The Pharmaceutical and Chemical Journal, 2017, 4(1):32-40

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Phytochemical screening and assessment of anti-sickling activity of total methanolic extracts of different organs of *Curcuma longa* L. (Zingiberaceae)

Clément M. Mbadiko[1], Koto-te-Nyiwa Ngbolua[1*], Pius T. Mpiana[2], Mutambel ' Hity[3], Ruffin N. Kikakedimau[3], Gisèle K. Makengo[1], Aaron L. Pambu[1], Landry L. Kemfine[1], Gédéon N. Bongo[1], Théophile F. Mbemba[1]

[1]Department of Biology, Faculty of Sciences, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo
[2]Department of Chemistry, Faculty of Sciences, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo
[3]General Commissariat on Atomic Energy /Nuclear Center of Kinshasa, University of Kinshasa, P.O Box 868, Kinshasa XI, Democratic Republic of the Congo

Abstract The aim of this study was (1) to compare the phytochemistry of different organs of *Curcurma longa* and assess the antisickling activity of *C. longa* different apart from the rhizome. In the present study, it was shown that besides the rhizome, other organs of this plant can also have interesting pharmacological properties. The chemical screening of different organs of *C. longa* showed that secondary metabolites are not distributed in the same proportion in different parts of the plants and each part presents a different phytochemical profile. The rhizome is the storage organ of the plant and contains most of phytoconstituents (anthocyanins, bound quinones, alkaloids, tannins, triterpenoids and free quinones) as found in other organs of the plant. Nevertheless, an advanced phytochemical study is required in order to characterize the phytoconstituents of each organ and then highlight the similarity or not of these chemical compounds. The antisickling activity was evaluated using Emmel test, total methanolic extracts of rhizome, leaves, roots and floral parts (petals and sepals) used in this study showed a high activity. The minimal concentration of normalization was 31.25 µg/ml for the total methanolic extract of leaves and 62.5 µg/ml for other organs. This study demonstrated that *C. longa* could be used as an alternative for the treatment of sickle cell anemia. Bioguided fractionation studies are useful for identifying the (s) active ingredients and/or the synergy between different phytoconstituents.

Keywords Phytochemical screening, *Curcuma longa* L, antisickling activity, sickle cell anemia

1. Introduction

Curcuma longa is a perennial herb belonging to the family of Zingiberaceae. The intense cultivation of this plant is due to the use of rhizomes in the different recipes than medicinal [1-5]. The powdered rhizome is used as a food spice to enhance food flavor, food conservation and as a coloring agent for food and textiles, even in cosmetic products, dyes and medicines [12]. They are used in Ayurvedic medicine for treating asthma, allergies, hepatic disorders such as jaundice, anorexia, rheumatism, colds and sinusitis, etc. [1-4]. In Chinese medicine, they are used to treat abdominal pain and they are also recognized for their anti-inflammatory properties. Thus, centuries of rhizome uses of this plant as a food and traditional medicine have shown its safety [1-3].

In vitro and *in vivo* studies in animals and humans have shown that extracts of rhizomes of *C. longa* have broad pharmacological potential such as anti-cancer, anti-inflammatory, healing, cholesterol-lowering, hypoglycemic, anti-Alzheimer's, antiplasmodial, anti-inflammatory, antioxidant, antibacterial, antifungal, anti-venom, antipyretic, analgesic, inhibits the action of the integrase of HIV-1 replication and integrase protein of HIV-1, protects against diabetic retinopathy and numerous other pathologies [6-7]. The rhizomes of *C. longa* and other species of the genus are related to the presence of abundant terpene essential oils, as well as quasi-exclusive molecules and specific Zingiberaceae: the diarylheptanoids (curcuminoids) whose leader is curcumin that it is considered as responsible of most pharmacological actions [8-10]. Curcuminoids, the coloring principles of the drug, are also the most interesting compounds at the medicinal level [2, 12]. Other major curcuminoids are demethoxycurcumin, bis-demethoxycurcumin and cyclocurcumine which were recently identified [4]. The phytochemical screening studies by Boukri [11], revealed the presence of alkaloids, gallic tannins, flavonoids, cardiac glycosides, anthocyanins, anthraquinones, terpenoids and volatile compounds in the extracts of *C. longa* rhizomes [12].

