169-175.

- P.T. Mpiana, K.N. Ngbolua, V. Mudogo, D.S.T. Tshibangu, E.K. Atibu, D.D. Tshilanda, N.M. Misengabu, 2011. 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* 11 (3): 129-137.
- P.T. Mpiana, F.M. Misakabu, J.M. Kitadi, K.N. Ngbolua, D.S.T. Tshibangu, B.K. Lombe, P.V. Tsalu, E.K. Atibu, B.Z. Gbolo, C.L.Muanishay, 2014. Antisickling activity and physico-chemical stability of anthocyanin extracts from *Hypoxis angustifolia* Lam. (Hypoxidaceae) bulbs. M. Noboru (Ed.), in: *Anthocyanins: Occurrence, Structure, Biosynthesis and Health benefits Based on their Evidences of Phytochemicals in Vegetables and Fruits*, NOVA Science Publishers, Inc., New York, USA, Vol. 2, pp: 97-113.
- P.T. Mpiana, F.M. Misakabu, P.M. Yuma, D.S.T. Tshibangu, K.N. Ngbolua, C.L. Muanyishay, B.Z. Gbolo, N.M. Misengabu, J.S. Kayembe, 2014. Antisickling activity and physico-chemical stability of anthocyanin extracts from *Ipomoea batatas* leaves. *Journal of Life Medicine* 2(1): 25-31.
- P.T. Mpiana, F.M. Misakabu, D.S.T. Tshibangu, K.N. Ngbolua, D.T. Mwanangombo, 2014. Antisickling activity and membrane stabilization effect of anthocyanins extracts from *Adansonia digitata* L. bark on sickle blood cells. *International Blood Research and Reviews* 2(5): 198-212.
- P.T. Mpiana, M.T. Bokota, J.P. Mbula, K.N. Ngbolua, D.S.T. Tshibangu, E.K. Atibu, J.T.K. Kwembe, M.K. Kakule, 2013. Effet of Anthocyanins Extracts from *Justicia matammensis* and *Justicia laxa* on Sickle cells. In: *Anthocyanins, Structure, Biosynthesis and Health benefits (Series: Biochemistry Research Trends, Nutritional and Diet Research Progress)*; Noboru Motohashi (ed.), NOVA PUBLISHERS, New York, USA, pp. 111-124.
- P.T. Mpiana, B.L. Kimbadi, A.M. Ombeni, K.N. Ngbolua, D.S.T. Tshibangu, D.D. Tshilanda, N.M. Misengabu, C.L. Muanyishay, S.K. Muyisa, 2013. *In vitro* inhibitory effects and anti-sickle erythrocytes haemolysis of *Dicliptera colorata* C.B. Clarke, *Euphorbia hirta* L. and *Sorghum bicolor* (L.) Moench. *Open Journal of Blood Diseases* 3: 43-48.
- P.T. Mpiana, E.N. Dianzenza, K.N. Ngbolua, D.S.T. Tshibangu, B.M. Mbala, O.M. Shetonde, E.K. Atibu, M.K. Kakule, M.T. Bokota, 2012. Antisickling properties, thermal and photochemical degradations of anthocyanins extract from *Annona senegalensis* (Annonaceae), *Int. J. Biol. Chem. Sci.* 6(5): 2241-2251.
- G.E. Ekutsu, K.N. Ngbolua, M.B. Bolaa, P.T. Mpiana, B.P. Ngoy, A.C. Masengo, G.N. Bongo, 2016. Enquête sur la pharmacopée des bonobos (*Pan paniscus*, Primates) dans un foyer endémique et Mise en évidence de l'activité anti-drépanocytaire chez un taxon végétal (*Treculia africana* Decne ex Trécul, Moraceae) testé *in vitro*. *International Journal of Innovation and Applied Studies* 14 (2): 315-326.
- K.N. Ngbolua, D.S.T. Tshibangu, P.T. Mpiana, S.O. Mihigo, B.K. Mavakala, M.C. Ashande, L.C. Muanyishay, 2015. 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 2(1): 10-19.
17. K.N. Ngbolua, P.T. Mpiana. The Possible Role of a Congolese polyherbal formula (Drepanoalpha<sup>®</sup>) as source of Epigenetic Modulators in Sickle Cell Disease: A Hypothesis. J. of Advancement in Medical and Life Sciences. V2I1. DOI: 10.15297/JALS.V2I1.02, 2014.