An overview of previous studies on *Curcuma longa*, has allowed us to see that the majority of these studies focused only on the rhizomes of the plant. Studies on other parts of the plant: leaves, roots and floral pieces remain poor or unrealized. What justifies the realization of this study of which the purpose was to compare the phytochemistry of different organs C. *longa*. Later, this simple phytochemical screening could raise the interest characterizing the secondary metabolites of various organs of *C. longa*. Furthermore the bioguided fractionation of these extracts from different organs of *C. longa* could open doors to the discovery of new active ingredients. Most of these studies were focused only on the rhizome extracts of the plant and then no studies on antisickling activity have been reported. This justifies our interest in evaluating the antisickling activity of extracts from various organs of *C. longa* L.

Sickle cell disease is a hereditary and genetic blood disorder in which the normal hemoglobin A is replaced by sickle hemoglobin S. The affected red blood cell takes the form of a holly leaf, sickle and solidifies. Transmission is autosomal recessive i.e. the two copies of the same gene (one from each parent) must be carriers of the abnormality for the disease to be expressed. In this case, parents are generally not affected by sickle cell disease, but they hold the line and have a normal gene that compensates for the mutated gene.

At the molecular level, it is a point mutation caused by the substitution on the α-chain of globin in position 6, a glutamic acid (negatively charged) by valine (neutral). This mutation reduces the affinity of hemoglobin for oxygen and substantially reduces the solubility of hemoglobin S in its non-oxygenated form (deoxyHbS). Thus, once the oxygen partial pressure drops, the hemoglobin S becomes very slightly soluble. Then it polymerizes with other hemoglobin S molecules and crystallizes in the red blood cell becoming sickle. Sickling predisposes red blood cells to early hemolysis [18]. In fact, the sickle red blood cells are stopped by the filter which is the spleen where they are all destroyed. This destruction causes a decrease red blood cell and thus a regenerative anemia, called sickle cell anemia [12].

In addition, the sickle red blood cells can clog small blood vessels and then block blood flow. A poorer blood oxygenation and blood vessel occlusion in sickle cell patients lead to acute chronic pains, severe bacterial infections and necrosis [13]. Chronic manifestations of sickle cell disease involve a delay of height and weight as well as nutritional deficiencies [12]. The disease is reported in infants but is usually not manifested at birth because the red blood cells of newborns still contain 50-90% of fetal hemoglobin. The symptoms of this disease can appear from the age of six months [12]. It is estimated that every year in the world, and mostly in low- and middle-income, more than 300,000 children are born with sickle cell disease. About 5% of the world population are healthy carriers of the sickle cell gene. This percentage reaches 25% in some areas (tropical regions of Africa) [13]. Sickle cell disease that was formerly considered a disease from tropical regions only is now throughout the world due to the population migration [13, 14, 25].

The epidemiological status of the disease in the world today is characterized by several major facts: the lack of effective antisickling chemotherapy at the clinical level and predictive genetic or early clinical marker of individual severity and the basic care of sickle cell patients based on heavy treatments with risks such as toxicity, erythrocyte incompatibility reaction graft against host [23-24]. Because of the high loss of human lives in the world due to sickle cell disease [14, 21] whose the most vulnerable layer is made of children under 5 years old [14]. Several treatment

methods have been used such as bone marrow transplantation, gene therapy, blood transfusion and the taking of hydroxyurea in order to relieve patients. It turns out that these treatments are not only ineffective and very expensive for the poor African populations but can also be a risk of HIV/AIDS contamination [14, 17, 24].

Henceforth, the need for an alternative therapy is required. Nowadays, the best solution is to incorporate medications based on plants in the health system as a therapeutic alternative. Currently, herbal medicine is used as an alternative in Africa precisely in DRC giving relief to sickle cell patients. As a matter of fact, natural products are a possible source of new types of drugs that can generally fight against several diseases and particularly against sickle cell anemia.

The positive impact of medicinal plants in the treatment of sickle cell disease is well established nowadays. In Democratic Republic of the Congo, precisely in the natural products and medicinal chemistry laboratory where the phytochemical investigation of Professor Mpiana's research team of University of Kinshasa confirmed the antisickling activity of more than 100 Congolese plants. This activity would have been due to anthocyanins [14-22].

2. Materials and Methods

2.1. Plant material

The following plant materials: rhizomes, roots, leaves and flower parts (sepals and petals) of *C. longa* were used. The plant was identified at herbarium of the National Institute of Studies and Agronomic Researches, Faculty of Sciences, University of Kinshasa.

2.2. Collection and sample conditioning

First, the plant was cultivated in monoculture in an experimental setting and different organs were harvested. The harvest of rhizomes and roots was made at the beginning of July 2015. Besides, rhizomes were used to cultivate new plant in order to harvest the leaves and floral parts. The floral parts were harvested in November 2015; and it was observed that *C. longa* renews its petals everyday i.e. each 24 petals degrade in order to be replaced by other newly formed. Thus the removal of the petals was performed every day (between 22 November and 12 December 2015). The leaves were collected 5 months after the cultivation of the plant.

Different ways were used for drying the samples:

- Rhizomes and roots were dried at laboratory temperature under shade. First, they were cut into small pieces in order to increase the contact surface with heat. The drying process for rhizomes lasted up to a month. Leaves were also cut into small pieces and dried at 30 °C in the incubator (Melag Nur Für Wechselstrom).
- The floral parts were only dried at laboratory temperature under shade. After drying samples, these were ground using an electric grinder.

2.3. Phytochemical screening.

The phytochemical screening is a chemical screening that includes a number of qualitative analysis that allows their identification of secondary metabolites present in a certain sample. The detection of these chemical groups is performed through color and precipitation reactions occurring with the addition of specific reagents [12, 13].

2.4. Assessment of the antisickling activity

2.4.1. Biological Material

Blood samples used in our experiments were taken from Centre de MédecineMixteetd'Anémie SS (CMMSS) from sickle cell patients. In the lab, the blood samples obtained were stored in the refrigerator and performed Emmel's test 24 h later to ensure that these blood samples obtained from the hospital were really taken from sickle cell patients.

2.4.2. Obtaining total methanol extracts

Using a balance, 25 g of roots and rhizomes, 20 g of leaves, 3.69 g of sepals and 5 g of petals were weighed respectively and then were macerated in 75 ml of methanol for 24 hours. The macerated were filtered using a filter paper and then put in an oven for 5 days. The antisickling activity of total methanolic extracts from different parts of *C. longa* was evaluated by Emmel's test, as described by several authors, including [14-24].

3. Results and discussion

3.1. Phytochemical screening.

The experimental results of phytochemical tests on different extracts of *C. longa* organs are indicated in Table 1 below.

Table 1: Phytochemical screening on different organs of *C. longa*

Search of phytochemical compound		**Used parts**				
Aqueous Extract	**Chemical groups**	**Rhizomes**	**Roots (bulbs)**	**Leaves**	**Sepals**	**Petals**
Aqueous	Polyphenols	+++	+++	+++	+++	+
	Flavonoids	-	-	-	-	-
	Anthocyanins	++	-	++	++	-
	Leucoanthocyanins	-	-	-	-	-
	Bound Quinones	++	-	-	-	-
	Alkaloids	+++	+++	+++	+++	++
	Tannins	+++	+++	-	++	-
	Saponins	-	-	-	-	-
Organic	Steroids and triterpenoids	+++	++	++	++	-
	Free Quinones	+++	+++	-	-	-

Legend:

+++ indicates a very strong concentration
++ indicates an average concentration
+ indicates a weak concentration
- indicates zero presence

The analysis of results as reported in Table 1 shows that:

- Secondary metabolites are not distributed in the same proportion in the tissues of *C. longa* organs. Almost the majority of phytoconstituents detected in extracts of leaves, roots, floral parts (petals and sepals) were also highlighted in the extracts of rhizomes. This could be justified by the fact that the rhizome is the place of storage of metabolites. Nevertheless, it has to be noted that phytoconstituents (Flavonoids, Anthocyanins, Leucoanthocyanins, Bound quinones, Alkaloids, Tannins, Saponins, Steroids, triterpenoids and Free Quinones) distributed in different organs of *C. longa* are not necessarily identical at the structure level. Henceforth, the need for advanced phytochemical analyzes.
- Regarding steroids and triterpenoids, Table 1 indicates the existence of a mixture of two phytoconstituents in an average amount in roots and leaves extracts. This presence is certified by a violet color of extracts just after the addition of Lieberman Burchadat reagent. On the contrary, the presence of large quantities of individually triterpenoids was revealed in the rhizomes while that of steroids has been demonstrated in the sepals. As mentioned above, the triterpenoids have a purple color while steroids have a green color after the addition of Lieberman Burchadat reagent in plant extracts.
- A strong presence of total polyphenols and alkaloids was revealed in rhizomes, roots, leaves and sepals extracts while this presence was low in petal extracts.
- The average content of anthocyanins content was revealed in extracts of rhizomes, leaves and sepals. This presence is relatively zero in roots and petals extracts.