  18. P.T. Mpiana, V. Mudogo, K.N. Ngbolua, D.S.T. Tshibangu, E.K. Atibu, 2019. *In vitro* antisickling activity of anthocyanins extracts from *Morinda lucida* Benth (RUBIACEAE) in Medicinal Plants: Phytochemistry, Pharmacology and Therapeutics, VK Gupta, GD Singh, Surjeet Singh and A Kaul (Eds): DAYA PUBLISHING HOUSE, NEW DELHI, Vol. 1, pp. 330-337.
  19. P.T. Mpiana, V. Mudogo, K.N. Ngbolua, D.S.T. Tshibangu, P.K. Mangwala, E.K. Atibu, M.K. Kakule, L.K. Makelele, M.T. Bokota, 2010. Antisickling Activity and Thermodegradation of an Anthocyanin fraction from *Ocimum basilicum* L. (LAMIACEAE). Comp. Bio. Nat. Pro. Vol. 3. Effects, Safety & Clinical (Pt II), pp. 278-287.
  20. P.T. Mpiana, V. Mudogo, Y.F. Kabangu, D.S.T. Tshibangu, K.N. Ngbolua, E.K. Atibu, K.P. Mangwala, M.B. Mbala, L.K. Makelele, M.T. Bokota, 2009. Antisickling Activity and Thermostability of Anthocyanins Extract from a Congolese plant, *Hymenocardia acida* Tul. (Hymenocardiaceae), Int. J. Pharmacol. 5 (1): 65-70.
  21. P.T. Mpiana, E.K. Balangayi, A.B. Kanangila, E.M. Kalonda, K.N. Ngbolua, D.S.T. Tshibangu, E.K. Atibu, J.B.S. Lumbu, 2009. Activité anti-drépanocytaire et thermo-dégradation des anthocyanes extraits de *Sterculia quinqueloba* et *Ficus capensis*, Int. J. Biol. & Chem. Sci. 3 (3): 551-560.
  22. P.T. Mpiana, V. Mudogo, K.N. Ngbolua, D.S.T. Tshibangu, E.K. Atibu, E.K. Kitwa, A.B. Kanangila, 2009. *In vitro* antisickling activity of anthocyanins extracts of *Vigna unguiculata* (L.) walp., In Recent progress in Medicinal Plants: Chemistry and Medicinal Value, JN Govil, VK Singh (Eds): DAYA PUBLISHING HOUSE, NEW DELHI, Vol.25, pp. 91-98.
  23. P.T. Mpiana, V. Mudogo, L. Nyamangombe, M.K. Kakule, K.N. Ngbolua, E.K. Atibu, M.B. Mbala, A.K. Mbongo, J.N. Ntumba, 2009. Antisickling Activity and Photodegradation Effect of Anthocyanins Extracts from *Alchornea cordifolia* (SCHUMACH & Thonn.) and *Crotalaria retusa* L. Ann. Afr. Med. 2 (4): 240-245.
  24. P.T. Mpiana, V. Mudogo, D.S.T. Tshibangu, K.N. Ngbolua, E.K. Atibu, E.K. Kitwa, A.B. Kanangila, L.K. Makelele, 2009. Activité antifalcémiant et thermodégradation d'une fraction d'anthocyanes extraits de *Zizyphus mucronata*, Ann. Afr. Med. 2 (2): 91-97.
  25. P.T. Mpiana, V. Mudogo, D.S.T. Tshibangu, K.N. Ngbolua, D.D. Tshilanda, E.K. Atibu, 2009. Antisickling activity of anthocyanins extract of *Jatropha curcas* L. In Recent progress in Medicinal Plants: Chemistry and Medicinal Value, JN Govil, VK Singh (Eds): DAYA PUBLISHING HOUSE, NEW DELHI, Vol.25, pp. 104-108.
  26. P.T. Mpiana, K.N. Ngbolua, D.S.T. Tshibangu, D.T. Mwanangombo, P.V. Tsalu, 2015. Antisickling and radical scavenging activities of anthocyanin extracts from the leaves of *Gardenia ternifolia* subsp. *jovis-tonantis* (Welw.) Verdc. (Rubiaceae). In: Sickle Cell Disease: Genetics, Management and Prognosis. NOVA PUBLISHERS, New York, USA, pp: 61-77.