- A strong presence of free quinines was revealed in rhizomes and roots extracts. However, this presence is zero in leaves and floral parts extracts (sepals and petals). The presence in average amount of free quinones was revealed only in the rhizome extracts. The presence of saponins was null for all the samples.

The petals extracts analysis revealed that total polyphenols were in small quantities and alkaloids in average quantity. Other phytoconstituents are virtually absent. We believe this may be due to the influence of conditioning and drying this section of the plant after harvest. Indeed, in our study, we noticed that after the harvest of the petals where these are not well conditioned (cut into small pieces and spread in a less humid and ventilated), they softened a few hours after. And then when drying the petals cut beforehand to small pieces and spread on a nylon bag at laboratory temperature became soft and reduce the intensity of their coloring. We believe that this decrease in color and consistency is due to the degradation of these phytoconstituents. The results of this study go along with [11] but have some divergence. The phytochemical screening conducted by [11] revealed the presence of alkaloids, gallic tannins, flavonoids, cardiac glycosides, anthocyanins, anthraquinones, terpenoids and volatile compounds.

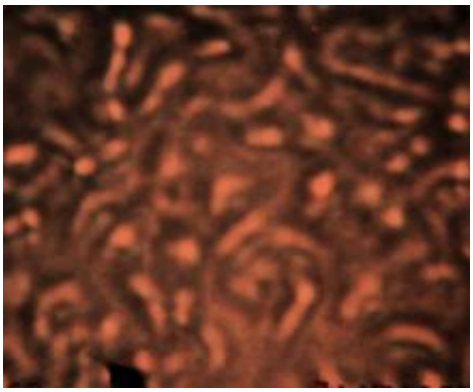
3.2. Evaluation of the antisickling activity of different extracts

3.2.1 Extraction yield

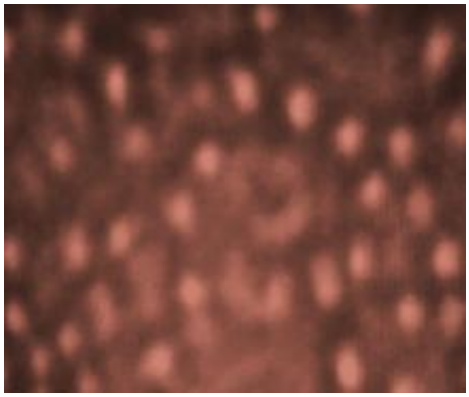
After extraction with methanol and drying in the oven, we obtained 0.71 g and 0.4 g of rhizomes and roots respectively. Then, out of 20 g of leaves, 3.69g of sepals and 5 g of petals 5g, we obtained 1.12g, 0.23 g and 0.19 g of methanol extracts respectively.

3.2.2 Evaluation of the antisickling activity of different extracts

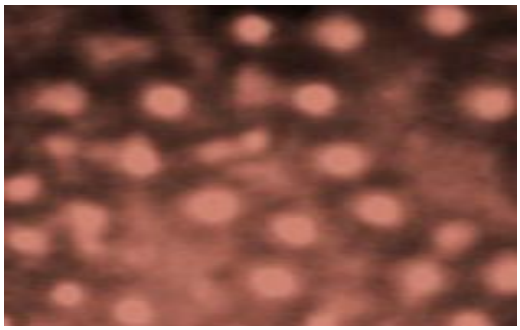
Figures below illustrate the morphology of the SS blood erythrocytes and their structure in the presence of total methanolic extracts from different organs of *C. longa*. Figure 1 shows that all cells have the sickle form under hypoxia conditions.



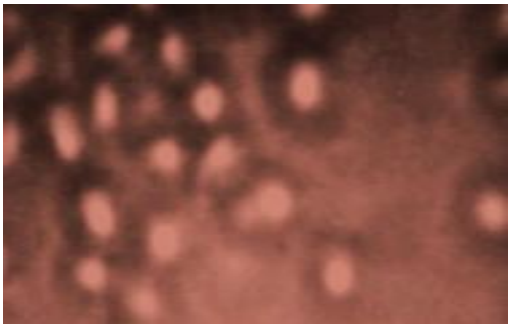
1a. Control (SS blood alone)



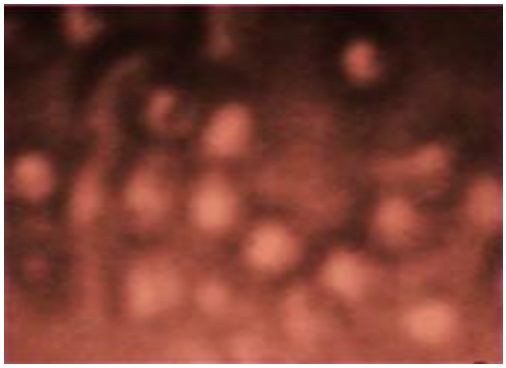
1b. SS blood + Total methanolic extract of the leaves (62,5 µg/ml).



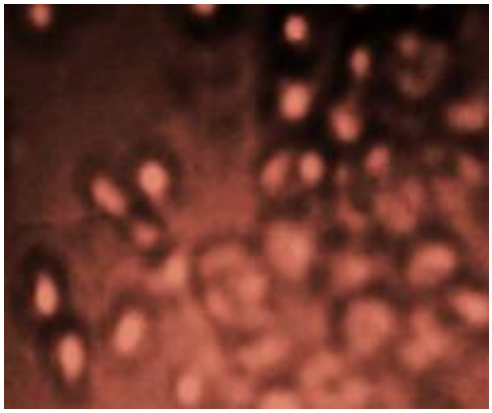
1c. SS blood + Total methanolic extract of the rhizomes (500 µg/ml).



1d. SS blood + Total methanolic extract of the petals (125 µg/ml)



1e. SS blood + + Total methanolic root extract (1 mg/ml)



1f. SS blood + + Total methanolic extract of the petals sepals (125 µg/ml)

Figure 1: Morphology of untreated sickle blood (1.a) or SS blood treated with plant extracts under hypoxia conditions (b-f) (X500), [NaCl 0.9%; Na2S2O5 2%].

On the contrary, remaining figures illustrate microscopic preparations with plant extracts showing different forms of sickle cells under the same conditions. The cells were found to have their normal or biconcave form. We believe that these changes result from the presence of total methanolic extracts of different organs of *C. longa*.

The extracts from all parts of the plant have shown an interesting antisickling activity. The minimum normalization concentration was of 31, 25µg/ml for the total methanolic extracts of the leaves and of 62.5 µg/ml for other organs. These results confirm our hypotheses: (i) extracts of *C. longa* rhizomes have antisickling activity and (2) besides the rhizome of *C. longa* considered as the interesting part pharmacologically, other organs of the plant as well have pharmacological properties.

Mpiana [15-22] and Ngbolua [24] reported the antisickling activity of plants is generally due to anthocyanins and organic acids from plants. Boukri[11] reported that rhizomes, leaves and sepals of *C. longa* have large amounts of anthocyanins after screening these organs. These chemicals could be at the basis of the antisickling activity of extracts of different organs of *C. longa*.

Besides the action of anthocyanins, we believe that the antisickling activity of the extracts from the organs of *C. longa* could also result from the presence of curcuminoids, including curcumin of which is attributed the responsibility for major pharmacological properties of this plant (such as anti-cancer, anti-inflammatory, healing, cholesterol lowering, hypoglycemic, anti-Alzheimer, antiplasmodial, antioxidant).In addition, it was reported also that curcumin and its derivatives inhibit the oxidation of hemoglobin at a low concentration, or 0.08 mM [26].