  27. P.T. Mpiana, V. Mudogo, K.N. Ngbolua, D.S.T. Tshibangu, O.M. Shetonde, B.M. Mbala, 2007. *In vitro* Antisickling Activity of Anthocyanins from *Ocimum basilicum* L. (Lamiaceae). Int. J. Pharmacol. 3 (4): 371-374.
  28. P.T. Mpiana, V. Mudogo, D.S.T. Tshibangu, K.N. Ngbolua, O.M. Shetonde, P.K. Mangwala, B.K. Mavakala, 2007. *In vitro* Antisickling Activity of Anthocyanins Extracts of a Congolese Plant: *Alchornea cordifolia* M.Arg. J. Med. Sci.7 (7): 1182-1186.
  29. K.N. Ngbolua, V. Mudogo, P.T. Mpiana, M.J. Malekani, H. Rafatro, R.S. Urverg, L. Takoy, H. Rakotoarimana, D.S.T. Tshibangu, 2013. Evaluation de l'activité anti-drépanocytaire et antipaludique de quelques taxons végétaux de la République démocratique du Congo et de Madagascar. Ethnopharmacologia 50: 19-24.
  30. K.N. Ngbolua, H. Rafatro, H. Rakotoarimana, V. Mudogo, P.T. Mpiana, D.S.T. Tshibangu, D.D. Tshilanda, 2015. *In vitro* anti-erythrocyte sickling effect of lunularic acid of natural origin. International Blood Research & Reviews 4(3):1-6.
  31. P.T. Mpiana, V. Mudogo, D.S.T. Tshibangu, E.K. Kitwa, A.B. Kanangila, J.B.S. Lumbu, K.N. Ngbolua, E.K. Atibu, M.K. Kakule, 2008. Antisickling Activity of Anthocyanins from *Bombax pentadrum*, *Ficus capensis*, *Zizyphus mucronata*: Photo degradation effect. J. Ethnopharmacol. 120: 413-418.
  32. D.S.T. Tshibangu, F.O. Shode, N. Koorbanally, V. Mudogo, P.T. Mpiana, K.N. Ngbolua, 2011. Antisickling triterpenoids from *Callistemon viminalis*, *Meulaleuca bracteata* var. *Revolution Gold* *Syzygium guineense* and *Syzygium cordatum*. The 14th NAPRECA Symposium and AAMPS Ethnoverterinary Medicine Symposium 8<sup>th</sup>-12<sup>th</sup> August. Internatiuonal Centre For Insect Physiology and Ecology (ICIPE) : Kasarani, Nairobi, Kenya., pp.296-300.
  33. D.D. Tshilanda, D.N.V. Onyamboko, P.T. Mpiana, K.N. Ngbolua, D.S.T. Tshibangu, M.B. Mbala, K.M. Bokolo, K.M. Taba, K.T. Kasonga, 2014. Antisickling activity of butyl stearate isolated from *Ocimum basilicum* (Lamiaceae). Asian Pacific Journal of Tropical Biomedicine 4 (5): 393-398.
  34. D.D. Tshilanda, D.N.V. Onyamboko, P.B. Babady, K.N. Ngbolua, D.S.T. Tshibangu, E.F. Dibwe, P.T. Mpiana, 2015. Anti-sickling Activity of Ursolic Acid Isolated from the Leaves of *Ocimum gratissimum* L. (Lamiaceae). Nat. Prod. Bioprospect 5: 215-221.
  35. K.N. Ngbolua, P.T. Mpiana, D.S.T. Tshibangu, P.P. Mazasa, Z.B. Gbolo, E.K. Atibu, J.N. Kadima, F.M. Kasali, 2014. *In vitro* anti-sickling and radical scavenging activities of a poly-herbal formula (Drepanoalpha<sup>®</sup>) in Sickle cell erythrocyte and acute toxicity study in Wistar albino rats. European Journal of Medicinal Plants 4(10): 1251-1267.
  36. WHO, 1999. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva.
  37. K.O. Folashade, E.He. Omoregie, A.P. Ochogu, 2012. Standardization of herbal medicines - A review. International Journal of Biodiversity and Conservation 4(3): 101-112.
  38. A. Rasheed, S.B. Reddy, C. Roja, 2012. A review on standardization of herbal formulation. Int. J. of Phytotherapy 2(2): 74-88.

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