The content of curcuminoids in total organic extracts rhizomes of *C. longa* ranges from 5-8%. Curcuminoids are pigmented principles of the rhizome of *C. longa* powder and they are rich in phenolic molecules of which 50 to 60% are represented by the curcumin mixture (diféruloylméthane) up to 70-76% of monodemethoxycurcumin (16%) and bisdemethoxycurcumin [1-4].

Conclusion and Recommendations

In view of the results found in this study, we think that besides the rhizome of *C. longa*, other organs of the plant also have interesting phytoconstituents that deserve a particular phytochemical exploration. These phytoconstituents could be used in the pharmaceutical and/or food processing. As well this study demonstrated for the first time that total methanolic extracts from different organs of *C. longa* have antisickling activity. At part from the remarkable pharmacological properties of *C. longa* reported by numerous studies, this plant could be used for the manufacture of herbal medicines that can be useful in the treatment of sickle cell disease.

Further studies are needed for:

The dosage of different phytoconstituents of *C. longa* different organs is required in order to determine the content of these compounds in the whole plant. As well, the separation, characterization and/or identification of these secondary metabolites through bioguided fractionation for *C. Longa* different organs are also required in order to highlight the similarity or the difference of the active compounds and extract anthocyanins, organic acids and curcuminoids. At last, the achievement of pharmacological studies in order to assess their antisickling, antibacterial, antiparasitic, antifungal, antioxidant, hypoglycemic activities and toxicological studies of the extracts from leaves, roots, floral parts of *C. longa*.

References

1. Hombourger C. Turmeric longa of spices him in the medicine. Doctoral thesis, University Henri Poincare-Nancy 1, Faculty of pharmacy. 2010; 222p. (http://www.culture.gouv.fr/culture/infos-pratique/droits/protection.htm)
2. Cheikh Z. A. Chemical and biological studies of *Aframomum spectrum* (Zingibéraceae) and the curcumine. Doctoral thesis, University Paris-sud, Faculty of pharmacy of Chatnay-Melabry.2013; 161pp. (http:// www.tel.archives-ouvertes.fr)
3. Perry Marie Claude M. C. Evaluation of the curcumine as cancer agent in the treatment of brain tumours. Memoire of Master's Degree in chemistry. University of Quebec in Montréal.2008; 97pp.

4. Vaquier R. L. A. Interest of nutricament in aimed anti-inflammatory in the the management of the locomotive disorders of horse. Aspects biblographiques and chemical studies. Veterinary school of Affort, Faculty of Medecine of Cretal. 2010;199pp.
5. Araujo C. C, L. L. Léon L. L. Biological activities of *Curcuma longa* L. Mem Inst Oswaldo Cruz. 2001; 96(5), 723-728 pp. (http://www.scielo.br).
6. Anbujeba Sunilson J. R, Suraj G, Rejitha, Anandrarajagopal K, Anita Gnana Kumari A. V, Promwichit P. *In vitro* antimicrobial evaluation of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* extracts as Natural food preservatives. American Journal of food technology. 2009; 4(5):192-200.
7. Sivabalan S, Anuradha C.V. A Comparative study on the antioxidant and glucose lowering effects of Curcumin Analogue trough *in vitro* assay. International Journal of pharmacology. 2010; 6(5): 664-669.
8. Niamsa N, Sittwet C. Antimicrobial activity of *Curcuma longa* aqueous extract. Journal of pharmacology and toxicology. 2009; 4 (4): 173-177
9. Nada G, Talet Y, Mujahid J, Nayyar J, Samia A. Study of the antibacterial effect of different fractions of *Curcuma longa* against urinary tract infections Isolation. Pakistan Journal of biological Sciences. 2004; 7(12): 2055-2060.
10. Bruneton J. Pharmacognosy, phytochemestry, medicinal plants. Rev. Medicinal International, 2009; Vol 6. 1288 pp.
11. Boukri Nour E.B. Contribution to the phytohemical study of the raw extracts of spices contained in the mixture Ras-el-hanout. Memoir Master's Degree, University of Sciences of t-he nature.2014; 99 p.
12. Harbone J.B. Phytochemical Methods. Chapman Hall, London. 1998; 60-66.
13. Tula Y.M. Activité anti drépanocytaire et stabilité des anthocyanes extraits des bulbes d'*Hypoxisangustifolia* et de feuilles d'*Ipomoeabutatus.* Mémoire de licence, Faculté des Sciences, Département de Chimie, Université de Kinshasa. 2011. (http//:www.memoireonline.com).
14. OMS. Drépanocytose autres hémoglobinopathies. Aide –mémoire. 2011; n°308. (www. Who.int/media centre).
15. Mpiana P.T, Tshibangu DST, Shetonde OM., Ngbolua KN. In vitro antidrepanocytary activity (anti-sickle cell anemia) of some Congolese plant.Phytomedecine.2007; 14: 192-194.
16. Mpiana P.T, Mudogo V, Tshibangu, D.S.T., Shetonde, O.M, Ngbolua, K.N, Mbala, M.B. Antisickling activity of some Congolese plants, in: Drug discovery rom African flora, The 12th Symposium of the Natural Product Research Network for Eastern and Central Africa, July 22-26; University of Makerere, Kampala, Uganda. 2007; p.45 (PS-6).
17. Mpiana P.T, Mudogo, V, Tshibangu, D.S.T, NgboluaK.N, Shetonde O.M, Mangwala, K.P, Mavakala B.K. *In vitro* antisickling activity of anthocyanins extract of a Congolese plant: *Alchornea cordifolia*. *M. Arg. J. Med. Sc.* 2007; 7(**7**), 1182-1186.
18. Mpiana P.T, Mudogo V, Tshibangu D.S.T, Kitwa E.K, Kanangila A.B, Lumbu J.B.S, Ngbolua K.N, Atibu E.K, Kakule M.K. Antisickling activity of anthocyanins from *Bombax pentadrum*, *Ficus capensisanv*, *Ziziphus mucronata*: Photodegradation effect. Ethnopharmacology. 2008; 120,413-418.
19. Mpiana P.T, Mudogo V, Tshibangu D.S.T, Ngbolua K.N, Atibu E.K, Kitwa E.K, Kanangila A.B. Antisickling activity of anthocyanins extracts of *Vigna unguiculata* (**L**.)Walp. Recent Progress in Medicinal Plants. 2009; 25, 91-98.
20. Mpiana P.T, Mudogo V, Tshibangu D.S.T, Ngbolua K.N, Tshilanda D.S.T, Atibu E.K. Antisickling activity of anthocyanins of *Jatropha curcas* L. Recent Progress in Medicinal Plants. 2009; 25, 101-108.
21. Mpiana P.T, Kitwa E.K, Kanangila A.B, Tshibangu DST, Ngbolua K.N, Atibu E.K, Kalonda E.M. (2009). Influence de la photodégradation sur l'activité antifalcémiante des anthocyanes extraits de quelques plantes de Lubumbashi (RD Congo). Ann. Fac. Sci. 2009 ; 1 : 45-53.
22. Mpiana P.T, Makelele L.K, Oleko R.W, Bokota M.T, Tshibangu D.S.T, Ngbolua K.N, Mbala M.B, Atibu E.K., Nsimba S.M. Antisickling activity of medicinal plants used in the management of Sickle cell Disease in Tshopo district, D.R.Congo, Australian Journal of Medical Herbalism. 2010; 22(**4**), 132-137.

23. Ngbolua, K.N. Evaluation de l'activité antidrépanocytaire et antipaludique de quelques taxons végétaux de la R.D. Congo et Madagascar. Thèse de Doctorat, Département de biologie, Faculté des Sciences, Université de Kinshasa. 2012.
24. Ngbolua, K.N, Mudogo V, Mpiana P.T, Malekani, M.J, Rafatro H, UrvergRatsimamanga S, Takoy L, Rakotoarimana H, Tshibangu D.S.T. Evaluation de l'activité antidrépanocytaire et antipaludique de quelques taxons végétaux de la République Démocratique du Congo et de Madagascar. J. Ethnopharmacologia 2013; 50 :19p.
25. Kaplan, JC, Delpech M. Biologie moléculaire et médecine, 2eme édition, Flammarion Médecine-Sciences, paris. 1993; p.360.
26. Unnikrishnan M.K., Rao M.N.A. Curcumin inhibits nitrogen dioxide induced oxidation of hemoglobin. Molecular and Cellular Biochemistry 1995, 146 (1): 35–37.